J. Crop Prot. **2016, 5 (3): 377-387**
doi: 10.18869/modares.jcp.5.3.377

Research Article

Soilborne and invertebrate pathogenic *Paecilomyces* species show activity against pathogenic fungi and bacteria

Sima Mohammadi[1†], Jalal Soltani[2†] and Khosro Piri[1*]

1. Department of Plant Biotechnology, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.
2. Department of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.

Abstract: The fungal genus *Paecilomyces* comprises numerous pathogenic and saprobic species, which are regularly isolated from insects, nematodes, soil, air, food, paper and many other materials. Some of the *Paecilomyces* species have been known to exhibit capabilities for curing human diseases. Here, bioactivities of metabolites from some soil inhabitant and invertebrate pathogenic *Paecilomyces* species were explored against a panel of target prokaryotic and eukaryotic microorganisms. First, Petri plate assays indicated that all tested *Paecilomyces* species were capable of producing diffusible metabolites and volatile compounds with antifungal activities against *Pyricularia oryzae* and *Saccharomyces cerevisiae*. Subsequently, the metabolites of the *Paecilomyces* species were extracted and the growth inhibitory and antimitotic effects of extra-cellular metabolites were shown using the yeast *S. cerevisiae* as a model. Further research indicated some antibacterial activity of extra-cellular metabolites from *Paecilomyces* species against human pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* (G^+) and *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (G^-). These findings indicate that the *Paecilomyces* species, either saprobic or pathogenic, have a strong arsenal of bioactive metabolites which show inhibitory or cytotoxic effects against other microorganisms, with a potential for application in agroforestry and medicine.

Keywords: *Paecilomyces fumoroseus*, *Paecilomyces lilacinus*, *Paecilomyces variotii*, Secondary metabolite, volatile compounds, antifungal, antibacterial, antimitotic

Introduction

The discovery of new biologically active secondary metabolites is of very high interest for both agrochemical and pharmaceutical bioindustries. Secondary metabolites (SM) are small bioactive molecules produced by many organisms specially plants, fungi and bacteria. These compounds are particularly abundant in the soil-dwelling microorganisms, which exist as multicellular communities competing with each other for ecological niches, nutrients, minerals and water. Among these, filamentous fungi are well established sources for such substances (Keller *et al.*, 2005).

Soil represents one of the main reservoirs of filamentous fungi. The fungal genus *Paecilomyces* comprises numerous pathogenic and saprobic species, which are regularly isolated from insects, nematodes, soil, air, food, paper and many other materials (Aguilar *et al.*,

Handling Editor: Naser Safaie

† JS and SM contributed equally to this work, and both should be considered as the first authors.

[*] **Corresponding author**, e-mail: Khpiri@gmail.com
Received: 25 July 2015, Accepted: 27 January 2016
Published online: 15 June 2016

1998; Fiedler and Sosnowska, 2007; Gupta *et al*., 1993; O'Day, 1977; Marti *et al*., 2006; Saberhagen *et al*., 1997; Tigano-Milani, *et al*., 1995; Westenfeld, *et al*., 1996). Although, there has been no comprehensive review of the genus yet, more than forty species have been recognized in the genus *Paecilomyces* (Luangsa-ard *et al*., 2004). A number of *Paecilomyces* species are known to produce a scintillating array of bioactive secondary metabolites of different chemical classes and with different biological activities (Wang *et al*., 2002).

Currently, sustainable agriculture is demanding innovative and environmentally friendly procedures to protect plants against biotic stresses. Moreover, there is an ongoing need for novel sources of bioactive metabolites for the treatment of human infections and cancer diseases. Some of the *Paecilomyces* species have been known to exhibit promising capabilities for curing diseases of human being (Furuya *et al*., 1983; Manabe *et al*., 1996; Choi *et al*., 1999). However, little is known about the biological activities of pathogenic *Paecilomyces* species that infect invertebrate organisms such as insects and nematodes. Here, antifungal, antimicrobial and antiproliferative activities of secondary metabolites from a few soil inhabitant and invertebrate pathogenic *Paecilomyces* species are explored against a panel of target prokaryotic and eukaryotic microorganisms.

Materials and Methods

Paecilomyces fungal species

The soliborne fungi *Paecilomyces variotii*, *Paecilomyces lilacinus* S1, and *Paecilomyces fumoroseus* isolated from the soil samples (Jamali and Banihashemi, Shiraz University, Fars, Iran, unpublished), the nematophagous *Paecilomyces lilacinus* N1 isolated from the potato cyst nematode *Globodera rostochiensis* (Giti M., Agriculture Research Center, Hamedan, Iran, unpublished) and the entomopathogenic *Paecilomyes* sp.1 and *Paecilomyces* sp.2 isolated from the Colorado potato beetles *Leptinotarsa decemlineata* (Say) (Assadollahpour *et al*., 2011) were obtained as pure cultures. The isolates were subcultured onto Potato Dextrose Agar (PDA) medium (Merck Co., Germany) and stored at 4 ºC.

Target microorganisms and culture condition

The bioactivity of *Paecilomyces* species were tested on a number of model target fungi and bacteria, *in vitro*. The filamentous fungus *Pyricularia oryzae* HS-1390 (Hosseyni-Moghaddam and Soltani, 2013) served as a model target in antifungal experiments. The fungus was brought into pure culture and maintained on PDA culture medium at 4 ºC. The budding yeast *Saccharomyces cerevisiae* PTCC5269 was also used as a model target for antifungal and antiproliferative/cytotoxicity assays. The yeast isolate was maintained on Yeast Extract-Peptone-Dextrose-Agar (YPDA) culture medium.

The target bacteria included six human pathogenic bacteria, i.e. the gram-positive bacteria *Staphylococcus aureus* PTCC118, *Bacillus subtilis* PTCC1159, *Streptococcus pyogenes* PTCC1447, and the gram-negative bacteria *Escherichia coli* PTCC1399, *Pseudomonas aeruginosa* PTCC1181 and *Salmonella typhi* PTCC1609. The strains were periodically subcultured on Nutrient Agar (NA) medium and stored at 4 ºC.

Antifungal bioassays using *Pyricularia oryzae*

Pyricularia oryzae HS-1390 was initially used as a model target to screen for antifungal activity of *Paecilomyces* species. The anti-*P. oryzae* activity of *Paecilomyces* species was examined in two manners. First, a dual culture of each *Paecilomyces* isolate and *P. oryzae* was performed on PDA Petri plates at 28ºC. Daily mycelia growth of *P. oryzae*, in the presence of each *Paecilomyces* isolate was compared to that of control. After 10 days, the diameter (D) of the inhibition zone was measured (mm) and growth inhibition rate was calculated by the following formula: Growth inhibition rate (%) = (D control – D treated / D control) × 100.

Second, the effect of volatile compounds (VOC) produced by *Paecilomyces* isolates were examined on the mycelia growth of *P.*

oryzae, *in vitro*. To this end, each *Paecilomyces* isolate, as well as, the target *P. oryzae* were subcultured simultaneously on separate PDA Petri plates. The caps of Petri plates were removed and the plates were put on each other and sealed by parafilm. Daily mycelia growth of *P. oryzae*, was compared to that of control, and after 8 days, the diameter (D) of the inhibition zone was measured (mm) as explained before.

Antifungal bioassays using *Saccharomuces cerevisiae*

The budding yeast *Saccharomyces cerevisiae* PTCC5269 was also used as a model target in antifungal bioassays. The anti-yeast activity of *Paecilomyces* species was examined in two manners. First, a bilayer culturing system was applied. For this, each *Paecilomyces* species was cultured on a PDA Petri plate and incubated at 28 ºC for 10 days, until the hyphae covered 3/4 of the Petri plate. Then a cooled YPDA culture medium was poured on each *Paecilomyces* culture to make a bilayer culture medium, and was let be solidified. Then, the Petri plates were incubated at 28 ºC for 24 hours to let the *Paecilomyces* metabolites diffuse in the upper YPDA layer. Subsequently, the yeast cells were spread over the YPDA layer, and the plates were incubated at 28 ºC for 18 hours. The emergence and growth of yeast colonies were evaluated compared to the control plates in a quantifiable manner.

Second, the effect of volatile compounds (VOC) produced by *Paecilomyces* isolates were examined on the emergence and growth of yeast colonies, *in vitro*. To this end, each *Paecilomyces* isolate was subcultured on a separate PDA Petri plate, and let grow and cover the whole plate. When, the yeast was cultured on YPDA, the caps of Petri plates were removed and the *Paecilomyces* plates were put on each yeast plate and sealed by parafilm. After 24 hours at 28 ºC the emergence and growth of yeast colonies were compared to that of control. The experiment was repeated two times.

Intra-and extra-cellular metabolite extraction from *Paecilomyces* species

The fresh *Paecilomyces* species were inoculated into Potato Dextrose Broth (PDB) in Erlenmeyer flasks and incubated for 12 days at 28 ºC, 120 rpm, under dark condition. After the fermentation processes, each individual culture broth was extracted with methanol (MeOH).

To obtain extracellular metabolites, the fermentation broth was filtered. Then, equal volumes of the organic solvent MeOH (1:1) was added to each individual culture broth. The extract was transferred to 4 ºC for 12 hours to remove waxy materials. Subsequently, the solvent was removed by evaporation under 50 ºC. The dried MeOH extracts were re-dissolved in double distilled water or DMSO to obtain a final concentration of 250 mg ml^{-1}. Finally, the concentrated extracts were passed through a filtration membrane (d = 0.22 µm) before their bioactivities were assayed.

To obtain intracellular metabolites, the fresh *Paecilomyces* species were inoculated into Potato Dextrose Broth (PDB) in Erlenmeyer flasks (2 liter) and incubated for 30 days at 28ºC, the mycelia biomasses were harvested, thoroughly washed, and were macerated in MeOH (1:5, 2 days). Then the mycelia were homogenized thoroughly. The supernatants were further treated as explained above for the extracellurar metabolites.

Until used for bioassays, the secondary metabolites were kept at -20 ºC.

Antimitotic/cytotoxic bioassays

Saccharomyces cerevisiae was recruited as a model target microorganism to screen for antimitotic/cytotoxic activity of *Paecilomyces* extracellular metabolites. For this, the *S. cerevisiae* was grown overnight in YPD broth medium to obtain 1.5×10^8 CFU mL^{-1}. A 5 mL aliquot was seeded into each well of a 96 well microtiter plate containing 95 µl YPD. The sample extract (100 µL) was added to each well in a serially diluted manner to yield the final concentrations of 100, 50, 25 and 15 mg mL^{-1}. The assay plates were incubated at 27 ºC for 24 h. The growth of yeast was observed and compared with that of the control to determine the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). The experiments were performed in triplicate and were repeated three times.

Moreover, to track the cell number changes, *S. cerevisiae* (1.5×10^8 CFU.mL) was seeded (250 µL) into test tubes containing 10 mL YPD. Then, the sample extract (1 mL) was added to each test tube in a serially diluted manner to yield the final concentrations of 100, 50, 25 and 15 mg mL^{-1}. The assay tubes were incubated at 27 ºC for 18 h, 120 rpm. The experiments were followed as described above. The growing numbers of yeast cells (OD_{620}) were recorded at OD_{620}.

Antimicrobial bioassays

Six human pathogenic bacteria were employed as model target bacteria in our antimicrobial assays, as mentioned above. Extra-cellular secondary metabolites from *Paecilomyces* isolates were evaluated for their antimicrobial bioactivities. For this, bacteria were grown overnight in Nutrient Broth (NB) medium to obtain 1.5×10^8 CFU ml^{-1}. Then, 5 mL bacterial suspensions were seeded into each well of a 96 well microtiter plate that contained 95 µl of Nutrient Broth medium. The extracellular extract (100 µl) was added to each well in a serially diluted manner to yield the final concentrations of 100, 50, 25.15 mg ml^{-1}. The assay plates were incubated at 37 ºC for 24 h. The growth of target bacteria was observed and compared with that of the control to determine the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC). The experiments were performed in triplicate and were repeated three times.

Statistical analysis

SAS procedure and programs were used for statistical analysis. In case where the F-test showed significant differences among means, the differences among treatments were compared using least significant differences (LSD) test at 1% significance level.

Results

Antifungal activity of *Paecilomyces* species against *Pyricularia oryzae*

The rice blast pathogen *Pyricularia oryzae* is used as a model target for primary screening of antitumor and antifungal agents (Kobayashi *et al*. 1996, Dong *et al*., 2008; Xu *et al*., 2009; Hosseyni-Moghaddam *et al*., 2013; Hosseyni-Moghaddam and Soltani, 2014a, 2014b; Pakvaz and Soltani, 2016; Soltani and Hosseyni-Moghaddam, 2014a, 2014b, 2015; Soltani *et al*., 2016). As shown in Table 1, all *Paecilomyces* isolates inhibited the mycelial growth of the model fungus *P. oryzae*, in Petri plate dual culture assays. However, *Paecilomyces variotii* and *P. lilacinus* isolates were significantly more bioactive (54.3-61% growth inhibition) than the other isolates in this assay.

We further investigated the potential of volatile compounds (VOCs) production by *Pecilomyces* isolates and their possible biological effects. As shown in Table 2, all *Pecilomyces* isolates produced bioactive volatile compounds which inhibited the mycelia growth of the model fungus *P. oryzae*, in Petri plate assays. However, volatile compounds produced by *Paecilomyces variotii* and *P. lilacinus* S1 were significantly more bioactive than the other isolates (53.5-68% growth inhibition) in this assay.

Antifungal activity of *Paecilomyces* species against the yeast *Saccharomyces cerevisiae*

The antifungal activity of the *Paecilomyces* isolates against the yeast was shown in two ways. First, the bilayer technique indicated that the diffusible metabolites of the *Paecilomyces* isolates moderately inhibited the emergence and growth of yeast colonies (Table 3). However, the metabolites of *Paecilomyces lilacinus* S1, *Paecilomyces fumoroseus* and *Paecilomyces* sp.2 were more bioactive than the others in this assay.

Second, the inhibitory effects of volatile compounds (VOCs) produced by *Pecilomyces* isolates was shown, *in vitro*, on yeast. Observations indicated that all *Pecilomyces* species were capable of producting bioactive VOCs (Table 4). However, the VOCs of *Paecilomyces fumoroseus* were more bioactive than the other isolates, and severely decreased the emergence and growth of yeast colonies.

Table 1 Antifungal bioactivity of *Paecilomyces* isolates against the mycelia growth of *Pyricularia oryzae* in Petri plate dual culture assays.

Fungus isolate	Mycelia growth of *P. oryzae*	Growth inhibition (%)
Paecilomyces variotii	1.4 ± 0.15 [d]	61.0
Paecilomyces lilacinus N1	1.5 ± 0.11 [d]	57.0
Paecilomyces lilacinus S1	1.6 ± 0.10 [d]	54.3
Paecilomyces sp.1	1.8 ± 0.32 [cd]	48.5
Paecilomyces sp.2	2.1 ± 0.15 [c]	40.0
Paecilomyces fumoroseus	2.5 ± 0.26 [b]	28.5
Control	3.5 ± 0.10 [a]	-

Data are averages (± standard deviation) of three replications. Similar letters indicate no significant differences (LSD test, P < 0.01).

Table 2 *Pyricularia oryzae* mycelia growth inhibition activity of volatile compounds (VOCs) produced by *Pecilomyces* isolates, *in vitro.*

Fungus isolate	Mycelia growth of *P. oryzae*	Growth inhibition (%)
Paecilomyces variotii	0.86 ± 0.02 [d]	68.0
Paecilomyces lilacinus S1	1.26 ± 0.25 [cd]	53.5
Paecilomyces sp.1	1.40 ± 0.26 [c]	48.0
Paecilomyces lilacinus N1	1.43 ± 0.20 [c]	47.0
Paecilomyces fumoroseus	1.56 ± 0.11 [bc]	42.3
Paecilomyces sp.2	1.90 ± 0.10 [b]	29.6
Control	2.70 ± 0.02 [a]	-

Data are averages (± standard deviation) of three replications. Similar letters indicate no significant differences (LSD test, P < 0.01).

Table 3 Antifungal bioactivity of *Paecilomyces* isolates against the emergence and growth of the yeast *S. cerevisiae* colonies in Petri plate bilayer culture assays.

Fungus isolate	The 1st experiment	The 2nd experiment
Paecilomyces variotii	+ + -	+ + -
Paecilomyces lilacinus S1	+ - -	+ - -
Paecilomyces fumoroseus	+ - -	+ - -
Paecilomyces lilacinus N1	+ + -	+ - -
Paecilomyces sp.1	+ + -	+ + -
Paecilomyces sp.2	+ - -	+ - -
Control	+ + +	+ + +

Symbols: (+ + +): No inhibition (as the control); (- - -): Complete inhibition of yeast colonies; (+ + -) Moderate growth of yeast colonies; (+ - -): Low growth of yeast colonies. The observations were averages of two repeats.

Table 4 Antifungal bioactivity of volatile compounds (VOCs) produced by *Paecilomyces* isolates against the emergence and growth of the yeast *S. cerevisiae* colonies.

Fungus isolate	The 1st experiment	The 2nd experiment
Paecilomyces variotii	+ + -	+ - -
Paecilomyces lilacinus S1	+ + -	+ + -
Paecilomyces fumoroseus	+ - -	+ - -
Paecilomyces lilacinus N1	+ - -	+ + -
Paecilomyces sp.1	+ + -	+ + -
Paecilomyces sp.2	+ + -	+ + -
Control	+ + +	+ + +

Symbols: (+ + +): No inhibition (as the control); (- - -): Complete inhibition of yeast colonies; (+ + -): Moderate growth of yeast colonies; (+ - -): Low growth of yeast colonies. The observations were averages of two repeats.

Anti-fungal and anti-mitotic bioactivities of intra-and extra-cellular *Pecilomyces* metabolites tested on the yeast *Saccharomyces cerevisiae*

The budding yeast *Saccharomyces cerevisiae* (an ascomycetous fungus) was used in our assays as a model target organism to test for *Paecilomyces* isolates metabolite bioactivity. The concentrations of 5, 15, 25, 50 and 100 mg mL^{-1} of the intra- and extra-cellular metabolites from *Paecilomyces* isolates were investigated for their MIC and MFC efficiencies against the yeast *S. cerevisiae*. As shown in Table 5, except for *Paecilomyces* sp.1, at the provided concentrations the intra-cellular metabolites of *Paecilomyces* isolates didn't show any anti-yeast activity. However, the extra-cellular metabolites of all isolates showed inhibitory activities against *S. cerevisiae*, mainly at MIC concentrations of 50 mg mL^{-1}. Moreover, only the extra-cellular metabolites of *Paecilomyces* sp.1 had a fungicidal effect against *S. cerevisiae*, at an MFC concentration of 50 mg mL^{-1}.

Accordingly, the antimitotic bioactivity of extra-cellular metabolites from *Paecilomyces* isolates was investigated, as measured by the growing numbers of yeast cells (OD_{620}) after 18 hours of incubation at 27 °C. As shown in Table 6, all extra-cellular metabolites showed some degree of the antimitotic/cytotoxic activities. However, at the lowest metabolite concentrations of 15 and 25 mg mL^{-1}, the isolates *Paecilomyces variotii*, *Paecilomyces lilacinus* S1, and *Paecilomyces* sp.2 showed the most potent antimitotic activities. Increasing the metabolite concentration increased the antimitotic/cytotoxic activity. In this regard, the highest cytotoxic activity (87.1%) was seen for the 100 mg mL^{-1} concentration of *Paecilomyces lilacinus* S1 metabolite.

Antibacterial activity of extra-cellular metabolites from *Pecilomyces* species

Antimicrobial activities of extra-cellular *Pecilomyces* metabolites (5, 15, 25, 50 and 100 mg mL^{-1}) were further tested on six bacterial species, i.e. *B. subtilis*, *S. aureus*, *S. pyogenes E. coli*, *P. aeruginosa*, and *S. typhi*. The results of MIC and MBC experiments are presented in Table 7. As seen, in the range of the provided metabolite concentrations all extracts showed inhibitory effects. The results of MIC experiments indicated that, in general, the extra-cellular extracts of *Paecilomyces* sp.1 and *Paecilomyces* sp.2 could inhibit the growth of the target bacteria at lower concentrations than the other species. The highest MICs were observed for the metabolites of *P. variotii* and *Paecilomyces* sp.1 against *E. coli* and *B. subtilis*, respectively (Table 7).

However, as the results of MBC experiments indicated (Table 7), the extra-cellular extracts were rarely bactericidal in the range of the provided metabolite concentrations. In this respect, the metabolites of *P. variotii* and *Paecilomyces* sp.2 showed bactericidal activities at 25 and 50 mg mL^{-1} against *P. aeruginosa* and *E. coli*, respectively.

Table 5 Antifungal activities of extra and intra-cellular metabolites (5, 15, 25, 50 and 100 mg mL^{-1}) from *Paecilomyces* isolates against the yeast *Saccharomyces cerevisiae*.

Fungal isolate	Effective extract concentration (mg mL^{-1})			
	MIC		MFC	
	Exteracellular	Intacellular	Exteracellular	Intacellular
Paecilomyces variotii	50	NE	NE	NE
Paecilomyces lilacinus S1	50	NE	NE	NE
Paecilomyces fumoroseus	100	NE	NE	NE
Paecilomyces lilacinus N1	50	NE	NE	NE
Paecilomyces sp.1	50	100	50	NE
Paecilomyces sp.2	25	NE	NE	NE

Data were obtained from three replications. Abbreviations: MIC: The minimum inhibitory concentration, MFC: The minimum fungicidal concentration, NE: Not effective at concentrations up to 100 mg mL^{-1}.

Table 6 Antimitotic/cytotoxic activity of extra-cellular metabolites (5, 15, 25, 50 and 100 mg mL^{-1}) from *Paecilomyces* isolates against the yeast *Saccharomyces cerevisiae* proliferation.

Fungus isolate	Yeast cell numbers ($\times 10^6$)[1]			
	15 mg mL^{-1}	25 mg mL^{-1}	50 mg mL^{-1}	100 mg mL^{-1}
Paecilomyces variotii	12 (61.3%)	7 (77.4%)	8 (74.2%)	5 (83.9%)
Paecilomyces lilacinus S1	16 (48.4%)	11 (64.5%)	9 (71%)	4 (87.1%)
Paecilomyces sp.2	17 (45.2%)	12 (61.3%)	8 8 (74.2%)	7 (77.4%)
Paecilomyces lilacinus N1	17 (45.2%)	12 (61.3%)	8 (74.2%)	6 (80.1%)
Paecilomyces sp.1	21 (32.2%)	15 (51.6%)	11 (64.5%)	6 (80.1%)
Paecilomyces fumoroseus	22 (29%)	20 (35.5%)	13 (58.1%)	9 (71%)
Control	31	31	31	31

[1] The percentage of antimitotic activity in patenthesis.

Table 7 Antibacterial activity of extra-cellular metabolites (5, 15, 25, 50 and 100 mg mL^{-1}) from *Paecilomyces* species against human pathogenic G^+ and G^- bacteria.

Target bacterium	The effective concentration (mg mL^{-1})											
	Peacilomyces variotii		*Peacilomyces lilacinus* S1		*Peacilomyces fumosoroseus*		*Peacilomyces lilacinus* N1		*Peacilomyces* sp.1		*Peacilomyces* sp.2	
	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC	MBC	MIC	MBC
Bacillus subtilis	NE	50	NE	NE	NE	50	NE	15	5	NE	25	100
Staphylococcus aureus	NE	50	NE	NE	NE	100	NE	25	15	NE	15	NE
Streptococcus pyogenes	NE	50	NE	NE	NE	100	NE	50	25	NE	15	NE
Escherichia coli	NE	5	NE	NE	NE	50	100	25	15	NE	25	50
Pseudomonas aeruginosa	25	15	NE	50	NE	50	NE	15	25	NE	15	NE
Salmonella typhi	NE	25	NE	NE	NE	50	NE	50	15	NE	15	NE

Data were obtained from three replications. Abbreviations: MIC: The minimum inhibitory concentration, MBC: The minimum bactericidal concentration, NE: Not effective at concentrations up to 100 mg mL^{-1}.

Discussion

Recently, fungal secondary metabolites have attracted considerable attention from the scientific community (Shwab and Keller, 2008). The fungal genus *Paecilomyces,* which is widespread in nature, has adapted different life styles as saprobic, endophytic, enthomopathogenic, mycoparasitic, nematophagous, as well as opportunistic human pathogen (Aguilar *et al*., 1998; Fiedler and Sosnowska, 2007; Gupta *et al*., 1993; O'Day, 1977; Marti *et al*., 2006; Saberhagen *et al*., 1997; Tigano-Milani, *et al*., 1995; Westenfeld, *et al*., 1996). *Paecilomyces* species are of high interest in agrobioindustry for biological control of insects and plant pathogenic nematodes (Anonymous, 2016). Furthermore, some *Paecilomyces* species are capable of producing cytotoxic metabolites with potential application in cancer therapy (Huang *et al*., 2001; Shim *et al*., 2000; Xu *et al*., 2009). Here, we aimed at evaluating the potential of several soliborne, entomopathogenic and nematophagous *Paecilomyces* species for the production of secondary metabolites with antifungal, antibacterial and antimitotic activities.

Our data indicated that the *Paecilomyces* species were highly bioactive against the filamentous fungus *Pyricularia oryzae* and the yeast *Saccharomyces cerevisiae*, as they produced diffusible metabolites and volatile compounds with strong inhibitory effects against these model target fungi (Tables 1-4).

This indicated the promising potential of *Paecilomyces* secondary metabolites as antifungal agents. Then, intra- and extra-cellular metabolites of the *Paecilomyces* species were extracted and applied against a range of target fungi and bacteria. Data indicated that in contrary to intra-cellular metabolites, the extra-cellular metabolites of all *Paecilomyces* species inhibited the growth of *S. cerevisiae* at concentrations of 50 mg mL^{-1} (Table 5). At this concentration, the extra-cellular metabolite of *Paecilomyces* sp.1 also exerted fungicidal effect. Moreover, based on the growing numbers of yeast cells, all extra-cellular metabolites showed some degrees of the antimitotic/cytotoxic activity (Table 6). Hence, the extra-cellular metabolites of the *Paecilomyces* species were capable of both antifungal and antimitotic activities.

Furthermore, the antibacterial effects of the *Paecilomyces* extra-cellular metabolites were demonstrated against a panel of gram-positive and gram-negative human pathogens. Indeed, at the provided concentrations most extracts inhibited the growth of bacteria, but rarely had bactericidal activity (Table 7).

Taken all together, our findings indicate that the *Paecilomyces* species, either saprobic or pathogenic, have a strong arsenal of bioactive metabolites which show inhibitory or cytotoxicity effects against other microorganisms. Among these, *P. variotii* is a common environmental fungus that is widespread in composts, soils and food products, and has been emerged as an opportunistic human pathogen (Pitt and Hocking, 2009; Steiner *et al.*, 2011). *Paecilomyces lilacinus* colonizes a wide range of habitats including solis, insects, nematodes and is an infrequent cause of human disease (Domsch et al., 2007; Saberhagen *et al.* 1997). *Paecilomyces fumoroseus* is an entomophatogenic fungus, infecting over twenty five different families of insects and many species of mites, and is in use as a biocontrol agent (Zimmermann, 2008). In agreement with this diverse range of lifestyles and bioactivities, our finding further supports and extends the scope of Paecilomyces bioactivity against microorganisms such as pathogenic fungi and bacteria, as well. Such antifungal, antibacterial and antimitotic activities could find application in agroforestry, medicine, and bioindustry. Future research has to investigate the molecular and chemical basis behind these bioactivities.

Acknowledgements

We are thankful to Samad Jamali and Zia Banihashemi (Shiraz University, Shiraz, Iran), as well as Marzieh Asadollahpour (Bu-Ali Sina University, Hamedan, Iran) for their generous gift of *Peacilomyces* fungal species. This work was financially supported by a grant from Bu-Ali Sina University to Kh.P. Jalal Soltani dedicates this work to the memory of Majid Yalfani.

References

Aguilar, C., Pujol, I., Sala, J. and Guarro, J. 1998. Antifungal Susceptibilities of *Paecilomyces* Species. Antimicrobial Agents and Chemotherapy, 42: 1601-1604.

Anonymous, 2016. IBMA Members Directory. 66 pp. Available on: http://www.ibma-global.org/en/members-directory-2016 (Accessed January, 2016)

Assadollahpour, M., Zafari, D. and Zare, R. 2011. Insects Hyphomycetous fungi and their pathogenesis on Colorado potato beetle in Hamedan. Journal of Plant Protection, 24: 465-470. (In Persian).

Choi, Y. J., Hwang, H. K. and Lee, W. H. 1999. The production of artificial fruiting body of *Paecilomyces japonica*. The Korean Journal of Mycology, 27: 87-93.

Domsch, K. H., Gams, W., and Anderson, T. H. 2007. Compendium of Soil Fungi, 2nd Ed. IHW-Verlag, Eching, 672 pp.

Dong, Q. F., Wang, J. L., Zhang, S. F., Wang, Z., Zhang, C. X., Gao, H., Zhang, H. M. and Zhang L. 2008. Antifungal activity of crude extracts and fat-soluble constituents of *Holotrichia diomphalia* larvae. Bioresource Technology, 99: 8521-8523.

Fiedler, Z. and Sosnowska, D. 2007. Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson is also a biological agent for control of greenhouse insects and mite pests. Biocontrol, 52: 547-558.

Furuya, T., Hirotani, M., and Matsuzawa, M. 1983. N6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species. Phytochemistry, 22: 2509-2512.

Gupta, S. C., Leathers, T. D., and Wicklow, D. T. 1993. Hydrolytic enzymes secreted by *Paecilomyces* lilacinuscultured on sclerotia of *Aspergillus flavus*. Applied Microbiology and Biotechnology, 39: 99-103.

Hosseyni Moghaddam, M. S., and Soltani, J. 2013. An Investigation on the effects of photoperiod, aging and culture media on vegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae*. Progress in Biological Sciences, 3: 135-143.

Hosseyni Moghaddam, M. S. and Soltani, J. 2014a. Bioactivity of endophytic *Trichoderma* fungal species from the plant family Cupressaceae. Annals of Microbiology, 64: 753-761.

Hosseyni Moghaddam, M. S. and Soltani, J. 2014b. Psycrophilic endophytic fungi with bioactivity inhabit Cupressaceae plant family. Symbiosis, 63: 79-86.

Hosseyni Moghaddam, M. S., Soltani, J., Babalhavaeji F., Hamzei J., Nazeri S. and Mirzaei S. 2013. Bioactivities of endophytic Penicillia from Cupressaceae. Journal of Crop Protection, 2: 421-433.

Huang, Y. J., Wang, J. F., Li, G. L., Zheng, Z. H. and Su, W. J. 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants, *Taxus mairei*, *Cephalataxus fortunei* and *Torreya grandis.* FEMS Immunology and Medical Microbiology, 31: 163-167.

Keller, N. P., Turner, G. and Bennett, J. W. 2005. Fungal secondary metabolism - from biochemistry to genomics. Nature Reviews Microbiology, 3: 937-47.

Kobayashi, H., Namikoshi, M., Yoshimoto, T. and Yokochi, T. 1996. A screening method for antimitotic and antifungal substances using conidia of *P. oryzae*, modification and application to tropical marine fungi. The Journal of Antibiotics, 49: 873-879.

Luangsa-ard, J., Hywel-Jones, N. L. and Samson, R. A. 2004. The polyphyletic nature of *Paecilomyces* sensu lato based on 18S-generated rDNA phylogeny. Mycologia, 96: 773-780.

Manabe, M., Sugimoto, M., Azuma, Y., Takemoto, N., Yamashita, A., Tsuboi, H., Tsunoo, A., Kinjo, N., Huang, N. L. and Miyamoto, H. 1996. Effects of the mycelial extract of cultured *Cordyceps sinensis* on *in vitro* hepatic energy metabolism in the mouse. The Japanese Journal of Pharmacology, 70: 85-88

Marti, G. A., Lastra, C. C., Pelizza, S. A. and García, J. J. 2006. Isolation of *Paecilomyces lilacinus* (Thom) Samson (Ascomycota: Hypocreales) from the Chagas disease vector, *Triatoma infestans* Klug (Hemiptera: Reduviidae) in an endemic area in Argentina. Mycopathologia, 162: 369-72.

O'Day, D. M. 1977. Fungal endophthalmitis caused by *Paecilomyces lilacinus* after intraocular lens implantation. American Journal of Ophthalmology, 83: 130-1.

Pakvaz, S. and Soltani, J. 2016. Endohyphal bacteria from fungal endophytes of the Mediterranean cypress (*Cupressus sempervirens*) exhibit *in vitro* bioactivity. Forest Pathology, doi: 10.1111/efp.12274.

Pitt, J. L. and Hocking, A. D. 2009. Fungi and Food Spoilage, 3rd ed. Springer-Verlag US.

Saberhagen, C., Klotz, S. A., Bartholomew, W., Drews, D. and Dixon, A. 1997. Infection due to *Paecilomyces lilacinus*: a challenging clinical identification. Clinical Infectious Diseases, 25: 1411-3.

Shim, J. S., Min, E. G., Chang, H. R., Lee, C. Y., Kim, S. S. and Han, Y. H. 2000. Cytoctxicity against human cancer cell lines by *Paecilomyces tenuipes* DGUM 32001. The Korean Journal of Microbiology, 36: 312-315.

Shwab, K. and Keller, N. P. 2008. Regulation of secondary metabolism in filamentous ascomycetes. Mycological Research, 112: 225-230.

Soltani, J. and Hosseyni Moghaddam, M. S. 2014a. Antiproliferative, antifungal and antibacterial activities of endophytic *Alternaria* species from Cupressaceae. Current Microbiology, 69: 349-356.

Soltani, J. and Hosseyni Moghaddam, M. S. 2014b. Diverse and bioactive endophytic Aspergilli inhabit Cupressaceae plant family. Archives of Microbiology, 196: 635-644.

Soltani J. and Hosseyni Moghaddam, M. S. 2015. Fungal endophyte diversity and bioactivity in the Mediterranean cypress *Cupressus sempervirens*. Current Microbiology, 70: 580-586.

Soltani, J., Zaheri-Shoja, M., Hamzei, J., Hosseyni-Moghaddam, M. S. and Pakvaz, S. 2016. Diversity and bioactivity of endophytic bacterial community of Cupressaceae. Forest Pathology, doi: 10.1111/efp.12270.

Steiner, B., Valerio, R. A., Alessandra, A. P., Lucia, M., Alexandre, Z. and Luciano, Z. G. 2011. Apophysomyces variabilis as an emergent pathogenic agent of pneumonia. Hindawi Diseases, 17: 134-135.

Tigano-Milani, M. S., Carneiro, R. G., de Faria, M. R., Frazão, H. S. and McCoy, C. W. 1995. Isozyme characterization and pathogenicity of *Paecilomyces fumosoroseus* and *P. lilacinus* to *Diabrotica speciosa* (Coleoptera: Chrysomelidae) and Meloidogyne javanica (Nematoda: Tylenchidae). Biological Control, 5: 378-382.

Wang, J., Huang, Y., Fang, M., Zhang, Y., Zheng, Z., Zhao, Y. and Su, W. 2002. Brefeldin *A. A* cytotoxin produced by *Paecilomyces* sp. and *Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*. FEMS Immunology and Medical Microbiology, 34: 51-57.

Westenfeld, F., Alston, W. K. and Winn, W. C. 1996. Complicated soft tissue infection with prepatellar bursitis caused by *Paecilomyces lilacinus* in an immunocompetent host: case report and review. Journal of Clinical Microbiology, 34: 1559-62.

Xu, L. L., Han, T., Wu, J. Z., Zhang, Q. Y., Zhang H., Huang, B. K., Rahman, K. and Qin, L. P. 2009. Comparative research of chemical constituents, anti-fungal and antitumor properties of ether extracts of *Panax ginseng* and its endophytic fungus. Phytomedicine, 16: 609-616.

Zimmermann, G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Science and Technology, 18: 865-901.

فعالیت زیستی گونه‌های خاکزی و بیماریزای پسیلومایسس علیه قارچ‌ها و باکتری‌های بیماریزا

سیما محمدی[1]، جلال سلطانی[2] و خسرو پیری[1*]

۱- گروه بیوتکنولوژی گیاهی، دانشکده کشاورزی، دانشگاه بوعلی سینا، همدان، ایران.
۲- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه بوعلی سینا، همدان، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: Khpiri@gmail.com
دریافت: ۳ مرداد ۱۳۹۴؛ پذیرش: ۷ بهمن ۱۳۹۴

چکیده: جنس قارچی Paecilomyces مشتمل بر گونه‌های بیماریزا و ساپروفیتی می‌باشد که به‌طور معمول از حشرات، نماتدها، خاک، هوا، غذا، کاغذ و بسیاری از مواد دیگر جدا می‌شود. مشخص شده است که برخی گونه‌های Paecilomyces می‌توانند در کشاورزی ارگانیک، و نیز در درمان بیماری‌های انسان کاربرد پیدا کنند. در پژوهش حاضر، فعالیت زیستی شماری از گونه‌های خاکزی و بیماریزای Paecilomyces علیه برخی میکروارگانیسم‌های پروکاریوت و یوکاریوت بررسی گردیده و نشان داده شده است. نخست، اثرات ضدقارچی متابولیت‌ها و مواد فرّار گونه‌های Paecilomyces علیه دو قارچ *Pyricularia oryzae* و *Saccharomyces cerevisiae* در شرایط درون پلیت نشان داده شد. سپس، متابولیت‌های گونه‌های Paecilomyces استخراج گردیده و اثرات بازدارندگی از رشد و آنتی‌میتوز آنها علیه *S. cerevisiae* اثبات گردید. پژوهش‌های تکمیلی نشان از اثرات ضدباکتریایی عصاره‌های برون‌سلولی گونه‌های Paecilomyces علیه باکتری‌های گرم مثبت و گرم منفی بیماریزای انسان، از جمله *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* و *Salmonella typhi* داشت. این یافته‌ها نشان می‌دهد که گونه‌های Paecilomyces، چه ساپروفیت و چه بیماریزا، تولیدکننده‌ی متابولیت‌هایی هستند که ازنظر زیستی فعال بوده و دارای اثرات بازدارندگی یا کشندگی علیه دیگر میکروارگانیسم‌ها می‌باشند. این نتایج قابلیت کاربرد در کشاورزی و علوم دارویی را دارند.

واژگان کلیدی: *Paecilomyces fumoroseus*، *Paecilomyces lilacinus*، *Paecilomyces variotii*، متابولیت ثانویه، مواد فرّار، ضدقارچی، ضدباکتریایی، آنتی‌میتوز