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# *Chaenomeles speciosa* fruit endophytic fungi isolation and characterization of their antimicrobial activity and the secondary metabolites composition

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## Abstract

**Background:** Endophytes promote the survival of the host plants affected by unfavorable environment. To confirm the endophytes role in *Chaenomeles speciosa* pathogenic resistance, fungal isolates were derived from both fruit peel and pulp, and presumptively identified using macroscopic and microscopic techniques. Antifungal potential of the isolates was assayed by dual culture method and agar disc method against *Alternaria alternata*, *Fusarium culmorum*, and *Fusarium oxysporum* strains. Two most active fungal isolates were cultured in Czapek's liquid medium to obtain metabolites. The crude ethyl acetate extracts of metabolites were characterized for antibacterial activity against *Bacillus subtilis* and *Erwinia dissolvens*, and for component composition by GC–MS technique.

**Results:** Nine fungal endophytic isolates were derived from the peel and pulp of *C. speciosa* fruits and tentatively attributed to *Penicillium* spp. (seven isolates), *Aspergillus* spp. (one isolate), *Mucor* spp. (one isolate). Two fungal isolates, one each of the fruit peel and pulp, were designated as *Penicillium* sp. I and *Penicillium* sp. II respectively, and selected for further research. Both isolates showed similar activity against *A. alternata*; however, *Penicillium* sp. I activity against *F. culmorum* and *F. oxysporum* exceeded the activity of *Penicillium* sp. II. Cultural medium ethyl acetate extracts of both endophytes exhibited higher antibacterial activity against Gram-positive *B. subtilis*, while mycelium extracts were more active against Gram-negative *E. dissolvens*. In general, *Penicillium* sp. I antibacterial activity was higher in cultural medium extracts, while activity of *Penicillium* sp. II dominated in mycelium extracts. GC–MS analysis of the fungal metabolites component composition revealed the identity of 27 and 17 compounds, respectively in the ethyl acetate extracts of *Penicillium* sp. I and *Penicillium* sp. II cultural medium. Basic compounds produced by the first isolate, were represented by 3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo, Diisooctyl phthalate, 11-Hexadecyn-1-ol, and Propanedioic acid, dihydroxy. At the same time, Phthalic acid diisooctyl ester and other phthalates constituted the main part of the second isolate metabolites, followed by Hexadecanoic acid, Eicosyl isopropyl ether, and 4-Butoxy-2-butanone at a lower content.

**Conclusions:** The findings showed that the antimicrobial potential of *Chaenomeles* fruits endophytic fungi is promising and deserves further investigation.

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## 1 Background

Endophytic microorganisms colonize the inner intercellular space of plant asymptotically, not harming the hosts, but optimizing their interaction with the environment using different mechanisms. This may be a direct effect of endophytic fungi on pathogen, which reducing plant leaves infestation [1], or the competition of endophytes with pathogens for substrates as well [2]. Co-evolution with endophytes in natural ecosystems has enriched plants with numerous bioactive compounds [3], which have been used by mankind for centuries as plant-inherent components. The discovery that anticarcinogenic agent taxol is a secondary metabolite of endophytic fungi, served as a powerful stimulus for the examination of other endophytes metabolic potential [4]. In the recent years, endophytic microorganisms have been isolated from plants of different taxa and ecological groups, as well as from different plant organs, including leaves, stems, and rhizosphere [5]. Despite the differences in the endophytes habitats, the compounds they produce are often useful and provide one or more therapeutic effects, including bactericidal [6], antifungal [7], cytotoxic [8], antimutagenic [9], and antitubercular [10]. It becomes obvious that the endophytes isolation and cultivation has opened up new possibilities for obtaining necessary biologically active compounds instead of traditional extraction from plants [11].

The plants of genus *Chaenomeles* Lindley (*Rosaceae* family, *Magnoliopsida* class) grow naturally in south-east Asia, and were the important resource in traditional medicine for their ability to improve human health [12], which is explained by the accumulation of polyphenols and triterpenes [13, 14]. In the steppe zone of Ukraine, several *Chaenomeles* species have been successfully adapted and bear fruit [15], accumulating high level of the phenolic compounds [16] and showing greater resistance to pathogens common in the region as compared to other fruit plants [17]. Similarly, minimal fungal diseases susceptibility was indicated for *Chaenomeles* species in the other different areas [18, 19], which may be associated with the protective function of endophytes. However, there is little information about the endophytic community of *Chaenomeles* plants, except for the great diversity of *C. speciosa* mycorrhizal fungi [20]. In a previous study, we found a quite long shelf life of *C. speciosa* fruits after harvesting [15], which also may suggest the endophytes contribution to antipathogenic resistance. The aim of this work was to isolate the endophytic fungi from the *C. speciosa* fruits, discover antimicrobial

potential of the isolates and identify metabolites produced by the endophytes.

## 2 Methods

### 2.1 Plant material collection

Fruits of the *C. speciosa* plants were taken from the Botanical Garden of Oles Honchar Dnipro National University (48°26'7 " N, 35°2'34" E, Dnipro city, Ukraine). Here, plants of the genus *Chaenomeles* Lindl. have been introduced more than 25 years ago in the steppe climate with low precipitation (473 mm average, but 265 mm in dry years) and sharp temperature changes. Ripe fruits of *C. speciosa* were collected in the first half of September 2020, packed in plastic containers and taken to the laboratory immediately. The samples were washed with warm water, and then sterilized by sequentially immersing in 70% ethanol for 30 s and 4% sodium hypochlorite solution for 30 s, rinsed with sterile distilled water, and finally fired in a burner.

### 2.2 Endophytes isolation and identification

Portions of fruits peel and pulp (about 2.0 g each) were taken with a sterile scalpel and ground in a sterile mortar pestle with the addition of 10 ml of sodium chloride solution (0.5%). Aliquots (1 mL) of each suspension were inoculated on three different media, namely Potato Dextrose Agar (PDA), Meat Peptone Agar (MPA), and Gause's medium in sterilized Petri plates, followed by the plates were kept at  $28.0 \pm 0.2$  °C for 6 days. Then, the Petri plates were examined visually, and hyphae from distinct colonies were inoculated in the new Petri plates with PDA and incubated until colonies were formed. After incubation, pure cultures were transferred to PDA slants and stored at 4 °C until have been using for the further investigations. Macroscopic and microscopic techniques were the base for the identification of endophytic microorganisms. Macroscopic exploration of the endophytes morphology included revealing such features of colony as form, size, color, and hyphae. Micrography of the slides prepared from a fungal mycelium small portion and stained with methylene blue was carried out at 40× magnification using an Ulab XY-B2T LED microscope equipped with a trinocular attachment for connecting a digital camera-eyepiece.

### 2.3 Antagonistic activity bioassay by dual culture method

Antagonistic ability of the fungal isolates was evaluated by using dual culture method [21] against phytopathogenic fungi *Alternaria alternata* (strain KMB-F-16 from

the collection of Microbiology, Virology and Biotechnology Department of Oles Honchar DNU) and *Fusarium culmorum* (strain IMB-F-50716 provided by the Microbiology and Virology Institute named after Zabolotny, Kyiv, Ukraine). Mycelium discs (7 mm in diameter) of actively growing 6-day-old cultures of pathogens and isolated endophytes were placed in Petri plates with PDA medium at an equal distance from the periphery. Control plates were inoculated with pathogenic fungus only. Plates were incubated at  $28 \pm 0.2$  °C for nine days, and isolates antagonistic ability was measured as the percent inhibition (PI %) based on the equation  $PI \% = [(S_1 - S_2) / S_1] \times 100$ , where,  $S_1$  is the pathogen control colony area; and  $S_2$  is the pathogen colony area in presence of endophytic isolate.

#### 2.4 Antagonistic activity bioassay by agar disc method

Modified agar disc method [22] was used to estimate the antifungal activity of isolated endophytes against both the above-mentioned pathogens and also *Fusarium oxysporum* (strain IMB-F-54201 from Microbiology and Virology Institute named after Zabolotny). Spores suspension of each pathogen in saline was inoculated into Petri plates with Czapek's medium and kept for 4 h at room temperature, after which agar disks (7 mm in diameter), cut from an actively growing culture of isolates, were placed on the Czapek's medium surface. Plates were incubated at  $28 \pm 0.2$  °C for five days, and antagonistic ability of isolates was expressed as the inhibition zone diameter (mm). The isolates of endophytic fungi showed the highest inhibition ability in both antagonistic activity assays were selected for small scale fermentation.

#### 2.5 Cultivation and extraction of the fungal metabolites

The endophytic fungi isolated from the fruits peel (*Penicillium* sp. I) and pulp (*Penicillium* sp. II) were cultivated in submerged conditions. For this, a small piece of PDA slants containing fungal mycelium was transferred to 500 mL Erlenmeyer flask, containing 200 mL of Czapek's liquid medium of sterilized H<sub>2</sub>O, previously autoclaved at 121 °C for 40 min. After inoculation, the medium was incubated for 6 days at  $28 \pm 1$  °C with constant shaking of 160 rpm. Then, mycelia biomass was separated from the aqueous medium by filtration through a Whatman No. 1 filter, and filtrates were extracted three times with an equal volume ethyl acetate (EtOAc), using a separating funnel. Combined ethyl acetate extracts evaporated at 40 °C using rotary evaporator (IKA® RV 10, Germany), and obtained solid fraction stored at 4 °C prior to analysis. The mycelium biomass ground with the addition of ethyl acetate, was kept in the dark for 24 h, then filtered and evaporated to dryness. For bioassays and GC-MS

analysis, the residues were dissolved in ethyl acetate and filtered through a syringe filter.

#### 2.6 Antibacterial activity assessment

Antibacterial activity of crude ethyl acetate extracts of endophytic fungi was determined by the disc diffusion method [23]. Both endophytes cultural broth extracts and mycelium biomass extracts were screening for their antibacterial activity. Petri plates containing MPA medium were seeded with 10<sup>9</sup> cfu (colony forming units) suspension of Gram-positive test bacteria *Basillus subtilis* (environmental sample, strain 168) and Gram-negative *Erwinia dissolvens* (environmental sample, strain 170) both from the culture collection of Microbiology, Virology and Biotechnology Department of Oles Honchar DNU. Sterile paper discs (6 mm diameter) were impregnated with 20 µl of crude ethyl acetate extract and placed on the agar surface, followed by plates incubation at 37 °C for 24 h. Ofloxacin (5.0 µg per disc) and ceftriaxone (30.0 µg per disc) were used as the positive control. Inhibition zones produced by the EtOAc extracts were measured and antibacterial activity was expressed as the diameter of the inhibition zone (mm).

#### 2.7 Gas chromatography–mass spectrometry (GC–MS) analysis of the fungal metabolites content

Component composition of the crude ethyl acetate extracts was studied by the capillary gas chromatography connected to mass-spectrometry assay. GC-MS analysis of the extracts was performed using Shimadzu GCMS-QP 2020 EI equipped with Rxi®-5 ms column (30 m × 0.25 mm, film thickness 0.25 µm) containing 5% diphenyl/95% dimethyl polysiloxane as a fixed liquid phase. The column temperature was kept at 50 °C for 5 min, and then programmed temperature gradient increased to 300 °C at a rate of 15 °C per min and kept constant at 300 °C for 6.5 min. The carrier gas helium passed at a flow rate 54 ml/min. Injector temperature was 300 °C; sample volume was 1 µl. Peak areas of the separated compounds were integrated automatically. Mass Spectrum Library 2014 for GC-MS (O2125401310) was used to identify the separated compounds by comparing the mass spectra obtained with mass spectra of known compounds stored in the library database. The content of ethyl acetate extract individual components was expressed as a peak area percentage of total amounts.

#### 2.8 Statistical analysis

All bioassays were carry out in triplicate. The data obtained were calculated using Microsoft Excel XP 2007 and expressed as the Mean ± Standard Deviation, and the differences between means were tested with Tukey's

HSD. All differences were considered to be statistically significant at  $P < 0.05$ .

### 3 Results

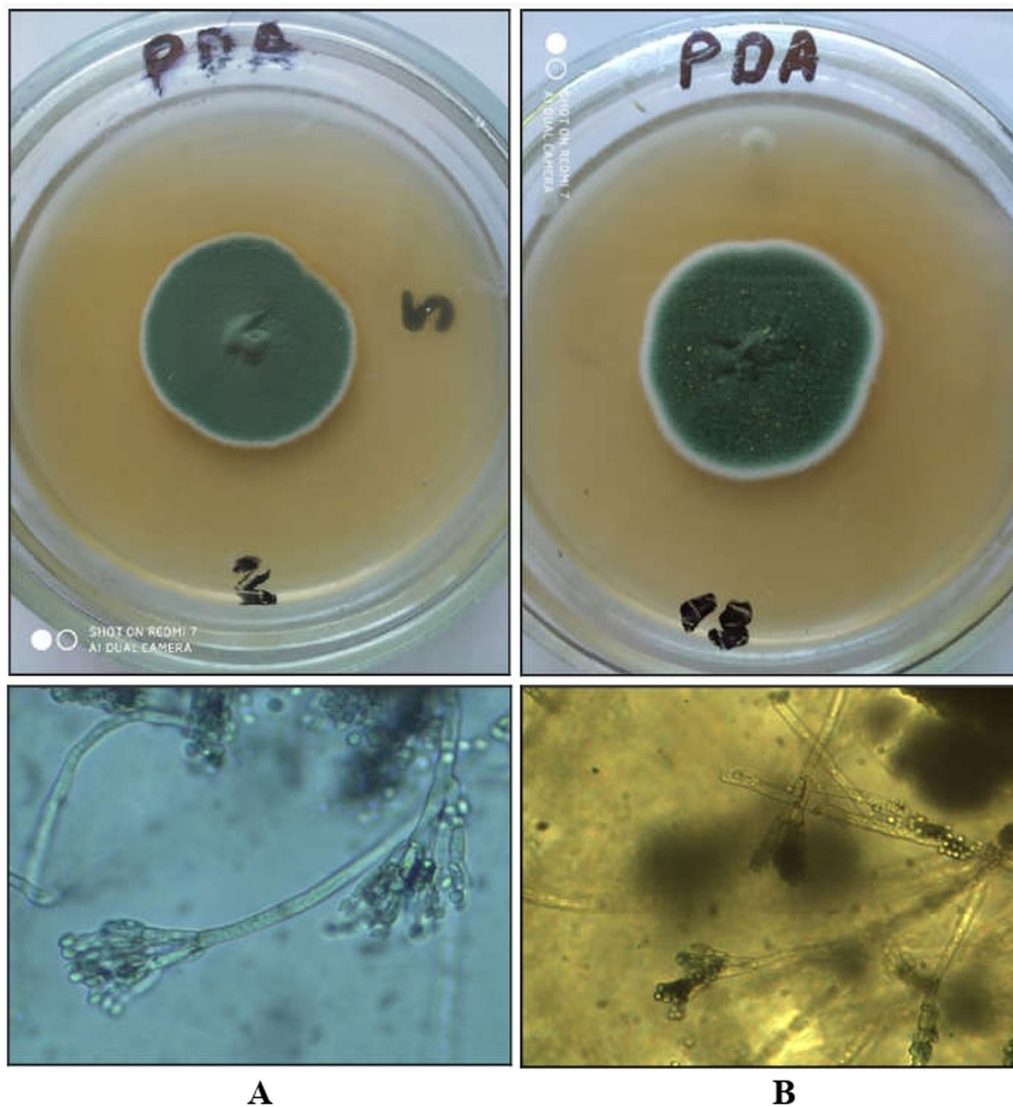
#### 3.1 Endophytes identification

Total, peel and pulp of the *C. speciosa* fruits yielded nine fungal endophytic isolates. On the base of tentative macroscopic and microscopic identification, the endophytes have been attributed to *Penicillium* spp. (7 isolates, both from peel and pulp), *Aspergillus* spp. (one isolate from the fruit peel), and *Mucor* spp. (one isolate from the fruit peel). In this study, antimicrobial ability of the fungal isolates attributed to *Penicillium* spp. was tested, and two isolates which showed higher activity against

more than one tested strains were designated as *Penicillium* sp. I (isolate from the fruit peel) and *Penicillium* sp. II (isolate from the fruit pulp). The colonies of both isolates had characteristic features of shape, color, and size. Microscopic observation of the stained mycelium slides confirmed the differences of selected *Penicillium* spp., in particular in the conidiophores morphology (including their length and width), and the conidia size (Fig. 1).

#### 3.2 Antagonistic activity evaluation

Results of dual culture bioassay on PDA medium showed the high antagonistic activity of *Penicillium* sp. I against both tested pathogenic fungi *A. alternata* and *F.*



**Fig. 1** Colonies and light micrographs of endophytic isolates from *C. speciosa* fruits. **A** *Penicillium* sp. I from peel. **B** *Penicillium* sp. II from pulp

*culmorum*, while antifungal activity of *Penicillium* sp. II was found only against *A. alternata* (Fig. 2).

In the case of *Penicillium* sp. I action in dual culture assay, inhibition of *A. alternata* colony growth averaged 77.82% of control, and a decrease in colony size of *F. culmorum* reached 76.24% compared to control. Antagonistic interaction of *Penicillium* sp. II with *A. alternata* caused the pathogenic colony growth inhibition above 84.71% of control (Table 1).

Study results of the endophytes interaction with pathogenic fungi assayed by agar disc method in Czapek's medium showed antagonistic activity of both *Penicillium* sp. I and *Penicillium* sp. II against all tested fungal pathogens (Fig. 3). The fungistatic potential of endophyte *Penicillium* sp. I was confirmed by the formation of inhibition zones with average diameter of 13.92 mm, 20.60 mm and 28.14 mm respectively in the colonies of *A. alternata*, *F. culmorum* and *F. oxysporum*. Antagonistic activity of *Penicillium* sp. II was found to be less noticeable producing the inhibition zones with a diameter of 12.90 mm, 15.41 mm, and 15.22 mm, respectively in the colonies of *A. alternata*, *F. culmorum*, and *F. oxysporum* (Table 1).

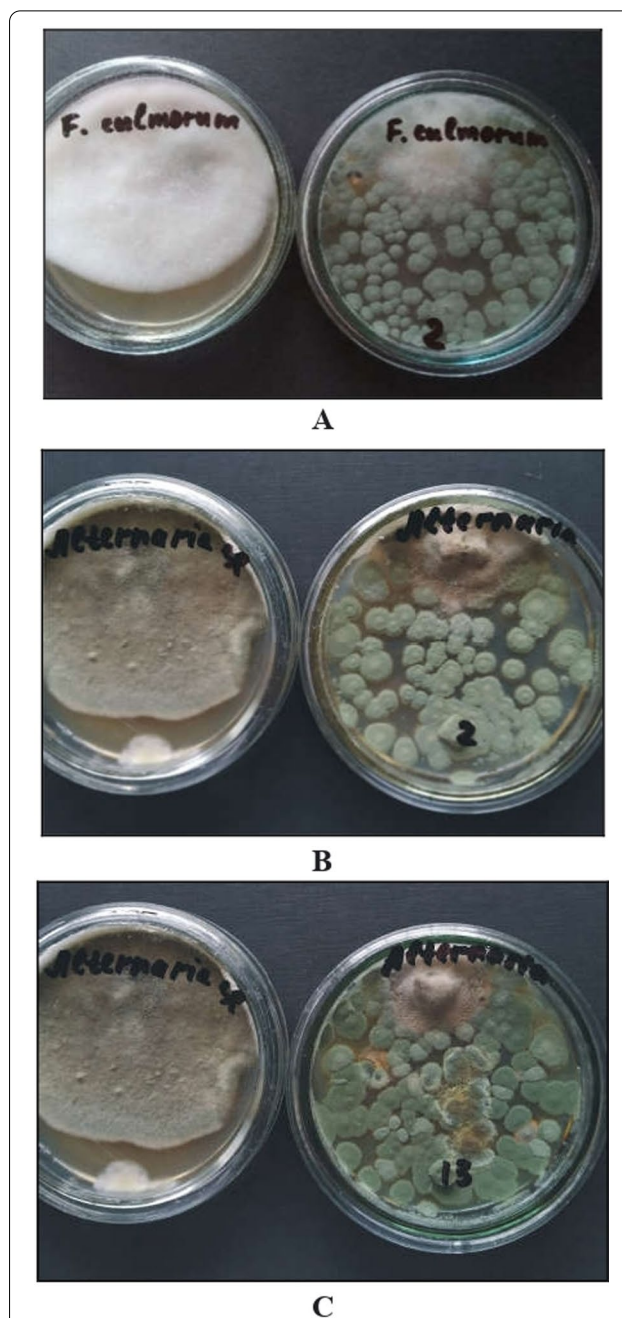
### 3.3 Antibacterial activity evaluation

Results obtained showed bioactivity of all tested crude ethyl acetate extracts of both endophytes against *Bacillus subtilis* and *Erwinia dissolvens* in the disc diffusion assay (Fig. 4).

Inhibition zones produced by *Penicillium* sp. I cultural medium extracts were more noticeable as compared to effect of *Penicillium* sp. II extracts, while the opposite result was shown by the mycelium biomass extract (Table 2). Antibacterial activity both of *Penicillium* sp. I and *Penicillium* sp. II cultural medium extracts exceeded the activity of mycelium extracts. Cultural medium extracts of both endophytes showed higher activity against Gram-positive *B. subtilis*, while mycelium biomass extracts were more active against Gram-negative *E. dissolvens*. Ethyl acetate individual action did not affect bacteria growth.

### 3.4 GC-MS analysis of the fungal secondary metabolites component composition

The results of GC-MS analysis of *Penicillium* sp. I cultural medium ethyl acetate extracts showed the identity of 27 compounds belonging to different chemical classes. The most abundant compounds (Table 3) were represented by 3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo- (49.95%), Diisooctyl phthalate (14.29%), 11-Hexadecyn-1-ol (13.19%), and Propanedioic acid, dihydroxy (9.09%). Among the 13 minor components of EtOAc extracts, which in total amounted to 2% of all only, the most notable were Cyclopentatrione, 3-butyl



**Fig. 2** Antagonistic activity of *C. speciosa* isolates as observed in dual culture assay on PDA medium. **A** *Penicillium* sp. I against *F. culmorum*. **B** *Penicillium* sp. I against *A. alternata*. **C** *Penicillium* sp. II against *A. alternata*

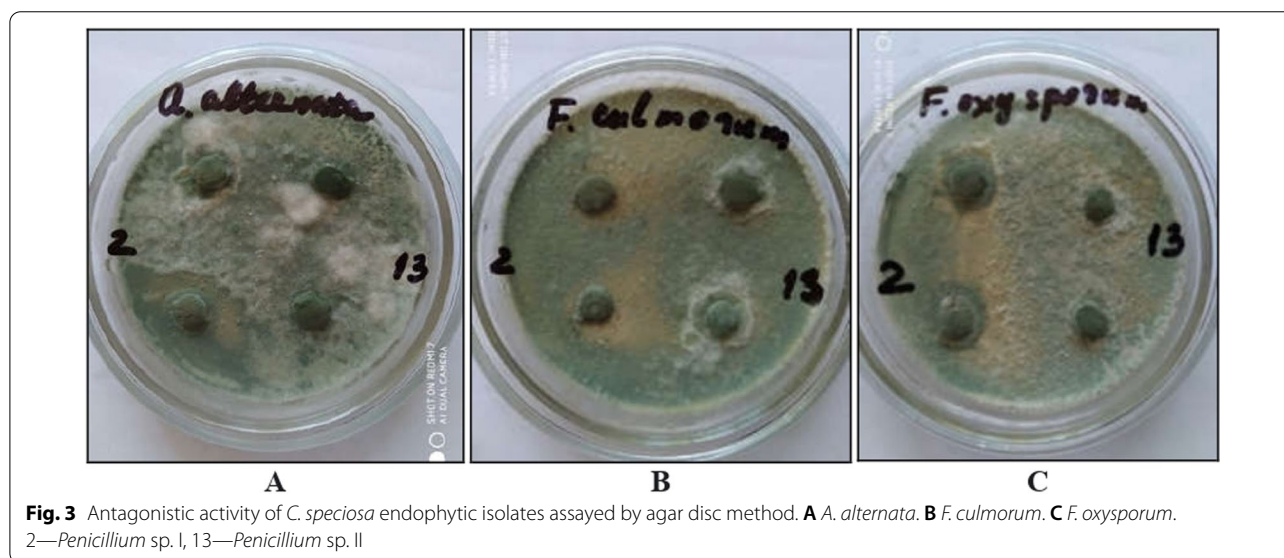
(0.12%), di-Mevalonic acid lactone (0.10%), Octadecanoic acid (0.10%), Ribitol (0.09%), Imidazole, 2-amino-5-[(2-carboxy) vinyl (0.08%), and 1H-Pyrazole, 3-Methyl (0.08%).

The GC-MS analysis of *Penicillium* sp. II ethyl acetate extracts predominantly revealed the presence of

**Table 1** Antifungal activity of endophytes isolated from *C. speciosa* fruits

Test pathogens	Percent of inhibition (PI %) by dual culture assay <sup>1</sup>		Inhibition zone diameter (mm) by agar disc method <sup>1</sup>	
	<i>Penicillium</i> sp. I	<i>Penicillium</i> sp. II	<i>Penicillium</i> sp. I	<i>Penicillium</i> sp. II
<i>Alternaria alternata</i> (KMB-F-16)	77.82 ± 2.91 <sup>a</sup>	84.71 ± 1.23 <sup>b</sup>	13.92 ± 0.34 <sup>a</sup>	12.90 ± 0.44 <sup>b</sup>
<i>Fusarium culmorum</i> (IMB-F-50716)	76.24 ± 3.12	NA	20.60 ± 1.92 <sup>a</sup>	15.41 ± 0.23 <sup>b</sup>
<i>Fusarium oxysporum</i> (IMB-F-54201)	NT	NT	28.14 ± 0.31 <sup>a</sup>	15.22 ± 0.31 <sup>b</sup>

<sup>1</sup> Data are expressed as the means of triplicate ± (SD). Values with different superscripts in each line are significantly different according to Tukey's test ( $P < 0.05$ ). NA no activity. NT not tested



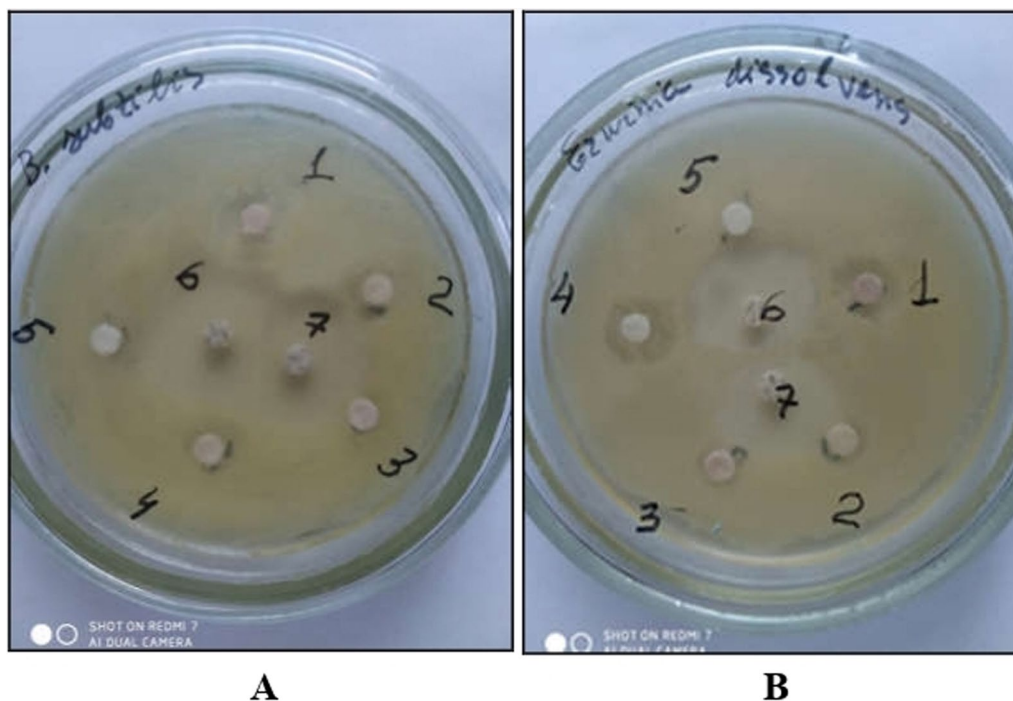
17 chemical compounds belonging to different classes, mainly to acids, esters and alcohols (Table 4). The main constituents of this EtOAc extracts were Phthalic acid diisooctyl ester (82.42%) and other phthalates, making up 87.72% of all identified compounds. Among other components, the most significant were Hexadecanoic acid (4.79%), Eicosyl isopropyl ether (1.85%), and 4-Butoxy-2-butanone (1.65%).

#### 4 Discussion

Despite a long history of the genus *Chaenomeles* species cultivation and the known tolerance to climatic conditions and pathogens, endophytic microorganisms associated with these plants have been little studied. To test the hypothesis about the possible endophytes contribution to the *C. speciosa* resistance to biotic and abiotic stimuli, we investigated antimicrobial potential of the fungal isolates from the fruits peel and pulp, as well as the secondary metabolites composition of isolates. Studies of the plant fruits endophytic microflora are few at the

moment. Known data are reports of endophytic fungus from the *Sapindus saponaria* fruits showed the ability to biotransform glycosides [24], and fungus from the *Melia azedarach* fruits which capable of synthesizing polyketide citrinin that inhibit *Leishmania* growth [25].

In our work, endophytic fungi were found both in the *C. speciosa* fruit peel and pulp and represented by three different genera. All of the fungal isolates attributed to the *Penicillium* genus were active against more than one test microorganism; however, potential of *Penicillium* sp. I from fruit peel and *Penicillium* sp. II from fruit pulp was more revealing. Both selected isolates inhibited growth of the phytopathogenic fungi *A. alternata* and *F. oxysporum*, while only *Penicillium* sp. I showed a noticeable antagonism against *F. culmorum*. In our study, *A. alternata* and *F. culmorum* strains were used as the test objects, since they parasitize on various fruit plants, including some *Chaenomeles* species [18]. At the same time, Wang et al. [6] reported *A. alternata* as the endophytic fungus isolated from *Camellia sinensis* branches.



**Fig. 4** Antibacterial activity of EtOAc extracts of *C. speciosa* fruit endophytic isolates. **A** *Bacillus subtilis*. **B** *Erwinia dissolvans*. 1—*Penicillium* sp. I cultural medium, 2—*Penicillium* sp. II cultural medium, 3—*Penicillium* sp. I mycelium, 4—*Penicillium* sp. II mycelium, 5—EtOAc, 6—ofloxacin, 7—ceftriaxone

**Table 2** Antibacterial activity of ethyl acetate extracts of *C. speciosa* fruit endophytic isolates<sup>1</sup>

Endophyte name	Extractive substrate	Extract content, µg per disc	<i>Bacillus subtilis</i>	<i>Erwinia dissolvans</i>
<i>Penicillium</i> sp. I	Cultural medium	20.0	19.0 ± 3.61 <sup>a</sup>	14.67 ± 3.06 <sup>a</sup>
	Mycelium biomass	6.0	9.33 ± 0.58 <sup>b</sup>	9.52 ± 0.50 <sup>b</sup>
<i>Penicillium</i> sp. II	Cultural medium	38.0	14.0 ± 1.00 <sup>ab</sup>	11.33 ± 1.15 <sup>ab</sup>
	Mycelium biomass	6.0	12.33 ± 1.53 <sup>b</sup>	14.00 ± 2.00 <sup>ab</sup>
Ofloxacin			25.33 ± 2.08 <sup>c</sup>	23.33 ± 1.15 <sup>c</sup>
Ceftriaxone			25.33 ± 0.58 <sup>c</sup>	24.33 ± 2.08 <sup>c</sup>
Ethyl acetate			NA	NA

<sup>1</sup> Data on the inhibition zone diameter (mm) are expressed as the means of triplicate ± (SD). Values with different superscripts in each column are significantly different according to Tukey’s test ( $P < 0.05$ ). NA no activity

Like this, *F. culmorum* as the endophyte can confer salt tolerance to the coastal plant *Leymus mollis* [26]. There is no contradiction here, since fungal species are characterized by the ability to enter into different forms of symbiosis, expressing either a mutualistic or pathogenic lifestyle in different host plants [27].

Antibacterial activity against Gram-positive *B. subtilis* and Gram-negative *E. dissolvans* was found in the ethyl acetate extracts of culture medium and mycelium of both studied isolates; however, antibacterial activity of *Penicillium* sp. I was higher, as was its antifungal potential. In

general, the study results are consistent with the known data on a wide spectrum of antimicrobial activity of the endophytic fungi secondary metabolites, shown by *Aspergillus fumigatus* [7], *Botryosphaeria dothidea* [8], and *Aspergillus clavatonanicus* [21].

According to GC–MS assay, both *Penicillium* sp. I and *Penicillium* sp. II produced the pool of different chemical compounds, including fatty acids, esters and ethers, alcohols, and aldehydes. The study results confirm the known ability of the genus *Penicillium* for the various secondary metabolites biosynthesis [28]. The

**Table 3** Main metabolites content of isolate *Penicillium* sp. I as identified by GC–MS analysis

Peak no	RT <sup>1</sup> , min	Compound name	Area, %	Molecular formula	Class
1	1.46	Propanedioic acid, dihydroxy	9.09	C <sub>3</sub> H <sub>4</sub> O <sub>6</sub>	Acid
2	2.23	2-Butenoic acid, 3-Methyl	0.30	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	Acid
3	4.24	Pentanedioic acid, 3-(1,2-diphenyl-propyliden), monomethyl ester	1.61	C <sub>21</sub> H <sub>22</sub> O <sub>4</sub>	Ester
4	4.51	Propanedioic acid, phenyl	0.57	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	Acid
5	5.23	Benzenamine, 3-Methoxy-4-[3(1-piperidinyl)propoxy]-	2.45	C <sub>15</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub>	Aromatic amine
6	5.68	Benzeneethanol, 4-hydroxy	1.31	C <sub>8</sub> H <sub>10</sub> O <sub>2</sub>	Alcohol
7	6.03	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	49.95	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	Acid
8	6.22	11-Hexadecyn-1-ol	13.19	C <sub>16</sub> H <sub>30</sub> O	Alcohol
9	6.32	7-Hexadecenal	0.42	C <sub>16</sub> H <sub>30</sub> O	Aldehyde
10	8.26	Hexadecanoic acid	0.95	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Fatty acid
11	8.35	Phthalic acid, butyl tridecyl ester	0.64	C <sub>25</sub> H <sub>40</sub> O <sub>4</sub>	Ester
12	8.65	Henicosanal	2.32	C <sub>21</sub> H <sub>42</sub> O	Aldehyde
13	9.04	Octadec-9-enoic acid	0.51	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Fatty acid
14	10.88	Phthalic acid, diisooctyl ester	14.29	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	Ester

Data on compounds content are expressed as peak area (% of total amounts)

RT Retention time

**Table 4** Main metabolites content of isolate *Penicillium* sp. II as identified by GC–MS analysis

Peak no	RT <sup>1</sup> , min	Compound name	Area, %	Molecular formula	Class
1	1.84	4-Butoxy-2-butanone	1.65	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	Ketone
2	4.57	9-Oxononanoic acid	0.30	C <sub>9</sub> H <sub>16</sub> O <sub>3</sub>	Acid
3	5.99	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	0.38	C <sub>12</sub> H <sub>16</sub> O <sub>5</sub>	Acid
4	6.07	9-Methyl-10-pentadecen-1-ol	0.31	C <sub>16</sub> H <sub>32</sub> O	Alcohol
5	6.20	8,10-Hexadecadien-1-ol	0.34	C <sub>16</sub> H <sub>30</sub> O	Alcohol
6	7.00	1-Hexadecanol	0.38	C <sub>16</sub> H <sub>34</sub> O	Alcohol
7	7.62	Tetradecanoic acid, 1-methylethyl ester	0.63	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	Ester
8	7.92	Bis(tridecyl) phthalate	0.40	C <sub>34</sub> H <sub>58</sub> O <sub>4</sub>	Ester
9	8.04	Octanoic acid, oct-3-en-2-yl ester	0.36	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	Ester
10	8.26	Hexadecanoic acid	4.79	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Fatty acid
11	8.35	Phthalic acid, butyl tridecyl ester	1.78	C <sub>25</sub> H <sub>40</sub> O <sub>4</sub>	Ester
12	8.80	Nonadecanol-1	0.54	C <sub>19</sub> H <sub>40</sub> O	Alcohol
13	9.05	Octadec-9-enoic acid	0.75	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Fatty acid
14	9.59	Phthalic acid, isobutyl octadecyl ester	1.55	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	Ester
15	10.95	Phthalic acid, diisooctyl ester	82.42	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	Ester
16	12.02	Phthalic acid, di(2-ethylhexyl) ester	1.57	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	Ester
17	12.96	Eicosyl isopropyl ether	1.85	C <sub>23</sub> H <sub>48</sub> O	Ether

Data on compounds content are expressed as peak area (% of total amounts)

1 RT Retention time

Furanacetic acid derivative (3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-) which is a significant component of both *Penicillium* sp. I and *Penicillium* sp. II ethyl acetate extracts, may presumably exhibit biological activity similar to other furancarboxylic acid derivatives having the antibacterial and antifungal potential [29]. Alcohol

11-Hexadecyn-1-ol, produced by *Penicillium* sp. I, was characterized [30] as plant essential oil component having high antioxidant potential. Phenylmalonic acid contained in the *Penicillium* sp. I ethyl acetate extract, was reported [31] as bioactive compound. Antifungal activity was shown by acetic acid, 2-methylpropyl ester [32]



and benzene ethanol [33], which were identified in our work in the cultural medium of isolate *Penicillium* sp. I. Imidazole, 2-amino-5-[(2-carboxy) vinyl] revealed in the *Pencillium* sp. I ethyl acetate extract, was reported [34] as metabolite of pome fruits pathogen *Penicillium expansum* showed a wide range of antibacterial activity. Both fruit isolates *Penicillium* sp. I and *Penicillium* sp. II exhibited the ability to synthesize Cis-9-octadecenoic acid, which is known due to versatile health benefits including cancer preventive, hypocholesterolemic, anti-inflammatory, and choleric action [35].

The high content of phthalic acid esters produced by both *Pencillium* sp. I and *Pencillium* sp. II was not expected in our work, but at the same time it is not a unique finding. Bhimba et al. [23] designated Dibutyl phthalate and Mono (2-ethylhexyl) phthalate produced by the marine fungi *Phoma herbarum* among the major components of fungal filtrates, which exhibited the wide spectrum of antibacterial activity. Phthalic acid, butyl undecyl ester was identified in the methanolic extract of *Penicillium expansum* [34]. Lotfy et al. [22] reported Di(2-ethylhexyl) phthalate as the most abundant bioactive metabolite produced by fungus *Aspergillus awamori*, exhibited activity against *Candida albicans* and the Gram positive bacteria *Sarcina lutea*, and also cytotoxic activity against some carcinoma cell lines. A specific feature of the *Pencillium* sp. I and *Pencillium* sp. II isolated from the *C. speciosa* fruit consists of the variety of phthalic acid esters produced by these fungi. In total, five different phthalates have been identified in the fungal ethyl acetate extracts. Among them, Diisooctyl phthalate and Butyl tridecyl phthalate were synthesized by both isolates, with the first compound dominance in the metabolites. Only *Pencillium* sp. II produced Bis(tridecyl) phthalate, Isobutyl octadecyl phthalate, and Di(2-ethylhexyl) phthalate. These data allow us to consider the action of metabolites with known biological activity as a possible basis for the antimicrobial ability of *Penicillium* sp. I and *Penicillium* sp. II. However, further research is needed in order to carry out large-scale cultivation of the fungal isolates, to obtain the synthesized individual compounds and to identify their bioactivity.

## 5 Conclusions

In our study, nine fungal endophytes were isolated from *C. speciosa* fruits. All the fungal isolates attributed to *Penicillium* genus were moderately active against more than one tested pathogens; however, the antimicrobial potential of the fruit peel isolate *Penicillium* sp. I was more pronounced than of fruit pulp isolate *Penicillium* sp. II. The secondary metabolites of both cultured isolates contain several compounds with known antimicrobial activity, which indicates the further study advisability of the

fruit-associated endophytes biosynthetic potential. The findings also indicate that the endophytic community of fruit plants resistant to pathogens and adverse environment can be a rich source of new bioactive compounds.

### Abbreviations

EtOAc: Ethyl acetate; CFU: Colony Forming Units; GC–MS: Gas Chromatography–Mass Spectrometry; MPA: Meat Peptone Agar; PDA: Potato Dextrose Agar; PI: Percent Inhibition; RT: Retention Time.

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### Authors' contributions

Concept developing: YVL and NOK. Experiments and methodology: OAD, AOA, TVS, OOD, and NOK. Data and statistical analysis: OOD, AOA, and TVS. Writing draft manuscript: NOK and OAD. Writing – review and editing manuscript: YVL, NOK, TVS, and OOD. Supervision: YVL, NOK and OOD. All authors have read and approved the final manuscript.

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### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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