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In vitro evaluation of antifungal activity of *Artemisia* species essential oils (*A. absinthium, A. dracunculus* and *A. vulgaris*)

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SUMMURY

Due to the trend to reduce the use of synthetic pesticides and the increasing attention to environmental protection, research in the field of allelopathy, especially allelochemicals that can be used for the synthesis of biopesticides, has been intensified in recent decades. The genus *Artemisia* belongs to the family Asteraceae and includes a large number of aromatic and weedy species (about 500), which are known as a significant source of biologically active compounds characterized by allelopathic effects. Therefore, the aim of this study was to investigate the effect of essential oils from three species of the genus *Artemisia* (*A. absinthium*, *A. dracunculus* and *A. vulgaris*) on the mycelial growth of different isolates of the phytopathogenic fungus *Colletotrichum orbiculare*, the causal agent of anthracnose of Cucurbitaceae hosts *in vitro*.

The antifungal activity of the essential oils in *in vitro* tests was determined using the disc volatilisation method. The essential oils were applied directly in amounts of: 1, 5, 10, 15 and 20 µl for *A. dracunculus* and *A. vulgaris*, i.e. 10, 15, 20, 25, 30 µl for *A. absinthium*. The concentrations of essential oils achieved in the air phase were 0.035, 0.17, 0.35, 0.53 0.70 µl ml^{-1} for *A. dracunculus* and *A. vulgaris* and 0.35, 0.53, 0.70, 0.88, 1.05 µl ml^{-1} for *A. absinthium.* The average IC_{50} values were 0.16, 0.25 and 0.62 µl ml^{-1} of air phase for *A. vulgaris*, *A. dracunculus* and *A. absinthium*, respectively. According to the IC_{50} values obtained, the essential oil of *A. vulgaris* showed the strongest antifungal potential, while the essential oil of *A. absinthium* showed the weakest inhibitory effect.

Keywords: allelopathic potential, antifungal activity, essential oils, *Artemisia* sp.

INTRODUCTION

Allelopathy includes processes caused by secondary metabolites (allelochemicals) produced by plants and other living organisms. Allelopathic processes are direct and indirect interactions between living organisms through the synthesis and release of chemical compounds into the environment that can have a harmful or beneficial effect on other living organisms (Rice, 1984). The reasons for the increase in research in the field of allelopathy are numerous: the negative effects of pesticides on the environment and human health, the increase in organic production, pest resistance, the phasing out of the use of numerous of active substances, the trend towards reducing the use of pesticides and towards integrated pest management in order to establish sustainable agricultural production etc.

Allelochemicals are natural compounds, products of secondary metabolism, which belong to numerous chemical groups. The positive chemical properties of many allelochemicals are reflected in their rapid degradation in the environment (Soytis et al., 2013), their complete or partial solubility in water and the absence of synthetic ring structures (Dayan et al., 2009). Many researchers have referred to allelochemicals as natural pesticides because they are characterized by a broad spectrum of biological activities Jabran et al., 2007; Dayan et al., 2009; Cheema et al., 2009; Kordali et al., 2009). The main chemical groups of allelochemicals that are most studied are phenolic components and terpenoids.

Terpenoids are the main constituents of essential oils, which are mainly synthesised in the trichomes on leaves and flowers of aromatic plants. Essential oils are volatile, complex natural mixtures that can contain around 20-60 different components in varying concentrations. Essential oils have a wide range of biological activities that depend on the chemical composition of the oil. The chemical constituents of the essential oil in plant species vary qualitatively and quantitatively depending on the geographical area and environmental conditions (Bakkali et al., 2008). Pedro et al. (2016) also pointed out that the synthesis of allelochemicals varies according to the stress conditions to which the plant is exposed, but also depending on the species itself. The complex mixture of substances with different mechanisms of action, such as essential oils, often has synergistic effect and can effectively prevent the emergence of resistant strains of phytopathogens (Martinez, 2012; Basaid et al., 2021). The chemical compounds of essential oils can penetrate the cell walls of fungi due to their lipophilic nature and thus influence the enzymes involved in cell wall synthesis. This leads to morphological changes in the fungi, which in turn lead to lysis of the fungal cell wall (Tian et al., 2012). They can also dissolve in the membranes and increase the permeability of the cell membrane (Cox et al., 2000).

The genus *Artemisia* belongs to the family *Asteraceae* and includes of about 500 species, small herbs and shrubs, from South Asia, North America and European countries (Abad et al., 2012). The Asteraceae family is rich in plants yielding essential oils and *Artemisia* species occupy a top position in bioprospecting (Pandey and Singh, 2017). Most species of the genus *Artemisia* are perennial herbs with a strong and aromatic odor due to of high concentration of volatile constituents. Due to their biological and chemical diversity, *Artemisia* species have numerous applications in the treatment of plant and human diseases, cosmetics and pharmaceutical industries. *Artemisia* species are known for their use in the treatment of diseases such as malaria, hepatitis, cancer, inflammation and infections caused by fungi, bacteria and viruses (Willcox, 2009). *Artemisia* species exhibit significant intraspecific and interspecific variation in the yield and the terpene constituents of their essential oils. An important role in the production of terpenes is played by the presence of glandular trichomes, which are the main source and site of their synthesis. Tojić and Rančić (2023) observed much fewer glandular trichomes in *A. vulgaris* than in *A. absinthium* collected in Serbia, which may indicate lower production of volatile compounds. The quality and yield of essential oils of *Artemisia* species are also influenced by harvest time, fertilizer and soil pH, choice and stage of drying conditions, geographical location, chemotype or subspecies, choice of plant part or genotype, or extraction method (Abad et al., 2012).

It is pointed out that essential oils of *Artemisia* species have allelopathic effects and could be used as biological control agents due to the antibacterial (Janaćković et al., 2015), antifungal (Jeremić et al., 2020), repellent (Wang et al., 2006), insecticidal (Zhang et al., 2022) and phytotoxic (Jiang et al., 2021) effects of the volatile compounds. The antifungal and antimycotic activity of *Artemisia* species, their plant extracts and essential oils, has been studied on phytopathogenic fungi that affect plants during their cultivation or after harvest, especially species of the genera *Aspergillus*, *Fusarium*, *Alternaria*, *Penicillium* etc. (Ivănescu et al., 2021). Several studies have investigated the inhibitory effect of essential oils from *Artemisia* species on *Colletotrichum* spp. – *C. acutatum*, *C. fragariae* and *C. gloeosporioides* (Meepagala et al., 2002; Huang et al., 2021). The genus *Colletorichum* is economically and scientifically important as it infects a wide range of crops. *C. orbiculare* is a fungus that causes watermelon anthracnose and has a significant impact on the yield and quality of produce. Therefore, the aim of this study was to investigate the antifungal activity of commercially available essential oils from three *Artemisia* species (*A. absinthium*, *A. dracunculus* and *A. vulgaris*) on different isolates of *C. orbiculare* in Serbia.

MATERIAL AND METHODS

The essential oils of *A. absinthium* and *A. dracunculus* were obtained from cultivated plants by steam distillation under industrial conditions at the Institute of Field and Vegetable Crops in Novi Sad, while the essential oil of *A. vulgaris* was obtained from Avena lab - Farmadria d.o.o. Vršac.

Watermelon fruit samples with typical symptoms of anthracnose were collected in 2023 in watermelon growing areas in Serbia (Vojilovo and Samarinovac). The isolates obtained were identified as *C. orbiculare* according to morphological, pathogenic and molecular characteristics. The antifungal effects of essential oils on mycelial growth for a total of ten isolates was assessed by disc volatilization method. The essential oils were added directly to sterile filter papers with a diameter of 5 mm placed in the center of the lids of Petri plates (Ø = 55 mm) in amounts of: 1, 5, 10, 15, 20 μl (for *A. dracunculus* and *A. vulgaris*) and 10, 15 20, 25, 30 μl (for *A. absinthium*). The achieved concentrations of essential oils in the air phase were: 0.35, 0.53, 0.70, 0.88, 1.05 µl ml^{-1} for *A. absinthium* and 0.035, 0.17, 0.35, 0.53, 0.70 µl ml^{-1} for *A. dracunculus* and *A. vulgaris.*

Previously dissolved PDA medium (55°C) was poured into Petri plates in the amount of 5 ml and seeded with a piece of fungal mycelium with a diameter of 5 mm. Petri plates which no essential oil had been added served as a control. The Petri boxes were wrapped with parafilm and incubated in an inverted position at a temperature of 25±1°C for 7 days. Three replicates were performed for each oil concentration and each control, and the entire experiment was repeated twice. The percentages of inhibition were determined by measuring the growth of the fungus at different concentrations of essential oils and comparing it with the control. To determine the IC_{50} (mean inhibitory concentration to give 50% inhibition) values, the probit analysis method was used. A difference between the fungicidal and fungistatic activity of the selected essential oil was found.

RESULTS AND DISCUSSION

Sensitivity of *C. orbiculare* isolates to *A. vulgaris, A. dracunculus and A. absinthium* essential oils are presented in Table 1.

The IC_{50} values for a total of ten isolates tested ranged from 0.04-0.30 µl ml^{-1} of air phase for *A. vulgaris*, from 0.17-0.30 µl ml^{-1} of air phase for *A. dracunculus* and from 0.52-0.80 µl ml^{-1} of air phase for *A. absinthium*. The average IC_{50} values were for *A. vulgaris* (0.16 µl ml^{-1} of the air phase), followed by *A. dracunculus* (0.25 µl ml^{-1} of the air phase) and *A. absinthium* (0.62 µl ml^{-1} of the air phase) (Figure 1). According to the IC_{50} values obtained, the essential oil of *A. vulgaris* showed the strongest antifungal potential (Picture 1), while the essential oil of *A. absinthium* showed the weakest inhibitory effect. The isolates collected in Samarinovac showed higher sensitivity to the essential oils used, especially to *A. vulgaris*. The isolate most sensitive to the essential oil of *A. vulgaris* was CO115 (0.04 µl ml^{-1} of the air phase), while the highest IC_{50} was obtained from isolate V4 (0.30 µl ml^{-1} of the air phase). The isolate most sensitive to the essential oil of *A. dracunculus* was V8 (0.17 µl ml^{-1} of the air phase), while the highest IC_{50} was observed for isolate CO124 (0.33 µl ml^{-1} of the air phase). In the case of *A. absinthium* essential oil, the lowest IC_{50} was for the isolates CO115 and CO117 (0.52 µl ml^{-1} of the air phase), while isolate V4 was the least sensitive (0.80 µl ml^{-1} of the air phase).

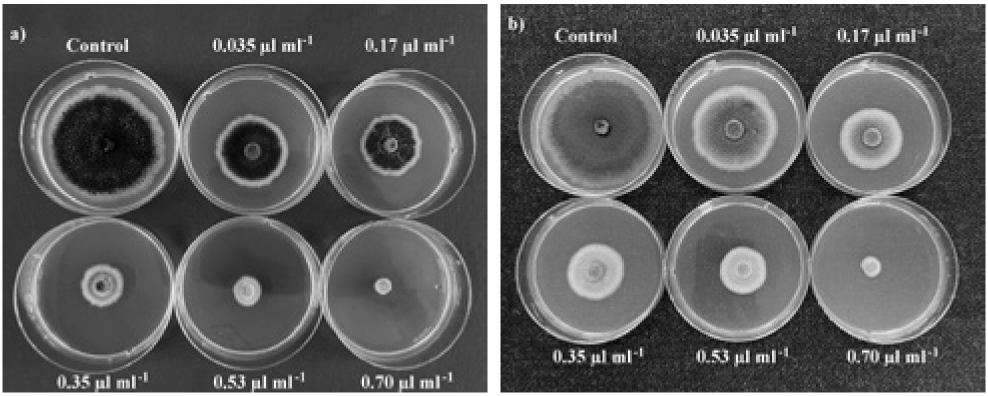
Numerous studies have investigated and comprehensively evaluated the antifungal properties of essential oils from various plants. There is no data on the influence of *Artemisia* species on *Colletotrichum orbiculare*, but there are other species of the genus *Colletotrichum*.

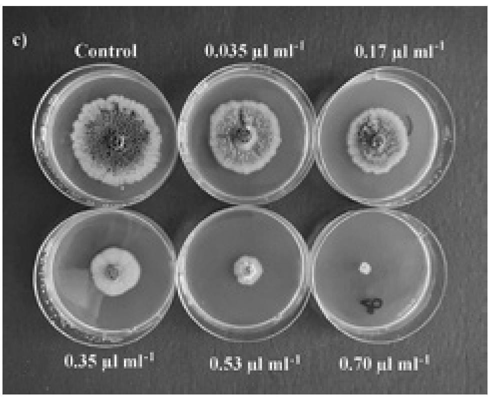
Meepagala et al. (2002) reported that the oil of *A. dracunculus* contains 5-phenyl-1,3-pentadiyne and capillarin, which have fungicidal activity against *Colletotrichum fragariae, C. gloeosporioides* and *C. acutatum*. According to Huang et al. (2021), the essential oils of *A. lavandulaefolia*, *A. scoparis* and *A. annua* have an antifungal effect against *C. gloeosporioides* in mangoes.

Table 1. Sensitivity of *C. orbiculare* isolates to essential oil of *A. vulgaris, A. dracunculus* and *A. absinthium*
Tabela 1. Osetljivost izolata *C. orbiculare* na etarsko ulje *A. vulgaris, A. dracunculus* i *A. absinthium*

Isolate* Izolat	Essential oil Etarsko ulje								
	Artemisia vulgaris			*Artemisia dracunculus*			*Artemisia absinthium*		
	IC$_{50}$ (μl ml^{-1}) Values[1]	Range[2]	*b*[3]	IC$_{50}$ (μl ml^{-1}) Values[1]	Range[2]	*b*[3]	IC$_{50}$ (μl ml^{-1}) Values[1]	Range[2]	*b*[3]
V4	0.30	0.14-0.62	1.47	0.20	0.08-0.49	1.35	0.80	0.72-0.88	4.17
V5	0.24	0.11-0.53	1.43	0.32	0.21-0.47	2.05	0.69	0.64-0.74	4.82
V10	0.22	0.14-0.36	1.89	0.27	0.14-0.50	1.59	0.69	0.62-0.74	4.15
V8	0.20	0.14-0.29	2.31	0.17	0.09-0.35	1.58	0.67	0.60-0.73	4.00
V9	0.25	0.18-0.36	2.30	0.20	0.14-0.30	2.26	0.63	0.57-0.69	4.19
CO124	0.08	0.03-0.20	1.56	0.33	0.16-0.71	1.46	0.58	0.53-0.64	4.20
CO115	0.04	0.01-0.21	1.33	0.30	0.18-0.49	1.80	0.52	0.49-0.55	6.02
CO117	0.08	0.04-0.18	1.68	0.19	0.07-0.48	1.33	0.52	0.50-0.55	6.80
CO122	0.12	0.05-0.27	1.52	0.27	0.18-0.41	2.04	0.54	0.50-0.57	5.23
CO105	0.08	0.04-0.16	1.82	0.25	0.09-0.67	1.26	0.57	0.53-0.61	5.22
Mean		0.16			0.25			0.62	

*Code of tested isolates from different sites (V-Vojilovo site; CO-Samarinovac site); [1]Mean inhibitory concentration to give 50% inhibition; [2]Confidence interval; [3]Expressing the relative sensitivity of the isolates;





Picture 1. Mycelial growth of different isolates at concentrations of 0.035, 0.17, 0.35, 0.53, 0.70 μl ml^{-1} of the air phase for *A. vulgaris*: a) isolate CO115, b) isolate CO124, c) isolate V10
Slika 1. Porast micelije različitih izolata na koncentracijama 0,035, 0,17, 0,35, 0,53, 0,70 μl ml^{-1} vazdušne faze za etarsko ulje *A. vulgaris*: a) izolat CO115, b) izolat CO124, c) izolat V10

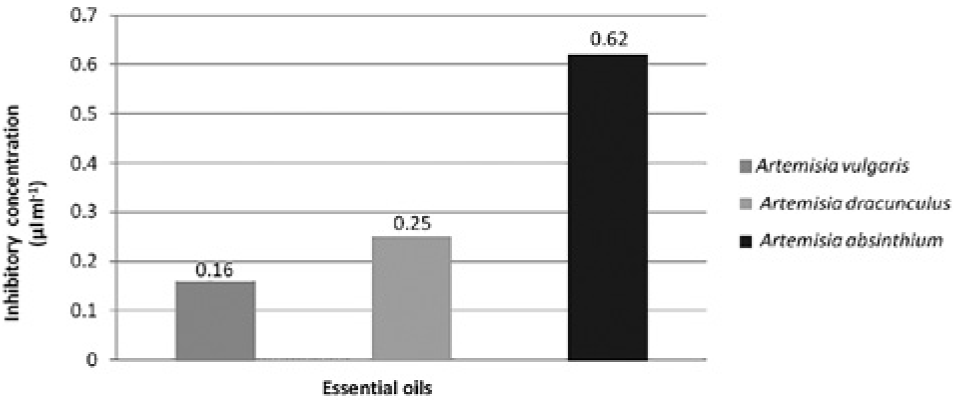


Figure 1. Mean IC_{50} values for *Artemisia* species essential oils
Grafik 1. Prosečne IC_{50} vrednosti za etarska ulja *Artemisia* vrsta

Kordali et al. (2005a, b) investigated the chemical composition and antifungal activity of essential oils extracted from four Turkish *Artemisia* species: *A. dracunculus*, *A. absinthium*, *A. santonicum* L., and *A. spicigera*. The oil of *A. dracunculus* showed weaker antifungal activity compared to the other *Artemisia* oils tested, but all essential oils tested strongly inhibited the growth of fungal species (about 34 fungal species).

Moreover, camphor, 1,8-cineole, and chamazulene isolated from the Turkish population were described as effective fungicides against the wilt fungi *F. solani* and *F. oxysporum* (Kordali et al., 2005b), and thujone-rich *A. absinthium* oil from Uruguay showed strong fungicidal activity against *Alternaria* sp. and *Botrytis cinerea* (Umpierrez et al., 2012). In addition, Badea and Delian (2014) discovered that the essential oils of *Artemisia* species, including *A. vulgaris* and *A. dracunculus*, reduced the growth of mycelia of *Sclerotinia sclerotiorum*, although the effects of inhibition varied depending on the origin and composition of the *Artemisia* species.

The percentage of some main ingredients and antifungal activity generally correlated. Numerous studies have shown that enhanced antifungal activity correlates with high levels of oxygenated monoterpenes. The oils that showed antifungal activity contained high concentrations of oxygenated monoterpenes (Kordali et al., 2005a, b).

CONCLUSION

Having shown a good inhibitory effect, the essential oils of *Artemisia* spp. (*A. absinthium, A. dracunculus* and *A. vulgaris*) could find application in future research. However, the antifungal properties of the main components of the essential oils were not investigated in our study, as these are only preliminary results. In order to properly utilize the potential of essential oils to control plant diseases, further research is needed to fully define the ingredients and conduct further screening. The efficacy, phytotoxicity and safety of these oils need to be further investigated, perhaps through *in vivo* trials.

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In vitro procena antifungalne aktivnosti etarskih ulja vrsta roda *Artemisia* (*A. absinthium*, *A. dracunculus* i *A. vulgaris*)

REZIME

Zbog trenda smanjenja upotrebe sintetičkih pesticida i sve veće pažnje usmerene ka zaštiti životne sredine, poslednjih decenija intenzivirana su istraživanja u oblasti alelopatije, posebno alelohemikalija koje se mogu koristiti za sintezu biopesticida. Rod *Artemisia* pripada familiji Asteraceae i obuhvata veliki broj aromatičnih i korovskih vrsta (oko 500), koje su prepoznate kao značajan izvor biološki aktivnih jedinjenja koja se odlikuju alelopatskim dejstvom. Stoga, cilj istraživanja ovog rada je bio da se ispita uticaj etarskih ulja tri vrste roda *Artemisia* (*A. absinthium*, *A. dracunculus* i *A. vulgaris*) na porast micelije izolata fitopatogene gljive *Colletotrichum orbiculare,* prouzrokovača antraknoze biljaka iz familije Cucurbitacea, u laboratorijskim uslovima.

Antifungalna aktivnost etarskih ulja u *in vitro* testovima određena je primenom makrodilucione fumigantne metode sa isečkom micelije. Etarska ulja dodavana su direktno u količinama od: 1, 5, 10, 15 i 20 µl za *A. dracunculus* i *A. vulgaris*, odnosno 10, 15, 20, 25, 30 µl za *A. absinthium*.

Postignute koncentracije etarskih ulja u vazdušnoj fazi su iznosile 0,035, 0,17, 0,35, 0,53, 0,70 µl ml^{-1} za *A. dracunculus* i *A. vulgaris*, a 0,35, 0,53, 0,70, 0,88, 1,05 µl ml^{-1} za *A. absinthium*. Prosečne IC_{50} vrednosti iznosile su 0,16, 0,25 i 0,62 µl ml^{-1} vazdušne faze za *A. vulgaris*, *A. dracunculus* i *A. absinthium*, istim redom. Prema dobijenim IC_{50} vrednostima, etarsko ulje *A. vulgaris* je pokazalo najjači antifungalni potencijal, dok je najslabije inhibitorno delovanje utvrđeno kod etarskog ulja *A. absinthium*.

***Ključne reči*:** alelopatski potencija, atifugalno dejstvo, etarska ulja, *Artemisia* sp.