ANTIMICROBIAL ACTIVITY OF THE VOLATIL CORIANDER OIL (*CORIANDRUM SATIVUM* L.)

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The results of chemical composition of industrially produced *Coriandrum sativum* L. essential oil and antimicrobial assessment are reported. The gas chromatography-mass spectrometry analysis allowed the identification of 32 components belonging to terpenes (26.43%), aliphatic compounds (71.91%) and heterocycles (0.99%). The essential oil of *C. sativum* exhibits high antibacterial activity for the species *Bacillus subtilis, Pseudomonas fluorescens, Xanthomonas campestris, Erwinia carotovora, E. amylovora* in the small concentrations – 0.0035–0.007% and antifungal activity for the species *Fusarium oxysporum, F. sporotrichiella, Drechslera sorokiniana* in concentrations 0.002; 0.01 and 0.05%.

Keywords: Coriandrum sativum L., essential oil, GC-MS analysis, antibacterial and antifungal activity.

INTRODUCTION

Plants produce a wide range of natural products called secondary metabolites. These compounds have important ecological functions, providing plants with protection against attacks by herbivores and various pathogens, and people harness these natural products in the different areas (Osbourn & Lanzotti, 2009). The rapid increase in harvests crops was possible due to the discovery and use of chemicals for the disease control but the adverse consequences of preparations on human health have led to the reduction in the number of synthetic pesticides applied in agriculture. Thus, the current paradigm on the exclusive role of synthetic pesticides in combating of the diseases and pests is reconsidered, which has spurred the discovery and promotion of new natural-based pesticides to substitute the excluded compounds because of the new registration requirements that stipulate that the preparations proposed for implementation in agriculture should not to be toxic. The application of numerous severe pesticides has led not only to adverse consequences for human health, but has also resulted in the emergence of many strains of

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microorganisms resistant to them, which is why identification or creation of the new substances / compounds / products / preparations with antimicrobial activity has become a topical objective for many countries (Dayan *et al.*, 2009).

The medicinal plant *Coriandrum sativum L.*, known since Antiquity, contains numerous secondary metabolites. Its phytochemical screening has demonstrated the presence of alkaloids, glycosides, flavonoids in the acetone and methanolic extracts.

The acetone extracts have been found to exhibit antibacterial and antifungal activities higher than the methanolic. However, the lack of effect on *Klebsiella pneumoniae* and *Saccharomyces cerevisiae* denotes the specificity of these extracts (Chaudhary *et al.*, 2014). Also known is the anticandidic activity (*Candida albicans*) of the essential oil of *C. sativum* plants (Freieres *et al.*, 2014).

The root rot in common wheat (*Triticum aestivum L.*) is one of the most widespread diseases in its cultivation areas, with manifestations specific to the ontogenetic phases: seeds rot, primary and secondary rootless, coleoptile, stem base, wilting in the plant phase, obstruction of the vessels with mycelium and grain growth, depigmentation of the stem and spike, empty spikes with rough pallets, old seeds and or with black embryos in mature plants. The disease presents one of the most devastating pathologies of the cereal crops in the 20th–21st centuries, being caused by several genes of ubiquitous fungi spread in the soil (Tunali *et al.*, 2008; Hajieghrari, 2009).

In cereal crops, root rot is produced by a broad set of fungus with facultative or obligate pathogenicity. Depending on the geographical region and the environmental conditions, the predominant species in the fungal complex are quite different: Fusarium (Toth B. *et al.*, 2008), Drechslera / Bipolaris (Lehmensiek *et al.*, 2010), Cochliobolus (Mathre *et al.*, 2018), Pythium (Higginbotham *et al.*, 2004), Rhizoctonia (Gill & Smettem, 2001). According to our research, in the Republic of Moldova conditions, the species of *Fusarium* and *Drechslera* are among of the most spread and virulent causative agents of the root rot in common wheat. *Fusarium oxysporum* is a ubiquitous pathogen spread in the soil, the fungus causing various diseases to a big number of crop plants, including cereals (Lupascu *et al.*, 2015).

Bacteriosis is a major factors, with a very serious impact on the achieving of the production potential of many plant species. For example, *E. carotovora*, a gram-negative bacterium, causes soft rot to economically important crops such as potatoes, tomatoes, or cucumbers. In the case of potato, stem and tuber soft rot occurs before and after harvesting, thus greatly reducing the production yield (Yap *et al.*, 2004; Benada *et al.*, 2018). *Erwinia amylovora* is the causative agent of bacterial fire – a devastating disease for a wide range of species within the Rosaceae family, presenting a major global threat to the commercial production of apples and pears (Vrancken *et al.*, 2014; Piqué *et al.*, 2015). *Xanthomonas campestris* pv. *vesicatoria*, a biotrophic gram-negative bacterium, is the causative agent of bacterial spot at tomatoes (*Solanum lycopersicum L.*) and pepper (*Capsicum annuum L.*). Bacterial symptoms include defoliation and necrotic and chlorothic lesions on leaves, stems, fruits and flowers, which subsequently lead to a reduced yield (Tamir-Ariel *et al.*, 2007).

The existing chemical preparations have poor efficacy for said pathogens or are undesirable from the point of view of the toxicological impact, which is why the extension of the spectrum of natural substances with antimicrobial effect presents an important goal at the present stage.

MATERIAL AND METHODS

The *Coriandru sativum* L. plant material was harvested in June–July of 2017 from the fields around the Pervomaysk village, Causeni district, the Republic of Moldova. The *Coriander* volatile oil (CO) was obtained industrially by hydrodistillation of fresh collected plants with immature seeds from the containers.

The sample of volatile oil was subjected to GC-MS analysis on an Agilent Technologies 7890A system. IR spectra were recorded on a Spectrum-100FT-IR spectrometer using the attenuated total reflection technique. ¹H and ¹³C NMR spectra were acquired in CDCl₃ on a Bruker Avance DRX 400 spectrometer (400 MHz). All chemical shifts are quoted on the δ -scale in ppm and referred to residual CHCl₃ (δ_H at 7.26 ppm) and as CDCl₃ (δ_C 77.00 ppm), respectively.

Microbiological tests. As test microorganisms for the evaluation of the antibacterial activity of coriander essential oil (*C. sativum* L.) were used the following microorganisms: non-pathogenic Gram-positive and Gram-negative strains of *Bacillus subtilis* CNMN BB-01 and *Pseudomonas fluorescens* CNMN-PFB-01, respectively, and phytopathogenic strains of *Xanthomonas campestris, Erwinia amylovora* and *Erwinia carotovora*.

As test microorganisms for the evaluation of the antifungal activity were used the following filamentous fungi: *Fusarium oxysporum*, *F. sporotrichiella* var. *tricinctum* and *Drechslera sorokiniana*.

For the testing of the antibacterial activity was used the successive double dilution method. For this, at the initial stage, 1 mL of peptone broth for test bacteria was introduced into a series of 10 tubes. Subsequently, 1 mL of the analyzed preparation was dropped into the first test tube. Then, the obtained mixture was pipetted, after which 1 mL of it was transferred to the next tube, so the procedure was repeated until the tube no. 10 of the series. Thus, the concentration of the initial preparation decreased 2-fold in each subsequent tube. At the same time, 24 hour test bacteria cultures were prepared. Initially, suspensions of test bacteria were prepared with optical densities (D.O.) of 2.0 for tested bacteria according to

the McFarland index. Subsequently, 1 mL of the obtained bacterial suspension was dropped in a tube containing 9 mL of sterile distilled water. The content of the tube was mixed, after which 1 mL was transferred to the tube no. 2 of the 5-tube series containing 9 mL of sterile distilled water. From the 5-th tubes of the series were taken 0.1 mL of the bacterial suspension, which represent the seeded dose and added to each tube with titrated preparation. Subsequently, the tubes with titrated preparation and the seeded doses of the bacteria were kept in the thermostat at 35 °C for 24 hours. On the second day, a preliminary analysis of the results was made. The last tube from the series in which no visible growth of bacteria has been detected is considered to be the minimal inhibitory concentration (MIC) of the preparation. For the estimation of the minimal bactericidal concentrations, the contents of the test tubes with MIC and with higher concentrations are seeded on peptone and Sabouraud agar from Petri dishes with the use of the bacteriological loop. The seeded dishes are kept in the thermostat at 35 °C for 24 hours. The concentration of preparation, which does not allow the growth of any colony of bacteria, is considered to be the minimal bactericidal (MBC) of the preparation (Methodical recommendations).

The screening of the antifungal activity of CO was performed by supplementing it at concentrations of 0.05; 0.01 and 0.002% to PDA (Potatoes Dextrosis Agar) (Tuite, 1969). As a control, served the PDA without CO. In the center of the Petri dish with solidified medium, the 5 mm diameter of mycelium was seeded, after which the dishes were kept at 23 °C. The colony growth was recorded on days 2, 4 and 6. The mycelial density was determined in the 3-step scale: 1 - poor, 2 - medium, 3 - normal. The experience was performed in 6 repetitions. The data obtained were processed in the STATISTICA 7 software package.

RESULTS

The content of essential oil in seeds and plants of *C. sativum* depends of geographical origin and climatic conditions. So, the yield of reported essential oil of Moldavian origin was about 0.3%.

According to GC-MS analysis in *C. sativum* essential oil thirty-two components were detected that corresponding to 99.32% of its total composition. Terpenic fraction (26.42%) includes monoterpenic hydrocarbons (3.07%) and their oxygenated derivatives (23.35%). The most abundant and varied is fraction of aliphatic compounds (71.91%). It consists of alkanes (0.57%), saturated alcohols (4.96%), unsaturated alcohols (15.86%), and both groups of saturated (8.43%) and unsaturated (42.09%) aldehydes. Also, *C. sativum* essential oil contains a small amount of heterocyclic compounds (~1%).

The essential oil of *C. sativum* exhibits high antibacterial activity in the range of concentrations of 0.0035–0.007% (Table 1).

Test-bacteria	Double successive dilutions (MBC, %)							
Test-Dacteria	0.25	0.12	0.06	0.03	0.015	0.007	0.0035	0.0017
Bacillus subtilis CNMN BB-01	-	-	-	-	-	-	+	+
(4.8 x 10 ⁸ CFU/mL)								
Pseudomonas fluorescens	-	-	-	-	-	-	-	+
CNMN-PFB-01								
(4.8 x 10 ⁸ CFU /mL)								
Xanthomonas campestris	-	-	-	-	-	-	-	+
(4.8 x 10 ⁸ CFU /mL)								
Erwinia amylovora	-	-	-	-	-	-	+	+
(4.8 x 10 ⁸ CFU /mL)								
E. carotovora	-	-	-	-	-	-	+	+
(4.8 x 10 ⁸ CFU /mL)								

Table 1

The antibacterial activity of the Coriandrum sativum essential oil

MBC- minimal bactericidal concentration.

The antibacterial action may be due to the presence in the extract of the linalool, *n*-decanal, (E)-dec-2-en-1-ol and other components, and they manifest their activity by likely mechanisms that include: structural damages of the cytoplasmic membrane, disruption of membrane proteins and the disturbance of the ionic transport process (Asgarpanah & Kazemivash, 2012).

At day 3 of cultivation (Table 2), strains of fungi sown on the PDA nutrient medium recorded a growth in almost all of the variants taken in the study – control and Coriander volatile oil (CO). The exception was the CO variant – 0.5%, in which the *F. oxysporum* and *D. sorokiniana* fungus showed no signs of growth and the diameter of the colonies of *F. sporotrichiella* var. *tricinctum* (21.1 \pm 5.8 mm) constituted 38.2% of the control. It should be noted that CO – 0.002% produced stimulation of the radial growth of *F. oxysporum* and *F. sporotrichiella* var. *tricinctum* with 17.9 and 20.1%, respectively, in comparison with the control.

On day 4, significant growth of *F. oxysporum* and *F. sporotrichiella var. tricinctum* in the variants "CO – 0.002%" and "CO – 0.01%", the diameter of the colonies exceeding 10.2 ... 16.7% of the control variant. At the 0.05% concentration, the diameter of the colonies of *F. oxysporum* and *F. sporotrichiella var. tricinctum* constituted 29.2 and 45.2%, respectively, from the control, and for *D. sorokiniana* there was no increase. It should be noted that on day 5, the colonies of *F. sporotrichiella var. tricinctum* reached the maximum growth rates (90 mm) in the control variants, CO – 0.002% and CO – 0.01%, and at the concentration 0.05% the diameter of the colonies (44.5 ± 6.4 mm) was 52.9% of the control (84.2 ± 1.0 mm).

On day 6, at the *F. oxysporum* fungus, the diameter of the colonies in the variants "CO – 0.002%" and "CO – 0.01%" recorded an exceeding of 24.5% of the control variant and in the variant "CO – 0.05%" the diameter of the colonies constituted 49.8% of the control variant. In the case of *D. sorokiniana*, it was found that in concentrations 0.002% and 0.001% UC, the diameter of the colonies constituted 86.9 and 76.0%, respectively, from the control, and at 0.05%, as in the previous days, growth was not recorded.

Table	2
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Influence of the Coriander volatile oil on the radial growth of fungi colonies

Variant	Concentration, %	Day 3	% Control	Day 4	% Control	Day 6	% control
F. oxysporum							
Control	-	40.2±0.8	-	53.1±1.3	-	72.3±0.9	-
CO	0.002	$47.4 \pm 0.5 *$	117.9	62.0±0.8*	116.7	90.0±0.0*	124.5
CO	0.01	43.3±1.0	107.7	58.5±1.2*	110.2	90.0±0.0*	124.5
CO	0.05	0.0±0.0	-	15.5±0.0*	29.2	36.0±0.0*	49.8
F.	sporotrichiella vai	. tricinctum	ı				
Control	-	55.3±1.8	-	71.7±2.9	-	-	-
CO	0.002	$66.4 \pm 1.6^*$	120.1	83.5±0.5*	116.5	-	-
CO	0.01	57.7±1.8	104.3	83.6±0.3*	116.6	-	-
CO	0.05	21.1±5.8*	38.2	32.4±8.3*	45.2	-	-
	D. sorokinia	na					
Control	-	38.0±0.9	-	48.4±0.3	-	76.2±0.5	-
CO	0.002	41.1±1.5	108.2	41.1±1.5	84.9	66.2±2.9	86.9
CO	0.01	31.8±2.7	83.4	31.6±2.6	65.3	57.9±3.3	76.0
СО	0.05	0.0±0.0*	-	0.0±0.0*	-	0.0±0.0*	-

*- difference with the control ($p \le 0.05$).

It should be noted that in all of the variants with CO, mycelium of fungi showed a much lower density compared to the control, the scarcity of the hyphae sometimes reaching maximum levels at the limit of normal development (Figure 1, Table 3).

Variant	Concentration, %	Day 3	Day 4	Day 6
		F. oxysporum		
Control	-	3.0±0.0	3.0±0.0	3.0±0.0
СО	0.002	0.8±0.3*	0.9±0.2*	0.9±0.2*
СО	0.01	0.8±0.3*	0.8±0.3*	0.8±0.3*
	0.05	0.7±0.2*	0.7±0.3*	0.6±0.3*
	F. sporot	richiella var. tricin	ictum	
Control	-	3.0±0.0	3,0±0.0	3.0±0.0
СО	0.002	0.8±0.3*	0,8±0.3*	0.8±0.3*
СО	0.01	0.7±0.3*	0.7±0.3*	0.7±0.3*
	0.05	0.6±0.3*	0.7±0.3*	0.6±0.3*
		D. sorokiniana		
Control	-	3.0±0,0	3.0±0.0	3.0±0.0
СО	0.002	1.2 ±0,3*	1.2 ±0.3*	1.1 ±0.2*
СО	0.01	1.2 ±0,3*	1.1 ±0.2*	1.1 ±0.2*
	0.05	-	-	-

Table 3

Influence of coriander volatile oil on the mycelial density of the phytopathogenic fungi

*- difference with the control, $p \leq 0.05$.

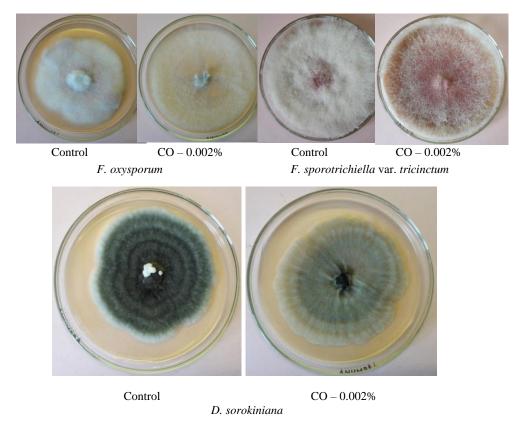
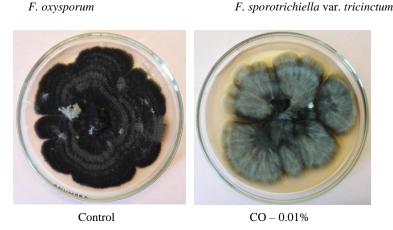


Fig.1. Influence of coriander volatile oil on the density and pigmentation of fungal mycelium.

For example, in the case of *F. oxysporum*, the mycelial density was 0.8–0.9; 0.7 ... 0.9; 0.6 ... 0.9 degrees; respectively on days 3, 4, 6; *F. sporotrichiella var. tricinctum*: 0.7 ... 1.0; 0.7 ... 0.9, respectively on days 3, 4; *D. sorokiniana*: 1.2 ... 1.3; 1.1 ... 1.2; 1.1 ... 1.2 degrees, respectively, on days 3, 4, 6.

Considering that the maximum mycelial density was 3, we can conclude that the test substances strongly inhibited the growth and development of the fungus under study.

In order to determine the degree of change of pigmentogenesis under the influence of the compounds, the cultures were reseeded on PDA nutrient medium, not supplemented with these compounds. It was found that at the cultures grown from changed pigmented mycelial fragments did not return the normal pigmentation, which indicates the irreversibility of pigmentogenesis modification (Figure 2).



D. sorokiniana

Fig. 2. Influence of biologically active substances on pigmentogenesis capacity at the reseeded cultures.

DISCUSSION

According to available literature data, the chemical composition of coriander essential oil obtained from different parts of the plant vary greatly (Mandal, 2015). The main fraction of the *C. sativum* essential oil extracted from ripe seeds belongs to terpenic fraction, more exactly, it includes terpenic hydrocarbons and their oxygenated derivatives like alcohols, aldehydes, ketones and esters. In contrast, the essential oil from the fresh herb consists mostly of aliphatic aldehydes.

The main fraction of investigated essential oil comprises a high and varied content of aliphatic compounds (~72%). Among them can be mentioned unsaturated alcohols (15.86%) and unsaturated aldehydes (42.09%) from the range of C10-C14, e.g.: dec-2-enol **1** (15.34%) and (*E*)-dec-2-enal **2** (23.40%).

Terpenic fraction is less abundant (~26%) and enclose monoterpene hydrocarbons (3.07%), e.g. α -pinene **3** (1.02%) and γ -terpinene **4** (0.93%) and oxygenated

Control

monoterpenes (23.35%) like linalool **5** (20.81%), camphor **6** (1.09%) and geranyl acetate **7** (0.35%). Heterocyclic compounds are represented by *n*-hexyl-2-furan **8** (0.34%) and *n*-octyl-furan **9** (0.65%) (Figure 3).

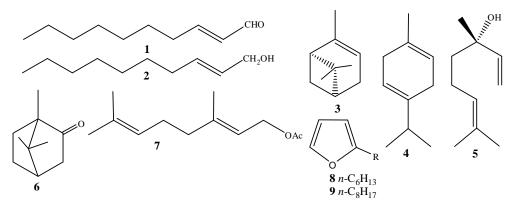


Fig. 3. The main constituents of Coriandrum sativum essential oil.

The presence of mentioned above components is confirmed by IR and NMR spectral analyses. In the IR spectra, there are absorption peaks that correspond to double bonds from molecules of components of *C. sativum* essential oil at 2957, 1637, 1457, 1377, 973 cm⁻¹ and confirm its highly unsaturated degree. Others absorption bands at 3451 and 1140 cm⁻¹ prove the presence of hydroxyl groups, and these at 1692 and 1457 cm⁻¹ show the presence of carbonyl groups. The ¹H NMR spectra of C. *sativum* essential oil includes signals of protons localized at exocyclic methylene groups and those adjacent to double bonds that are visible in the weaker field as doublets or multiplets from 4.99 ppm to 6.85 ppm. The presence of aldehyde protons are proved by singlet signals from 9.45–9.71 ppm.

In accordance with proton spectra, the ¹³C NMR spectra include signals of adjacent to double bonds carbon atoms (>C=CH, -CH=CH-, >C=CH₂) in the region 111.57-159.05 ppm, and these of carbonyl carbon atoms at 194.12 ppm.

Coriander volatile oil showed bactericidal activity in the concentration of 0.0035% for *P. fluorescens, X. campestris* and 0.007% for *B. subtilis, E. amylovora, E. carotovora,* therefore in low concentrations, which denotes high antibacterial activity of the compound and high opportunities to create effective preparations against severe bacteriosis produced by *X. campestris, E. amylovora, E. carotovora.*

One of the criteria of antifungal activity (in the case of filamentous fungi) is the influence of the preparation on the radial growth of the colonies on nutrient medium (Bilai, 1982). This implies the reduction of the diameter of the colonies in the variant with the test preparation in comparison with the control, but not necessarily the mycelial density.

In our research, *D. sorokiniana* fungus showed complete inhibition at 0.05% CO concentration and significant inhibition at 0.002 and 0.01% CO concentrations.

For the other 2 fungi in study – *F. oxysporum* and *F. sprotrichiella* var. *tricinctum* was found to significantly diminish the growth only at the maximum concentration of 0.05% CO. Concentrations of 0.002 and 0.01% of CO stimulated the radial growth of colonies, but this was accompanied by a strong decrease of the mycelial density, which is an evidence of inhibition of micellar growth of these fungi.

Filamentous fungi produce a wide range of secondary metabolites. Some of these are pigments, and color variability has been a long time used as a distinctive feature of strains. The role of fungal pigments for the viability of the microorganism is widely discussed in the literature. One of these is melanin, a multifunctional metabolite that protects against environmental stress, such as ultraviolet (UV) light, oxidizing agents and ionizing radiation, thus contributing to the ability of the fungus to survive to severe conditions. At the same time, pigment is also a factor of virulence (Eisenman & Casadevall, 2012).

Volatile coriander oil has affected not only the growth and development of fungi, but also the ability to synthesize pigments. Thus, in *F. oxysporum* and *D. sorokiniana*, under the action of the substances the mycelium had a pale color, and at *F. sporotrichiella* – more pronounced pigmentation – bright pink.

For Fusarium species, the most characteristic class of fungal pigments are carotenoids – a family of ubiquitous terpenoids in all major taxonomic groups. There are more than 750 natural carotenoids that produce typical yellowish, orange, reddish pigments. In this context, F. oxysporum fungus is considered a reference model in the research of fungal carotenoidogenesis. Biochemical pathways for carotenoid synthesis have many similarities to different fungi, but different regulatory mechanisms have been the basis for the induction of a high diversity of colors and shades, which makes it possible to respond more effectively to different conditions in natural habitats (Avalos et al., 2017). Fusarium colonies, including F. sporotrichiella var. tricinctum - a very toxic species widespread in the regions with the temperate climates (Wagacha et al., 2012) may have reddish tones due to the production of such pigments as aurofusarin or bicaverine (Sorensen et al., 2012). Cluster genes for biosynthesis (Schumacher et al., 2013) and aurofusarin (Gaffoor et al., 2005) are known. By high performance liquid chromatography, it has been found that the anthraquinone derivatives produced by Drechslera avenae fungus, which confer darkened mycelium, contain helminthosporin and cinodontin, capable of stimulating the growth of other pathogens or saprofits (Cegielko et al., 2011).

Thus, we can assume that by inhibiting the biosynthetic capacity of pigments in *D. sorokiniana, F. oxysporum, F. sporotrichiella* by UC, their ability to adapt to unfavorable environmental conditions – biotic and abiotic – may be reduced.

Acknowledgments

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CONCLUSIONS

- 1. As a result of quantitative (GC-MS) analyses of industrially obtained *Coriandrum sativum* L. essential oil thirty-two constituents belonging to monoterpens, aliphatic compounds and heterocyclic compounds with total content of 99.32% were identified. The qualitative (IR, ¹H and ¹³C NMR) analyses confirm its composition.
- 2. The essential oil of *C. sativum* exhibits high antibacterial activity for the species *Bacillus subtilis, Pseudomonas fluorescens, Xanthomonas campestris, Erwinia carotovora, E. amylovora* in the small concentrations 0.0035–0.007%. The antibacterial action, probably, is due to the presence in the extract of the linalool, *n*-decanal, (*E*)-dec-2-en-1-ol and other components that manifest their activity by likely mechanisms that include: structural damages of the cytoplasmic membrane, disruption of membrane proteins and the disturbance of the ionic transport process.
- 3. Supplementing of the nutrient medium with coriander volatile oil (CO) at concentrations of 0.002; 0.01 and 0.05% resulted in specific reactions of the test fungi (*F. oxysporum, F. sporotrichiella* var. *tricinctum, D. sorokiniana*) in the function of species and CO concentration.
- 4. Coriander essential oil has shown a pronounced toxicity in the concentration of 0.05% for the fungus under study there was no increase in *D. sorokiniana*, and at *F. oxysporum* and *F. sporotriochiella var. tricinctum* diameter of colonies consisted 29.2–49.8% and 38.2–45.2%, respectively, from the control. Concentrations of 0.002 and 0.01% stimulated the radial growth of *F. oxysporum* and *F. sporotrichiella var. tricinctum*, the values of the diameter of their colonies exceeding the control by 24.5 and 16.6%, respectively, but the stimulation of the radial growth of the fungi colonies was accompanied by a strong decrease in density and changing the color of the mycelium. By reseeding of the cultures in which the color of the mycelium has changed under the action of CO, it has been found that the compounds / coriander oil have irreversibly changed the synthesis capacity of fungal pigments.

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