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ALLELOPATHIC EFFECT OF *Elettaria cardamomum* ESSENTIAL OIL VAPOURS ON THE WINTER SEED MYCOFLORA AND GERMINATION

Simona NIȚĂ, Diana OBIȘTOIU, Anca HULEA, Lucian NIȚĂ, Ilinca Merima IMBREA, Ștefan Laurențiu BĂTRÎNA, Lucian BOTOȘ

University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului Street, Timisoara, Romania

Corresponding author email: dianaobistioiu@usvt.ro

Abstract

Developing agroecosystems to reduce yield losses, effective use of water resources, and environmentally friendly innovative biotechnological interventions to stop using more chemical pesticides and fertilisers have been the focus of several sustainable strategies. Our research aimed to identify the allelopathic in vitro effect of Elettaria cardamomum essential oil (ECEO) on wheat seeds regarding the antifungal efficacy and germination stimulation. Surface-sterilized seeds (n = 100) were transferred in sterile Petri dishes (Ø 120 mm), and the ECEO doses were 5 µL, 25 µL, 50 µL and 100 µL. The Petri dishes were transferred to a growth chamber and kept at 25 °C in the dark for 5 days. Consequently, the microflora and germination rate was assessed on the PDA medium. The results suggest that the minimum concentration needed for ECEO to affect the seeds contamination index was at 25µL, while values of 50µL and 100µL inhibited the germination, resulting in germination rates at 86% and decreasing to 43%. ECEO oil exhibited promising antifungal activity and seems to have a potent fumigant activity against wheat mycoflora and could be used as possible future natural agent in agriculture.

Key words: *antifungal, Elettaria cardamomum, essential oil, germination, wheat seed.*

INTRODUCTION

Researchers are focusing on finding alternative methods to effectively fight and control microbial infections while being environmentally friendly and not compromising plant germination (Hulea at al., 2022; Alexa et al., 2020). These methods can have a significant impact on agriculture by reducing the use of harmful pesticides and promoting sustainable farming practices. In addition, using plant growth-promoting microorganisms has emerged as a promising approach for sustainable food production. By harnessing the beneficial properties of microorganisms, such as *Eleteria cardamomum* and plant growth-promoting microorganisms, researchers aim to develop eco-friendly solutions that can effectively combat microbial infections without negatively affecting plant germination. This approach aligns with the intention to promote stable grain production, green development in agriculture, and sustainable farming practices. *Elettaria cardamomum* is an aromatic, herbaceous, perennial plant belonging to the *Zingiberaceae* family and *Elettaria* genus. Its essential oil (ECEO), produced by various methods, contains a combination of various volatile lipophilic compounds, in some cases more than 100, that possess several biological activities such as antioxidant, anticarcinogenic, and antimicrobial effects (Ivanović et al. 2021; Al-Zereini et al., 2022; Castillo et al., 2023).

The antimicrobial effect of ECEO was demonstrated against *Penicillium* spp., *Pacelomyces* spp., *Mucor* spp., and *Aspergillus* spp. (*Aspergillus nigerxerophilic*, *Aspergillus ochraceus, Aspergillus flavus, Aspergillus parasiticus)* (Saleh et al., 2011; Naz et al., 2023; Ibrahim et al., 2017). Although the antifungal activity of the extract of *Elettaria cardamomum* against *Fusarium* spp. has been demonstrated (Aliaa et al., 2016), there is no data in the specialised literature regarding the antifungal activity of the essential oil from this plant against this mycotoxigenic fungus.

The study aimed to highlight the antifungal activity of ECEO, and the ability to stimulate germination of wheat seeds.

MATERIALS AND METHODS

Treatment of wheat seeds

The fumigation method used in the present study was described by Bota et al., 2022. Briefly, the wheat seeds were washed with 1:9 (*v*/*v*) sodium hypochlorite solution and then rinsed three times with sterile distilled water. To obtain a relative humidity of 14%, the seeds were dried in an oven at 100°C. Seeds (n = 100) were transferred in sterile Petri dishes (Ø 120 mm), and exposed to different concentrations of ECEO (doTERRA (Pleasant Grove, Utah, USA) vapours.

The concentrations tested, respectively 5 µL, 25 µL, 50 µL and 100 µL were added to a sterile filter paper and inserted into each Petri dish with wheat seeds. The dishes were kept in the dark at 25°C, for five days (Figure 1).

Figure 1. Sterile Petri dishes with wheat exposed to different concentrations of ECEO vapours

Triplicate samples were performed.

Analysis of Fungal Contamination

Direct plating technique on PDA (Potato Dextrose Agar, CM0139, Oxoid, Thermo Fisher Scientific Inc.) medium was performed to detect the fungal growth.

After exposure to vapours of different concentrations of ECEO, the wheat seeds were transferred to Petri plates containing PDA medium and were kept in the dark at 25°C.

Fungi were isolated for their identification on the 5th day.

The following formula was used to calculate the frequency of each fungal genus from the total fungal genera:

$$Fr\% = NG/TNF * 100$$

where:

NG - number of colonies from the genera;

TNF - total number of fungi.

The seed contamination index was calculated according to the formula:

$$SCI = NCS/TNS$$

where:

NCS - number of contaminated seeds;

TNS - total number of seeds in a petri dish.

Percentage of germination

Germination was calculated on the 10th day according to the formula:

$$G\% = NGS/TNS$$

where:

NGS - number of germination seeds;

TNS - total number of seeds in a plate.

RESULTS AND DISCUSSIONS

The identified species presented in the control samples were represented by *Fusarium* spp. (60%), *Alternaria* spp. (10%), *Penicillium* spp. (10%), and others (10%).

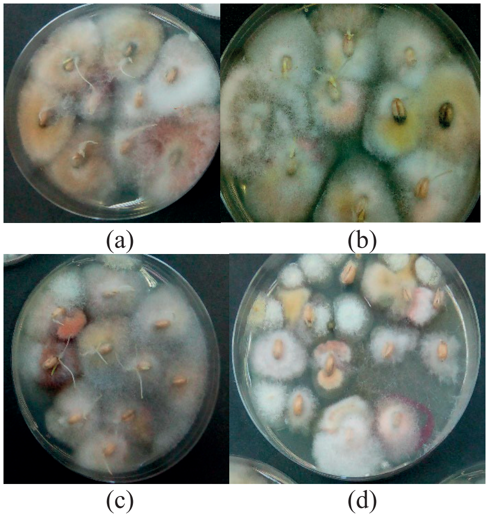

Figure 2. Petri dishes presenting the effect of different concentrations of ECEO on wheat seeds: (a) 5 µL; (b) 25 µL; (c) 50 µL; (d) 100 µL

The fungal contamination of wheat seeds after exposure to different concentrations of ECEO vapours is presented in Figures 2 and 3.

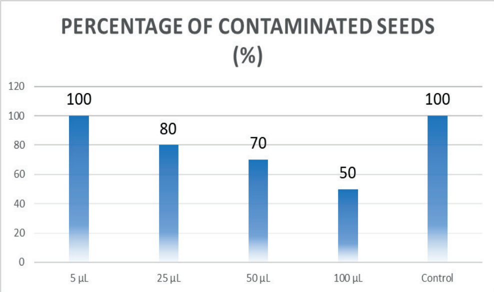

Figure 3. The percentage of contaminated seeds exposed to different concentrations of ECEO vapours

As is observed in the previous figure, the percentage of contaminated seeds in the presence of 5 µL of ECEO remains at the same value as the control samples, which means that this essential oil concentration doesn't have any antifungal activity. However, a concentration of 25 µL decreased the total number of contaminated seeds by 20%, while a concentration of 50 µL reduced the number of contaminated seeds by 30% compared with the control samples. The greatest reduction in the number of contaminated seeds was observed by exposure to a concentration of 100 µL, with the percentage reaching 50%.

Like the control sample, the dominant species was represented by *Fusarium* spp. followed by other species.

The germination percentage of the wheat seeds in the presence of the different concentrations of the ECEO is presented in Figure 4.

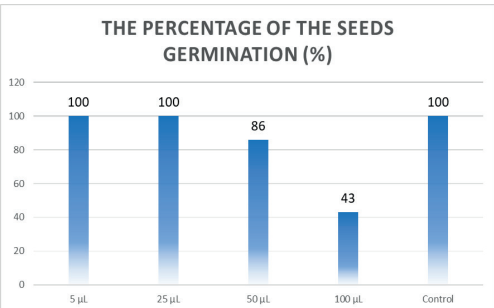

Figure 4. The percentage of seeds germination exposed to different concentrations of ECEO vapours

Regarding the germination rate, this had maximum values in the case of the control samples and those exposed to doses of 5 µL and 25 µL ECEO, respectively. The increase in the essential oil concentration determined a decrease in the germination percentage. Thus, 50 µL determined a germination percentage of 86%, and a concentration of 100 µL determined a reduction of the germination percentage by up to 43%.

The antifungal activity of essential oils has been demonstrated by numerous authors, who have shown that the effectiveness varies from one oil to another depending on its chemical composition and the fungal strain. Thus, Perczak et al., 2019, demonstrated that *Cinnamomum zeylanicum*, *Origanum vulgare, Cymbopogon martini, Thymus hiemalis, Mentha viridis, Foeniculum vulgare dulce*, and *Aniba rosaeodora* essential oil significantly inhibited the growth of *Fusarium graminearum* and *F. culmorum* (90.99-99.99%). In steed, orange essential oil inhibits the growth of *Fusarium* species in a smaller percentage, respectively of 68.33% (Perczak et al., 2019). Kedia et al., 2016, demonstrated that *Mentha spicata* essential oil produced 52.2% inhibition of *Alternaria flavus* at 1 µL/mL (Kedia et al., 2016). On the other hand, Li et al., 2016, demonstrated that *Litsea cubeba* essential oil determined 100% inhibition of *A. flavus* growth at 5 µL/g after 20 days (Li et al., 2016). *Rosmarinus officinalis* L. essential oil inhibited the growth of *A. flavus* at a percentage of 73.5% at 1.5 µL/mL (Prakash et al., 2015). The present study demonstrated that *Elletaria cardamom* essential oil had antifungal activity against *Fusarium* spp., *Penicillium* spp., *Alternaria* spp. All these prove that the antifungal effects of different essential oils vary depending on the chemical composition, which in turn depends on the geographical area and the climate in which the plant grew, as well as on the extraction method of the product.

The literature has shown that EO has antifungal activity against *Fusarium graminaerum* (Alexa et al., 2018). Furthermore, research has demonstrated that EO has an antifungal impact on *Aspergillius sp., Fusarium* sp., *Penicillium* sp., and *Verticillium dahliae* (Arslan et al., 2010; Kocic-Tanackov et al., 2013). EO has antifungal activity against *Fusarium graminaerum* (Alexa et al., 2018). Furthermore, research has demonstrated that EO has an antifungal impact on *Aspergillius* sp., *Fusarium* sp., *Penicillium* sp., and *Verticillium dahliae* (Arslan et al., 2010; Kocic-Tanackov et al., 2013). Also, studies show that the chemical composition of the essential oil can affect the germination rate, but most of them claim that an increase in oil concentration determines the decrease of this rate. Mirmostafaee et al., 2019 demonstrated that the germination rate of seeds exposed to *A. officinalis* essential oil varied, with an average of approximately 33% in control samples and slight modification, independent of seed age. The increase in essential oil concentration determined a decrease in the germination rate, indicating damage to seeds and embryos (Mirmostafaee et al., 2019). Post-ripening can improve seed germination rates and conditions. The germination rate and the conditions under which seeds germinate can increase during post-ripening (Stanisavljević et al., 2018). Terzić et al., 2023, demonstrated that treatment of seeds with *Althea officinalis* at a concentration of 0.02% showed full efficacy, the germination rate being increased by 13% in 3-year-old seeds. However, due to the high percentage of dormant seeds, the same treatment did not achieve full efficacy in 1- and 2-year-old seeds. (Terzić et al., 2023). The present study demonstrated that increased concentration of ECEO had as results a decreased of germination percentage.

CONCLUSIONS

Antifungal treatment of wheat seeds with *Eleteria cardamomum* has shown promising results in protecting the seeds from various fungal infections. The active compounds present in *Eleteria cardamomum* have demonstrated strong antifungal properties, making it a potential natural alternative to chemical fungicides.

Several studies have reported the efficacy of *Eleteria cardamomum* in inhibiting the growth of common fungi such as *Fusarium, Alternaria*, and *Aspergillus*, which are known to cause significant damage to wheat crops. The application of *Eleteria cardamomum* extract as a seed treatment has exhibited antifungal activity and the ability to enhance seed germination and promote healthy seedling development.

Moreover, using *Eleteria cardamomum* aligns with the increasing demand for sustainable and eco-friendly agricultural practices. By harnessing the natural antifungal properties of *Eleteria cardamomum*, farmers can reduce their reliance on synthetic fungicides, thereby minimising the environmental impact associated with chemical inputs.

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REFERENCES

Alexa, E., Sumalan, R.M., Danciu, C., Obistioiu, D., Negrea, M., Poiana, M.A., Rus, C., Radulov, I., Pop, G., Dehelean, C. (2018). Synergistic Antifungal, Allelopatic and Anti-Proliferative Potential of *Salvia officinalis* L., and *Thymus vulgaris* L. Essential Oils. *Molecules*, 23(1), 185-200.

Alexa, V.T., Szuhanek, C., Cozma, A., Galuscan, A., Borcan, F., Obistioiu, D., Dehelean, C.A., Jumanca, D. (2020). Natural Preparations Based on Orange, Bergamot and Clove Essential Oils and Their Chemical Compounds as Antimicrobial Agents. *Molecules*, 25, 5502. https://doi.org/10.3390/ molecules2 5235502 Mirmostafaee S, Azizi M, Fujii Y. Effects of essential oil of some medicinal plants on seed germination and seedling growth of lettuce as an indicator. J Plant Prot. 2019;33(4):475–91.

Aliaa, H., Mizhir Hiba, H., Hussein Raghad, K., Maih Alaa, H., Buthayna, Y., Hassoun, A. (2016). Evaluation of Antifungal Activity of Hot Water Extract on *Elettaria cardamomum* and *Cinnamomum* sp. against some Opportunism Fungi, Al-Kufa University, *Journal for Biology*, 328-333.

Al-Sohaibani, S., Murugan, K., Lakshimi, G., Anandraj, K. (2011). Xerophilic aflatoxigenic black tea fungi and their inhibition by *Elettaria cardamomum* and *Syzygium aromaticum* extracts, *Saudi Journal of Biological Sciences*, 18(4), 387-394.

Al-Zereini, W.A., Al-Trawneh, I.N., Al-Qudah, M.A., TumAllah, H.M., al Rawashdeh, H.A., Abudayeh, Z.H. (2022). Essential Oils from *Elettaria cardamomum* (L.) Maton Grains and *Cinnamomum verum* J. Presl Barks: Chemical Examination and Bioactivity Studies. *J. Pharm. Pharm. Res.*, *10*, 173–185.

Arslan, M., Dervis, S. (2010). Antifungal activity of essential oils against three vegetative-compatibility groups of *Verticillium dahliae. World J. Microbiol. Biotechnol*, 26, 1813–1821.

Bota, V., Sumalan, R.M., Obistioiu, D., Negrea, M., Cocan, I., Popescu, I., Alexa, E. (2022). Study on the Sustainability Potential of Thyme, Oregano, and Coriander Essential Oils Used as Vapours for Antifungal Protection of Wheat and Wheat Products. *Sustainability*, 14, 4298.

Castillo, N.E.T., Teresa-Martínez, G.D., Alonzo-Macías, M., Téllez-Pérez, C., Rodríguez-Rodríguez, J., Sosa-Hernández, J.E., Parra-Saldívar, R., Melchor-Martínez, E.M., Cardador-Martínez, A. (2023). Antioxidant Activity and GC-MS Profile of Cardamom (*Elettaria cardamomum*) Essential Oil Obtained by a Combined Extraction Method—Instant Controlled Pressure Drop Technology Coupled with Sonication. *Molecules,* 28, 1093.

Hulea, A., Obiștioiu, D., Cocan, I., Alexa, E., Negrea, M., Neacșu, A.G., Hulea, C., Pascu, C., Costinar, L., Iancu, I. (2022). Diversity of Monofloral Honey Based on the Antimicrobial and Antioxidant Potential. *Antibiotics,* 11, 595. https://doi.org/10.3390/antibiotics11050595

Ibrahim, F., Muhammad, A.A., Javed, I., Aftab, A., Abdul Basit, K. (2017). Inhibitory effects of natural spices extracts on *Aspergillus* growth and aflatoxin synthesis. *Australian Journal of Crop Science*, Volume, 11, 12, 1553-1558.

Ivanović, M., Makoter, K., Razboršek, M.I. (2021). Comparative Study of Chemical Composition and Antioxidant Activity of Essential Oils and Crude Extracts of Four Characteristic *zingiberaceae* Herbs. *Plants*, *10*, 501.

Kedia, A., Dwivedy, A.K., Jha, D.K., Dubey, N.K. (2016). Efficacy of Mentha spicata essential oil in suppression of *Aspergillus flavus* and aflatoxin contamination in chickpea with particular emphasis to mode of antifungal action. *Protoplasma*, 253:647–653. doi: 10.1007/s00709-015-0871-9.

Kocic-Tanackov, S.D., Dimic, G.R. (2013). Antifungal activity of essential oils in the control of food-borne fungi growth and mycotoxin biosynthesis in food. *Formatex*, 4, 838-849.

Li, Y., Kong, W., Li, M., Liu, H., Zhao, X., Yang, S., Yang, M. (2016). Litsea cubeba essential oil as the potential natural fumigant: Inhibition of *Aspergillus flavus* and AFB1 production in licorice. *Ind. Crops Prod*., 80:186–193. doi: 10.1016/j.indcrop.2015.11.008.

Naz, G., Anjum, A. A., Ali1, T., Nawaz, M., Iqbal, S., Manzoor, R. (2023). Activity of plant essential oils against ochratoxin a producing *Aspergillus ochraceus*. *Journal of Animal & Plant Sciences*, 33(5): 1115-1125 ISSN (print): 1018-7081; ISSN (online): 2309-8694 https://doi.org/10.36899/JAPS.2023.5.0705

Perczak, A., Gwiazdowska, D., Marchwińska, K., Juś, K., Gwiazdowski, R., Waśkiewicz, A. (2019). Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. *Arch Microbiol*, 201(8):1085-1097. doi: 10.1007/s00203-019-01673-5. Epub 2019 May 23. PMID: 31123790; PMCID: PMC6746685.

Prakash, B., Kedia, A., Mishra, P.K., Dwivedy, A.K., Dubey, N.K. (2015). Assessment of chemically characterised *Rosmarinus officinalis* L. essential oil and its major compounds as plant-based preservative in food system based on their efficacy against food-borne moulds and aflatoxin secretion and as antioxidant. *Int. J. Food Sci. Technol.,* 50:1792–1798. doi: 10.1111/ijfs.12822.

Stanisavljević, R., Poštić, D., Milenković, J., Đokić, D., Tabaković, M., Jovanović, S. (2018). Possibilities for improving the quality of alfalfa seed by applying temperature treatments before sowing. *J Process Energy Agric*., 2:76–9.

Terzić, D., Marijenka, T., Oro, v., Dobrivoj, P., Štrbanović, R., Filipović, V., Stanisavljević, R. (2023). Impact of essential oils on seed quality and seed-borne pathogens of *Althea ofcinalis* seeds of diferent ages. *Chem. Biol. Technol. Agric*., 10:33