Allelopathy of the essential oil of *Fortunella margarita* (Lour.) Swingle) in the germination on *Helianthus annuus* L. and *Hordeum vulgare* L.

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Abstract

Fortunella margarita known as 'Kin-Kan or kumquat orange' is a citrus species cultivated in South America with excellent adaptation to different climates, especially tropical. The fruits of *F. margarita* have a spectacular essential oil content. This study aimed to evaluate the allelopathic action of essential oil from ripe fruits of *F. margarita* in different concentrations on two large crops, sunflower (*Helianthus annuus*) and barley (*Hordeum vulgare*) in vitro. Ripe fruits of *F. margarita* were collected from an orchard in Rio Verde, Goiás, Brazil in January 2024. The skin of the fruits was removed and crushed to obtain the essential oil by Clevenger. The essential oil content obtained was expressed as a percentage (%). For the chemical profile, gas chromatography with sequential mass spectrometry (GC-MS) was used. For the allelopathic assay, varying concentrations of essential oil were produced. The allelopathic test was carried out in acrylic germination boxes and kept in a D.B.O. vegetation chamber. The results were expressed as percentage of germination µL mL^{-1}. The radicle length was determined using a digital caliper and expressed in centimeters (cm). Seven major volatile compounds were obtained (D-Limonene 22%, β-Phellandrene 12%, β-Pinene 11%, Germacrene D 9%, Bicyclosexquiphellandrene 8%, *Trans*-Arbuscolene and α-Guaiene both with 5%). The essential oil demonstrated strong allelopathic activity on sunflower germination rates, especially for concentrations greater than 12.5-100 µL mL^{-1}, between 54.40 and 12.80%. Barley seeds demonstrated resistance and a rate greater than 50% at the highest concentration (100 µL mL^{-1}). Radicle length also showed a significant difference ($P < 0.05$) in all essential oil concentrations. Again, sunflower was strongly affected, with average primary roots between 0.98 and 0.11 cm and between 2.77 and 0.11 cm for barley. The essential oil from the ripe fruits of *Fortunella margarita* has an allelopathic action on germination and a toxic effect on the development of primary roots in sunflower and barley seeds.

Keywords: *Fortunella* genus, allelopathy, sunflower, D-Limonene, biological effects, germination delay.

Alelopatia do óleo essencial de *Fortunella margarita* (Lour.) Swingle) na germinação de *Helianthus annuus* L. e *Hordeum vulgare* L.

Resumo

Fortunella margarita conhecida por 'laranja Kin-Kan ou kumquat' é uma espécie cítrica cultivada na América do Sul com excelente adaptação a diferentes climas, em especial, tropical. Os frutos de *F. margarita* apresentam espetacular teor de óleo essencial. Esse estudo teve por objetivo, avaliar a ação alelopática do óleo essencial de frutos maduros de *F. margarita* em diferentes concentrações sobre duas grandes culturas, girassol (*Helianthus annuus*) e cevada (*Hordeum vulgare*) *in vitro*. Frutos maduros de *F. margarita* foram coletados em um pomar em Rio Verde, Goiás, Brasil em Janeiro de 2024. A casca dos frutos foi retirada e triturada para obtenção do óleo essencial por Clevenger. O teor de óleo essencial obtido foi expresso em percentagem (%). Para o perfil químico, foi utilizado cromatografia gasosa com espectrometria de massas sequencial (CG-EM). Para o ensaio alelopático, concentrações variadas de óleo essencial foram produzidas. A ação alelopática foi realizada em caixas de germinação de acrílico e mantidas em câmara de vegetação do tipo D.B.O. Os resultados foram expressos em percentagem de germinação µL mL^{-1}. O comprimento da radícula foi determinado utilizando paquímetro digital e expresso em centímetros (cm). Foram obtidos sete compostos voláteis majoritários (D-Limoneno 22%, β-Felandreno 12%, β-Pineno 11%, Germacreno D 9%, Biciclosexquifelandreno 8%, *Trans*-Arbuscoleno e α-Guaieno ambos com 5%). O óleo essencial demonstrou forte atividade alelopática sobre as taxas de germinação para girassol em especial para as concentrações superiores a 12,5-100 µL mL^{-1} entre 54,40 e 12,80%. Sementes de cevada demonstraram resistência e taxa superior a 50% na maior concentração (100 µL mL^{-1}). O comprimento da radícula também apresentou diferença significativa (P < 0,05) em todas as concentrações de óleo essencial. Novamente o girassol foi fortemente afetado apresentando médias de raízes primárias entre 0,98 e 0,11 cm e entre 2,77 e 0,11 cm para cevada. O óleo essencial dos frutos maduros de *Fortunella margarita* apresenta ação alelopática sobre a germinação e efeito tóxico no desenvolvimento de raízes primárias em sementes de girassol e cevada.

Palavras-chave: gênero *Fortunella*, alelopatia, girassol, D-Limoneno, efeitos biológicos, atraso na germinação.

1. Introduction

Vegetables are natural factories of special metabolites with diverse biological activities. Among them, we have the allelopathic effect. The use of chemical products with a large number of synthetic molecules to control weeds is used annually on crops around the world and it is no different here in agricultural areas in Brazil. However, several synthetic molecules have a long-lasting residual effect in the soil and can be leached into forest areas, negatively influencing the germination and development of native plants and even in bodies of water (Mathiassen et al., 2021; Ganghi et al., 2021).

As a result, several studies are being carried out in an attempt to develop a natural allelopathic compound capable of acting as a herbicide, mainly on weed species that cause great damage to farmers and ranchers around the world (Bitencourt et al., 2021). Allelopathy is a chemical process where plants naturally release into the environment compounds from their special metabolism with the function of supplying, inhibiting and also providing the function of displacement on the establishment or development of other plants in their habitat (Barrales-Cureño et al., 2022). The allelopathic potential of some vegetable species presents encouraging results in the control of germination, development and patterns of defective plants or even in the inhibition of other plant species. This control has been used in favor of agricultural development in the production of large and small crops, where the aim is to control weeds capable of harming the establishment of crops for the production of food for humans and animals (Azirak; Karaman, 2008; Chu et al., 2014).

According to Barrales-Cureño and collaborators (2022), allelopathy is a phenomenon existing in a donor plant that releases chemical substances into the environment through a certain route (e.g. leaching, residue decomposition, among others), capable of causing a synergistic or antagonistic effect. in the physiology of germination, vegetative or reproductive development when incorporated by a recipient plant. In the study of the effects caused by allelopathic substances we have two types: eccrysodynamia, the influence of metabolites from autotrophic organisms, and saproccrinodynamia, the influence of metabolites produced by saprophytic organisms.

Most allelopathic compounds released by plants are considered ‘secondary’ metabolites produced by primary metabolic pathways (Hadacek, 2002). Depending on their phytotoxic action, concentration, persistence and destination in the environment in which they are released, they may present allelopathic activity (Inderjit, 2003). According to Inderjit & Olofsdotter (2002), the allelopathic effect produced by a specific vegetable is caused by the joint action of several allelochemicals, and not by the action of just a single compound.

Several studies have been published showing the formidable allelopathic action produced by essential oil (EO), which is one of the products of the special metabolism of several plant groups (Chu et al., 2014; Kong et al., 2021; Zheljazkov et al., 2021). Essential oils have a chemical constitution very rich in oxygenated, hydrocarbon and phenylpropanoid compounds with a volatile molecular mass range (Oliveira et al., 2021). The location of EO production and secretion varies greatly, according to each botanical family. Thus, EO can occur in glandular trichomes, leaves, flowers, roots, resins and fruit peel, the latter especially in citrus (*Fortunella margarita*) (Figure 1). Not only are plant-derived essential oils prized for their nutritional value, but also for their functional and technical properties. Furthermore, due to the presence of bioactive compounds with anti-diabetic, antioxidant, anti-inflammatory, analgesic, allellopathy and other properties, the quality of these fruit essential oils is enhanced (Lakache et al., 2022; Soni et al., 2022).

Fortunella spp. belongs to the *Citrus* genus and the Rutaceae family. *Fortunella* plants, including *F. japonica*, *F.*

bawangica, *F. margarita*, are often grown in the southern part of China, Japan, Philippines, Marocco, Corfu, and Corsica. In Brazil, *F. margarita* is known as 'kin-kan orange', where it has adapted well to different climates, especially the tropical one. In cooking, *F. margarita* is used in sweets, juices and consumed naturally. The skin of the green or ripe fruit produces a high content of EO. Chemical analysis of EOs of *F. margarita* revealed that the dominant compound was D-Limonene (84.2-96.3%), followed by Myrcene (1.3-12.9%) and Germacrene D (0.3-2.4%) (Fitsiou et al., 2016; Sutour et al., 2016; Mitropoulou et al., 2022).

Studies with the Rutaceae family demonstrate positive results with allelopathic action. Anaya et al. (2005) studied the allelochemical effect on *Stauranthus perforatus* from the Rutaceae family, where they verified allelopathic activity on weed species. Studies with *F. margarita* regarding the allelopathic effect promoted by the EO of the fruits are still lacking. In this sense, it is possible to extract three important characteristics of the allelopathic effect: 1) the release into the environment of a compound in charge of transmitting an effect, 2) its absorption by the receptor organism, and 3) an effect on growth.

Therefore, this study aimed to evaluate the essential oil of *Fortunella margarita* regarding its chemical profile and its allelopathic effect in different concentrations on the germination of sunflower (*Helianthus annuus* L.) and barley (*Hordeum vulgare* L.) seeds *in vitro*.

Figure 1. *Fortunella margarita* tree in fruiting (**A**) and (**B**) ripe fruits. Source: Authors, 2024.

2. Materials and Methods

2.1 Chemical reagents and equipment

Alkane standard solution C1-C40 (Sigma-Aldrich, USA), anhydrous sodium sulfate (FMaia, Brazil), dichloromethane (Hexis Científica, Brazil), dimethyl sulfoxide (DMSO) (Synth, Brazil), GC-MS grade dichloromethane (Merck, USA).

2.2 Plant collection and identification

Ripe fruits (5 kg) of *F. margarita* were collected in January 2024 in a rural area (Antônio Menezes & Filhos Farm) located in the municipality of Rio Verde, Goiás, Brazil. The species was identified by PhD. Antonio Carlos Pereira de Menezes Filho and an esxicata was deposited in the Herbarium of IF Goiano, Campus Rio Verde, Laboratory of Plant Systematics, Department of Biology with Voucher HRV: 14091.

2.3 Essential oil extraction and yield

The fruits were washed in distilled water and the skin was removed using a stainless steel knife. The peel (100 g) was crushed in a blender with 250 mL of distilled water for 5 min. The solution was then transferred to a 1 L round-bottom flask. The essential oil was extracted using a Clevenger-type system for 2 h. After this time, the hydrolate was collected and dissolved in 30 mL of dichloromethane. The solution was homogenized using a separating funnel and left to rest for 1 min, the organic phase was collected and dried with anhydrous sodium sulphate. The EO fraction obtained had its mass determined in percentage (%) as described by Menezes Filho & Castro (2019). The amount of EO produced was 4.41%. The oil was stored in an amber bottle and kept refrigerated at -12 ºC until analysis.

2.4 Chemical profile

The chemical profile (10 µL) of *F. margarita* EO was determined by gas chromatography with mass spectrometry (GC-MS) (Shimadzu GC Mod. QP 5000), fused silica capillary column (Optima®, 5-0, 25 µm (30 m x 0.25 mm)), with electron impact ionization (IE) mass spectrometer (70 e.V). The chromatographic run time was 60 min. GC-MS grade dichloromethane was used as solvent. The identification of the chemical compounds of the essential oil was based on the *Kovats* Index, using a series of *n*-alkanes (C-1 to C-40). The retention time comparison was carried out using the Nist 11 spectro library and literature Adams (2007).

2.5 Sunflower and barley cultivars

Sunflower seeds cultivar 'Multissol' were purchased from the company Sementes Caiçara, Brazil. Cultivar derived from the 'Catissol 01' cultivar, through classic mass selection, with a cycle of 115 and 130 days (grains), with planting time for normal harvest between August and December and off-season between January and March. Plant characteristics: erect size, height up to 1.80 m, without branches and equipped with rough hair. Moderately susceptible to alternaria spot, sclerotinia and moderately resistant to rust, mildew, powdery mildew and stem canker. Seeds of barley cultivar KWS Irina were collected in a production area in the State of Paraná, Brazil, harvest 2022/2023.

2.6 Allelopathic activity

Fortunella margarita EO was diluted in dimethyl sulfoxide (DMSO) at the following concentrations (100; 50; 25; 12.5 and 6.25 µL mL^{-1}) and the negative control was distilled water. The germination test was carried out with five replications containing 25 seeds of each species (sunflower and barley), which were sown in germination boxes where the substrate was three sheets of germination paper, moistened with a volume of distilled water equivalent to three times the dry paper mass. Right after sowing *H. annuus* and *H. vulgare*, 3 mL of the EO solution at each concentration were distributed on a germitest paper, added to the lid of the germination box, that is, on the top, avoiding direct contact of the seeds. seeds with EO. Then, the germination boxes were kept in a B.D.O type germination chamber with a temperature programmed at 25 ºC and a photoperiod of 12 h of light, under a light intensity of 25 µmol m^{-2} s^{-1} as described by Rosado et al. (2009) adapted.

The evaluation of the allelopathic potential of the essential oil was carried out by determining the germination percentage (%) and root length (cm) of sunflower and barley seedlings. The evaluation of the results was carried out on the first day that the plants began to germinate (radicle protrusion) and this occurrence was observed on the second day (sunflower) and third day (barley) after sowing. The experiment was evaluated for ten consecutive days where the number of germinated seeds (%), with radicle emission of at least (2 mm) in length were obtained daily.

The experiment to determine rootlet length was set up in a similar way to germination. After 48 h, 60 seedlings (five replications of 15) with 0.5 cm radicle length were selected. Then, these seedlings were transferred to *Petri* dishes containing the EO solutions per treatment and returned to the B.D.O. After four days of incubation, the radicle length of each seedling was measured, and the average results expressed in centimeters (cm) per seedling as described by Alves et al. (2004) modified.

2.7 Statistical analysis

The experimental design was completely randomized, with 5 replications, 5 treatments and two cultures, using DMSO as a negative control. The values obtained were subjected to analysis of variance, F test, and when treatment effects showed a significant difference ($P < 0.05$), the means were compared using the regression test. The statistical program used was SISVAR (Ferreira, 2019).

3. Results and Discussion

The chemical profile of the EO from ripe fruits (Table 1) showed 27 compounds with a total of 7 major compounds with (%) area above 5%, especially for the compounds β-Pinene with 11%, D-Limone with 22%, *β*-Phellandrene with 12%, Germacrene D with 9%, Bicyclosesquiphellandrene with 8% and *Trans*-Arbusculone and α-Guaiene with with 5% each. In another study evaluating the volatile chemical composition of the essential oil of green and ripe fruits of *F. margarita* Menezes Filho & Castro (2019) identified a total of 33 and 35 compounds respectively, regarding the majority compounds, our results corroborate this study with β-Pinene 20.19 and 11.42%, D-Limonene with 15.12 and 20.76%, β-Phellandrene with 11.81 and 12.30%, α-Guaiene with 6.14 and 5.88%, Germacrene D with 4.31 and 9.00% and Bicyclosesquiphellandrene with 6.25 and 8.07%.

Other studies demonstrate approximate results regarding the chemical profile of EO from *F. margarita*, Lakache et al. (2022) analyzed the chemical profile of *F. margarita* fruits harvested in Northern Algeria in 2021 where D-Limone presented a result of 86.31% belonging to the monoterpenehydrocarbons class and Germacrene D with 4.67% to the sesquiterpenehydrocarbons class. Still in this study, 96.79% of volatile molecules belong to non-oxygenated compounds and 2.30% to oxygenated compounds. Mitropoulou et al. (2022), evaluated *F. margarita* fruits collected between January and March on the Island of Corfu, Greece, where in chemical analysis the D-Limonene content was 93.78%. It is worth mentioning that the chemical profile of a species presents variation in the chemical constitution of volatile compounds, this is due to the region, season and time of collection, fruit maturation time, influence of the rainy season, soil nutrition and even chemotypes within of a plant species. However, our results corroborate the literature for the EO of *F. margarita* (Sharifi-Rad et al., 2017; Paw et al., 2020; Chambre et al., 2020).

Table 1. Chemical profile by gas chromatography with mass spectrometry (GC-MS) of the essential oil of ripe *Fortunella margarita* fruits.

Compound	**RI***	**%**
α-Pinene	936	0.28
β-Pinene	981	**11.64**
β-Myrcene	985	1.15
D-Limonene	1032	**22.08**
γ-Terpinene	1042	2.11
Trans-Arbusculone	1073	**5.34**
Humulan-1,6-dien-3-ol	1115	0.57
Cis-Ocimene	1131	0.20
σ-3-Carene	1151	0.66
Trans-β-Ocimene	1154	0.15
α-Terpineol	1183	0.93
β-Phellandrene	1616	**12.01**
Isoledene	1365	2.48
α-Copaene	1371	0.03
Geranyl oleate	1377	0.35
Longifolene	1408	0.79
β-Panasinsene	1411	0.04
α-Guaiene	1430	**5.80**
Aromadendrene	1439	2.05
Germacrene D	1477	**9.56**
Epizonarene	1509	0.35
Globulol	1581	1.67
Myrtenyl angelate	1608	0.21
10-*Epi*-γ-Eudesmol	1616	2.98

γ-Muurolene	1680	3.46
Bicyclosesquiphellandrene	1726	**8.53**
Seline-3,7(11)-diene	1792	0.84
Total (%)		**96.26**

Note: IR* = Literary Retention Index (Adams, 2007). FV** = Green Fruit and FM** = Ripe Fruit, in percentage of peak retention. Source: Authors, 2024.

According to Table 2 and Figure 2 (A), all germination data were significant. The classical germination percentage was 98% and 96% for sunflower and barley respectively. Dias et al. (2022) found a germination rate of 94%, corroborating our findings for the same cultivar Multissol. In other studies, with different sunflower cultivars, Rossetto et al. (2021) found germination rates of 98; 77 and 83% for the cultivars Catissol 01, Embrapa 122 and IAC Iaramã; and Morais et al. (2021) for the cultivar Hélio 253 germination rate of 95-91 in two lots of sunflower seeds evaluated. In Table 2 and Figure 2 (B) for barley, Pessenti et al. (2021) found a germination rate of 70% for the BRS Cauê cultivar. Our result for another cultivar KWS Irina was superior to that observed in this study by Pessenti and collaborators (2021). As for Ullah et al. (2022) using the barley cultivar AJJ obtained a germination rate of 95% close to that of our research.

Table 2. Germination rate by analysis of variance obtained from allelopathic assays at different concentrations of *Fortunella margarita* essential oil.

FC	Germination (%)	FC	Germiation (%)
Sunflower	316.89*	Barley	101.44*
CV %	8.51	CV %	4.73

Note: FC = F Calculated. *Significant. CV% = Coefficient of variation. Source: Authors, 2024.

Data on allelopathic action on sunflower and barley seeds showed a significant linear trend as the dose of EO increased, the germination rate decreased (Figure 2, A and B), respectively (P < 0.05). It was observed that sunflower seeds demonstrated low tolerance in the presence of EO from F. margarita, where at low doses the germination rates dropped sharply from 86 (6.25 µL mL^{-1}) to 12% (100 µL mL^{-1}) when compared the witness with 100%. For barley germination values, it is possible to verify that barley presents greater tolerance in the presence of *F. margarita* EO where at the lowest dose of 6.25 µL mL^{-1} there was a slight reduction in germination of 99%. At the highest dose of 100 µL mL^{-1} this is easily observed where there was a reduction of up to 52% of germinated seeds, demonstrating that this cultivar KWS Irina is resistant to the EO complex of volatile molecules (P < 0.05).

In the study by Kaya et al. (2013), researchers evaluated barley cultivars cv. Aydanhanim, from wheat cv. Tosunbey, lentil cv. Meyveci-2001 and sunflower Sanbro MR on the allelopathic action of sunflower EOs (stem and head) parts. As for the germination rate for dosages of 2.5; 5 and 10% values of 99.0 were obtained; 98.0 and 97.0% for EO stem and 99.0 for EO head; 97.5 and 97.0% for barley. For wheat, stem EO in the same doses provided germination rates of 100; 99.5 and 99.6 and for head EO 98.5; 98.5%. For lentils, stem EO provided germination of 99.0; 98.0 and 84.8% and for head EO with 94.5; 92.5 and 89.5%. And finally, stem and head EO showed the following results 92.5; 86.0 and 37.0 and 98.0; 96.0 and 96.5% for sunflower seeds. The allelopathic effect was observed only in the experiment using sunflower seeds as a test model.

In addition to volatile compounds, plant extracts also have a potential allelopathic effect, this is discussed in the studies by Silva & Carvalho (2009) where they verified allelopathic action on seeds and seedlings of sunflower cultivar Embrapa 122, evaluating doses of extracts of *Baccharis trimera* and *Symphytum officinale*. The aqueous extract of *S. officinale* did not demonstrate allelopathic activity on the percentage of seedling emergence when comparing the control. Regarding germination results, the extract demonstrated a significant difference with an increase in the number of normal seedlings up to a dosage of 200 g L^{-1} (82%), however, lower than the control with 84%. The researchers observed that this plant extract also provided growth stimulation and greater straw mass. For the aqueous extract of *B. trimera*, dosages greater than 100 g L^{-1} showed an emergence-stimulating effect. *In vitro*, the *B. trimera* extract at a dosage of 150 g L^{-1} caused toxic effects with a high rate of abnormal seedlings after the germination test.

For barley seed germination rate, Santiago et al. (2002) evaluated the action of *Impatiens walleriana* extract at concentrations of 0.46 and 0.23 mg mL^{-1} on two cultivars CEV 95033 and Embrapa 127 with germination of 63.3; 46.6 and 66.6; 60.0%, respectively. In this study, it is possible to observe that the dosages of *I. walleriana* leaf extract provided a promoting action, thus increasing the germination rate. In this case, no allelopathic effect was observed in the two barley cultivars evaluated.

Comparing our germination results with other crops, as described by Oliveira et al. (2021), where they evaluated the EO of *Blepharocalyx salicifolius* on the allelopathic action in *Sorghum bicolor* seeds where there was no significant difference between the doses and the positive and negative controls. As for *Lactuca sativa* seeds, they demonstrated a significant difference between treatments with EO at doses 5.0 and 7.5% compared to the control. At the highest concentration of EO 7.5% with only 80% germinated seeds. Our theory corroborates Oliveira et al. (2021) and Duke et al. (2002) that EOs are possible natural bioherbicides and can be effective in inhibiting the germination and development of weeds, as observed in this and other studies presented here.

Isolated volatile compounds with a high percentage in an EO, such as eucalyptol, have an action in inhibiting the sprouting of Solanum tuberosum tubers and are also involved in inhibiting the synthesis or action of gibberellins. Gibberellins are involved in the regulation of growth, stem elongation, germination, flowering, leaf sprouting, fruits and cellular senescence (Taiz; Zeiger, 2004; Suttle et al., 2016). The α-Pinene, β-Pinene, α-Phellandrene, β-Phellandrene, σ-3-Carene, Bornyl acetate, Citronellal, Camphor, 1,8-Cineole, Thymol, Geraniol, Menthol, Borneol, α-Terpineol and Terpi- nen-4-ol are also considered toxic compounds found in several EOs where they present allelopathic activity, inhibiting seed germination in numerous plant species, including those of agricultural interest (Abrahim et al., 2000; Zahed et al., 2010; Kennedy et al ., 2011).

As observed in our EO chemical profile results (Table1), D-Limonene as one of the major compounds in the skin of ripe *F. margarita* fruits is also reported by Zhao et al. (2016) as an inhibitor of cell development and photosynthetic reducer evaluated in *Chlorella vulgaris* algae. Other compounds such as *Trans*-Verbenol with 11.89%, Kaurene with 10.88%, Benzofuran with 13.65% and Calarene with 9.35% in sunflower essential oil (stem) demonstrated inhibition of germination in sunflower seeds.

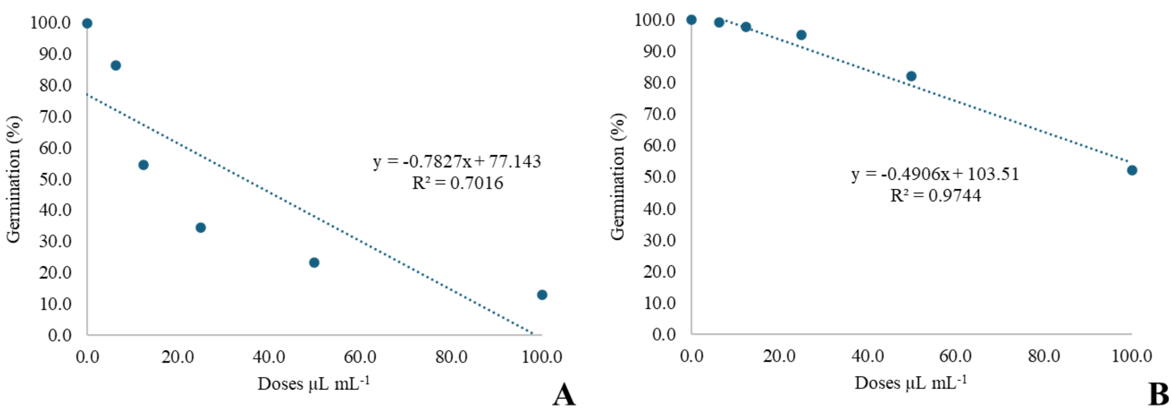

Figure 3. Germination percentage of sunflower (**A**) and barley (**B**) subjected to different concentrations of *Fortunella margarita* essential oil. Source: Authors, 2024.

The essential oil of *F. margarita* showed allelopathic action on the primary root data in sunflower and barley when compared with the controls. There was a significant difference with ($P < 0.05$) (Table 3). Dosages between 6.25 and 100 µL mL^{-1} of oil demonstrated a drastic reduction in root length, especially for sunflower with averages between 1.0 and 0.1 cm when compared to the control with 2.9 cm. For barley, the average variation was between 2.77 and 0.12 cm compared to the control with 3.40 cm (Figure 4).

Kaya and collaborators (2013) evaluated the allelopathic action of sunflower essential oil (stem and head) on the primary root development of barley and sunflower with results of 3.84; 2.61 and 2.18 and 3.49; 3.04 and 2.60 cm, and between 6.15; 1.87 and 1.72 and 4.89; 1.85 and 1.0 cm respectively for doses 2.5, 5 and 10%. The authors present as a theory about the allelopathic effect, the majority volatile compounds of sunflower essential oils which, as previously discussed, the majority compounds of the oils present allelopathic studies on the development of vegetables. In studies by Yoshimura et al. (2011) researchers discuss the action of the compound eucalyptol present as the main compound isolated in EO, where it showed activity in controlling the proliferation and elongation of cells in tobacco plants, detecting its inhibition. Another study I discussed on the allelopathic, and toxic activity of volatile compounds was developed by Sánchez-Muñoz et al. (2012) where they found that β-Caryophyllene has an allelopathic action in inhibiting root elongation in plants. These researchers studied this activity by evaluating this isolated volatile compound in *Physalis ixocarpa* and *Echinochloa crus-galli* plants between concentrations of 50 and 150 µg mL^{-1}.

Plant extracts also present interesting results on root development. Silva & Carvalho (2009) found a toxic effect on the primary root system in Empraba 122 sunflower seeds, using an aqueous extract of *S. officinale*. A high number of seedlings without primary roots was obtained at dosages greater than 100 g L^{-1}. For the abnormality, absence of secondary roots, curled hypocotyl, hard, dead and dormant seeds, the authors did not find statistical significance when compared to the control. The *B. trimera* extract at a dosage of 50 g L^{-1} showed a lower number of seedlings in the primary root. Regarding root length, Oliveira et al. (2021) did not observe a significant reduction in the root system in L. sativa in the EO doses of *B. salicifolius*, although for *S. bicolor* the EO showed a positive effect with allelopathic action for plant length and the root system in all doses 2.5 to 7.5%. It is possible to argue that the compounds of EO *B. salicifolius* do not present a positive interaction with a potential to reduce root development throughout the germination process.

Table 3. Root length by analysis of variance obtained from allelopathic assays at different concentrations of *Fortunella margarita* essential oil.

FC	Root length (cm)	FC	Root length (cm)
Sunflower	260.81*	Barley	330.56*
CV %	24.44	CV %	11.51

Note: FC = F Calculated. *Significant. CV% = Coefficient of variation. Source: Authors, 2024.

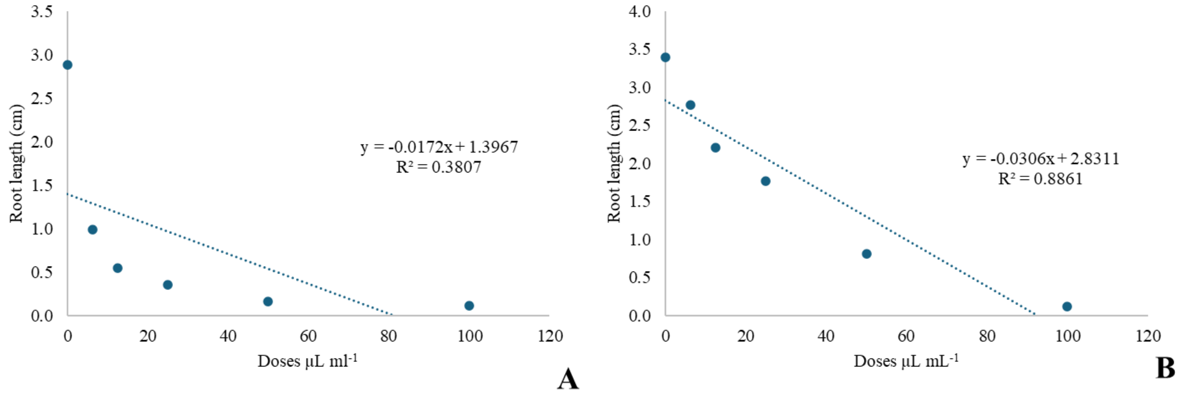

Figure 4. Root parameter (root length) on the allelopathic effect of the essential oil from the fruit of *Fortunella margarita* on (**A**) sunflower seeds and (**B**) barley seeds at different concentrations. Source: Authors, 2024.

4. Conclusions

The essential oil from the ripe fruits of *Fortunella margarita* presented a rich amount of volatile oxygenated and hydrocarbon compounds. As for allelopathic action, the different doses in particular (12.5-100 µL mL^{-1}) demonstrated high allelopathic activity on the germination rate, where sunflower seeds were highly susceptible to the action of the oil's volatile compounds. The same is observed for the length of the radicle where the essential oil of *F. margarita* demonstrated to be toxic with an inhibitory effect on the cellular propagation of the primary root.

5. Acknowledgments

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6. Authors' Contributions

Bárbara Mendes Cavalheiro: study design, writing the methodology, *in vitro* experimentation, extraction of the essential oil, statistical analysis, identification of volatile compounds and writing the article. *Anísio Correa da Rocha*: research coordinator, advisor, text correction, final evaluation and publication.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

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