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Bioprospecting endophytic *Diaporthe* species associated with *Pachystachys lutea* (Acanthaceae) with antagonistic effect against *Sclerotinia sclerotiorum*

Ana Paula Ferreira
Universidade Estadual de Maringá- UEM
Contato: apaulaf98@gmail.com

Natieli Jenifer Mateus
Universidade Estadual de Maringá- UEM
Contato: natijenifern2@gmail.com

João Arthur dos Santos Oliveira
Universidade Estadual de Maringá- UEM
Contato: joaoarthur_oliveira@hotmail.com

João Alencar Pamphile (*In memorian*)
Universidade Estadual de Maringá- UEM
Contato: prof.pamphile2@gmail.com

João Lúcio Azevedo
Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo- ESALQ-USP
Contato: jlazevedo@usp.br

Abstract: Endophytic microorganisms live inside the plant tissues symptomless. These microorganisms may present antagonistic activity against phytopathogens or produce metabolites with antifungal activity. We aimed to evaluate *in vitro* antagonism and competitive interactions between endophytic fungi of *Diaporthe* species against *Sclerotinia sclerotiorum.* We also carried out *in vitro* screening of antifungal activity of metabolic extracts of the two endophytes against the pathogen. The antagonism was performed by the paired-culture and two promising antagonists strains were selected for metabolic extraction from the fermented culture. Metabolic extracts were obtainment using two different organic solvents (Ethyl Acetate and Hexane) and their antifungal activity was carried out using the agar diffusion test. The *in vitro* antagonistic index ranged from 22.1 to 59.5%, with *Diaporthe* sp. PL03 (59.1%), *D. schini* PL40 (59.5%), *D. infecunda* PL63 (41.8%), *D. anacardii* PL64 (56.8%), with inhibition by mycelial contact. The endophytes PL01 (28.6%) and PL43 (28.5%), both *D. anacardii*, stood out blocking mycelial growth from a distance. In the antifungal assay, *D. anacardii* PL01 (31.7%) and *D. schini* PL40 (18.2%) acetate metabolite stood out. In summary, our results indicate a few *Diaporthe* endophytes able to antagonize a *S. sclerotiorum* pathogen under *in vitro* conditions.
Key words: Antagonism; Endophytic fungi; Metabolic extraction; Antifungal activity.

Bioprospecção de espécies de *Diaporthe* endofíticas associada à *Pachystachys lutea* (Acanthaceae) com efeito antagônico contra *Sclerotinia sclerotiorum*

Resumo: Microrganismos endofíticos vivem no interior dos tecidos das plantas sem causar prejuízos. Esses microrganismos podem apresentar atividade antagônica contra fitopatógenos ou produzir metabólitos com atividade antifúngica. Nós avaliamos o antagonismo *in vitro* e as interações competitivas entre espécies de fungos endofíticos de espécies do gênero *Diaporthe* contra *Sclerotinia sclerotiorum*. Também realizamos a triagem *in vitro* da atividade antifúngica de extratos metabólicos de dois endófitos contra o patógeno. O antagonismo foi realizado por meio da cultura pareada e duas linhagens promissoras antagonistas foram selecionadas para extração dos metabólitos da cultura fermentada. Os extratos metabólicos foram obtidos utilizando dois solventes orgânicos (acetato de etila e hexano) e sua atividade antifúngica foi avaliada pelo teste de difusão em ágar. O índice antagônico *in vitro* variou de

22,1 a 59,5%, com *Diaporthe* sp. PL03 (59,1%), *D. schini* PL40 (59,5%), *D. infecunda* PL63 (41,8%), *D. anacardii* PL64 (56,8%), com inibição por contato micelial. Os endófitos PL01 (28,6%) e PL43 (28,5%), ambos *D. anacardii*, se destacaram bloqueando o crescimento micelial à distância. No ensaio antifúngico, destacaram-se o metabólito acetato de *D. anacardii* PL01 (31,7%) e *D. schini* PL40 (18,2%). Em resumo, nossos resultados indicam alguns *Diaporthe* endofíticos capazes de antagonizar o patógeno *S. sclerotiorum* em condições *in vitro*.
Palavras-chave: Antagonismo; Fungos endofíticos; Extração metabólica; Atividade antifúngica.

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INTRODUCTION

Plants are hosts of populations of non-pathogenic microbial communities, for example, endophytic microorganisms that reside and may grow up within the plant tissues. Nevertheless, only a small part of these plants had their endophytic microbiota studied (ZHANG et al., 2014). Endophytic microorganisms are fungi and bacteria that live inside the plants without causing any damage to their hosts. These endophytes can come into the plant through natural openings and colonizing the inter- or intracellular spaces (BERNARDI-WENZEL et al., 2010; KUSARI et al., 2012; KUSARI et al., 2014).

Endophytes could be exploited for their ability to antagonize pathogens or produce secondary metabolites with antifungal activity (PARK et al., 2015). Studies have been evaluated the activity of endophytic fungi against phytopathogens (PARK et al., 2015; BONGIORNO et al., 2016; PRETO et al., 2017). Ribeiro et al. (2018) isolated several endophytic foliar fungi from the ornamental plant *Pachystachys lutea* (Acanthaceae). Some of these endophytes were identified as *Diaporthe* species with *in vitro* antagonistic activity against *Colletotrichum* sp. and *Fusarium oxysporum* pathogens.

Sclerotinia sclerotiorum fungus (white mold disease) is a pathogen of agricultural systems and causes a great deal of damage and economic losses (ABÁN et al., 2018). To control these pathogens in the agricultural system, chemical pesticides are commonly used, however, the continued use of these substances can cause harmful environmental effects in addition to acting as mutagenic agents (YING, 2018). As an alternative to these agrochemicals, biological control employing microorganisms, especially the endophytes, that are capable of antagonizing or producing antimicrobial compounds against these pathogens are excellent tools to guarantee agricultural production, mitigating the adverse effects of pesticides, being considered more sustainable (TERHONEN et al., 2018; TALAAT, 2019).

Considering that *Diaporthe* species have different biotechnological applications (GOMES et al., 2013; POLONIO et al., 2015; MEDEIROS et al., 2018; RIBEIRO et al., 2018; TANAPICHATSAKUL et al., 2018; WONG et al., 2018), in this work, we aimed to evaluate *in vitro* antagonism and competitive interactions between endophytes *Diaporthe* species and *Sclerotinia sclerotiorum*. We also carried out *in vitro* screening of antifungal activity of metabolic extracts of the two best endophytes against the pathogen.

MATERIAL AND METHODS

Endophytic fungi and pathogen

Fifteen *Diaporthe* sp., isolated as endophytes from healthy leaves of ornamental plant *Pachystachys lutea* (RIBEIRO et al., 2018) was retrieved from the Collection of Endophytic and Environmental Microorganism (CMEA) from the Laboratory of Microbial Biotechnology, State University of Maringá – Paraná, Brazil. The pathogen *S. sclerotiorum* also belongs to the CMEA and was isolated from soy symptomatic plants in Cambira, Paraná/Brazil. These fungi were cultured on PDA (Potato Dextrose Agar; pH 6.6) for 7

days at 28 ºC.

In vitro antagonism and competitive interactions

The dual culture method of Campanile et al. (2007) modified by Polonio et al. (2015) was used. The percentage of inhibition rate of mycelial growth was calculated following Polonio et al. (2015). Competitive interactions between endophytes and phytopathogen were determined according to the rating scale proposed by Badalyan et al. (2002), which considers three main types of interactions (A, B and C) and their subtypes. A = blocking mycelial growth with contact, B = blocking distance, C = endophytic growth on the initial pathogen without blocking; CA1 and CA2 = partial and complete endophyte growth on the pathogen after initial blocking with mycelial contact, and CB1 and CB2 = partial and complete endophyte growth on the pathogen after initial distance blocking. Five replicates were made as well the control with a phytopathogen plug inoculated in only one point of PDA dishes (Control 1) and a phytopathogen plug against the commercial fungicide (50 $mg.ml^{-1}$) (Control 2).

Metabolic extraction from the fermented culture

Two endophytic isolates were chosen based on statistical analysis and their competitive interactions against the pathogen and tested for their antifungal capacity. The endophyte PL01 (*Diaporthe anacardii*) was selected since blocking the phytopathogen by distance and PL40 (*Diaporthe schini*) showed inhibition by mycelial contact.

Three mycelia plugs (6-mm diameter) of seven-day-old cultures of each endophyte were inoculated into 500-ml Erlenmeyer flasks containing 500 mL of PDB (Potato Dextrose Broth; pH 6.8) and incubated at 28 ºC for 21 days under stationary condition. The metabolic extraction from the fermented culture broth, by using ethyl acetate and hexane as solvent, followed the protocol described by Polonio et al. (2015).

In vitro antifungal assay

To evaluate the bioactivity of crude extracts against *S. sclerotiorum*, five replicates of PDA dishes received 5-mm autoclaved filter paper plugs inoculated with 10 µL of metabolites (10 $mg.ml^{-1}$ in methanol) in an opposite position of phytopathogen plugs (6-mm diameter), and remained incubated at 28 ºC for seven days. For controls, the paper plugs received commercial fungicide (50 $mg.ml^{-1}$) (T1) or methanol (T2); a phytopathogen plug inoculated in only one point of PDA dishes (T3). The culture medium extracted with acetate and hexane also were made as controls. The percentage inhibition rate of mycelial growth was calculated according to Polonio et al. (2015).

Statistical analyses

Data from all experiments were compared by the Scott-Knott test ($p < 0.05$), using the statistical program SISVAR 5.6 (FERREIRA, 2011).

RESULTS AND DISCUSSION

Paired-culture assay

Table 1 shows the results obtained from dual culture assay between endophytes and phytopathogen. According to the Badalyan et al. (2002) scale, 86.67% of the interactions occurred by blocking mycelial growth of the phytopathogen with mycelial contact (Type A), and 13.33% blocking mycelial growth from a distance (Type B) (Table 1, Figure 1).

The percentages of inhibition ranged from 22.1% to 59.5%. The statistical analysis of antagonistic activities measured (Scott Knott with $p < 0.05$) yielded the strains into four groups, with the best performance observed for endophytes PL03 (*Diaporthe* sp.), PL64 (*D. anacardii*), and PL40 (*D. schini*) when compared statistically with the control (phytopathogen against the commercial fungicide) and second control (phytopathogen plug inoculated in only one point of PDA dishes), showing inhibition rates higher than the fungicide control (50.2%).

Table 1. Paired-culture assay between 15 endophytic fungi *Diaporthe* sp. against *Sclerotinia sclerotiorum.*

Endophytic strain	Antagonism index (%)*	Competitive interaction**
Control 1	0.0_{d}	---
Control 2	50.2_{b}	---
PL01 (*Diaporthe anacardii*)	28.6_{c}	B
PL03 (*Diaporthe* sp.)	59.1_{a}	A
PL09 (*Diaporthe* sp.)	44.4_{b}	A
PL18 (*Diaporthe* sp.)	31.7_{c}	A
PL39 (*Diaporthe infecunda*)	42.1_{b}	A
PL40 (*Diaporthe schini*)	59.5_{a}	A
PL43 (*Diaporthe anacardii*)	28.5_{c}	B
PL53 (*Diaporthe schini*)	49.7_{b}	A
PL61 (*Diaporthe* sp.)	31.9_{c}	A
PL63 (*Diaporthe infecunda*)	41.8_{b}	A
PL64 (*Diaporthe anacardii*)	56.8_{a}	A
PL66 (*Diaporthe infecunda*)	47.9_{b}	A
PL67 (*Diaporthe* sp.)	22.1_{c}	A
PL71 (*Diaporthe* sp.)	24.6_{c}	A
PL74 (*Diaporthe infecunda*)	44.6_{b}	A

*Means of five replicates. Means followed by the same letter in column do not differ by the Scott Knott test ($p < 0.05$). **Rating scale based on Badalyan et al. (2002). *The absence of competive interaction was indicated by (---).*

Bongiorno et al. (2016) also carried out a paired-culture technique to evaluate the antagonistic activity of endophytic fungi from an organic coffee tree against *S. sclerotiorum*. The authors reported that there were competitive interactions with pathogen-endophytes predominantly type A, with the inhabitation rates ranging from zero to 35%. Supporting our results, other studies have also been described the higher incidence of the interaction type A between endophytic fungi and phytopathogens (CAMPANILE et al., 2007; FELBER et al., 2016; OLIVEIRA et al., 2018).

Diaporthe fungi usually show expressive antagonistic rates. Perhaps it would be happening by the competition for space or nutrients during the paired culture. This competition mechanism might be used as an alternative pathogen biocontrol strategy (GHORBANPOUR et al., 2017). On the other hand, *in vitro* antagonistic interactions between fungal strains may be correlated with the production of secondary metabolites that have a toxic action on other microbial strains (BADALYAN et al., 2002; OLIVEIRA et al., 2018) and are what reinforce the need of subsequent tests with endophytic metabolic extracts produced by promising antagonists selected in dual culture experiments.

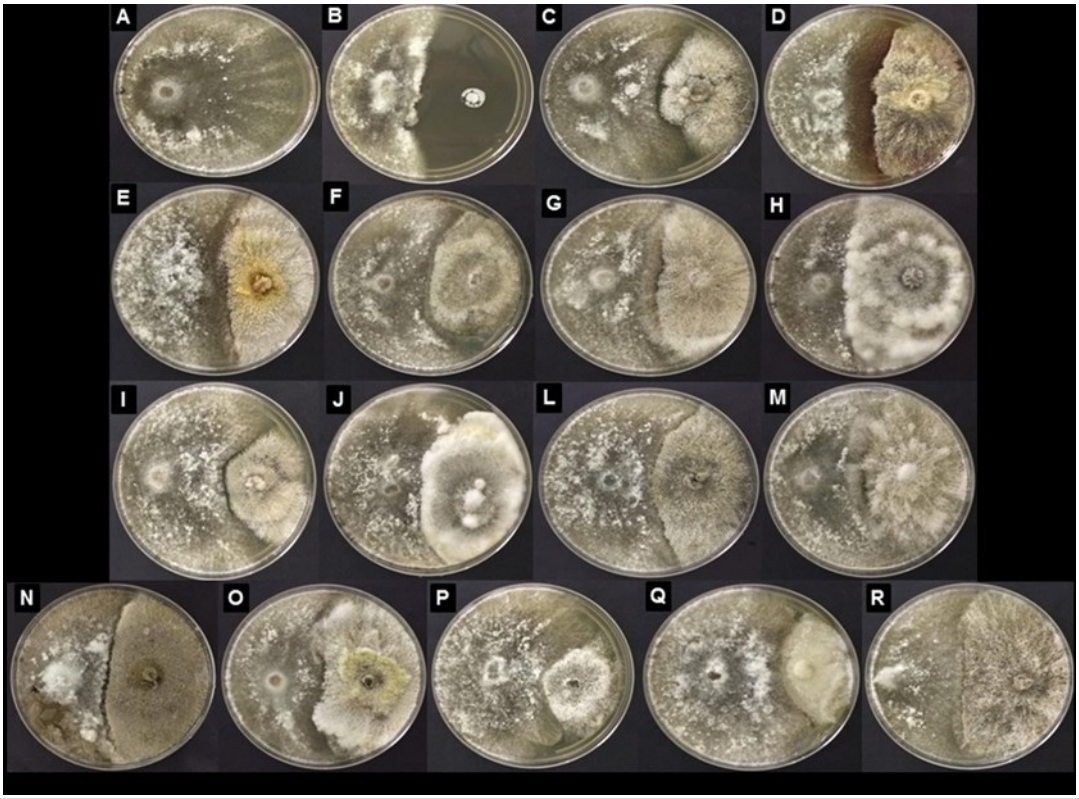

Figure 1. *In vitro* dual culture assay endophytes *Diaporthe* species (right) against *Sclerotinia sclerotiorum* (left). **A.** Control only the phytopathogen *S. sclerotiorum*. **B.** Phytopathogen control (left) against commercial fungicide (right). **C.** *D. anacardii* PL01, **D.** *Diaporthe* sp. PL03, **E.** *Diaporthe* sp. PL09, **F.** *Diaporthe* sp. PL18, **G**. *D. infecunda* PL39, **H.** *D. schini* PL40, **I.** *D. anacardii* PL43, **J.** *D. schini* PL53, **L.** *Diaporthe s*p. PL61, **M.** *D. infecunda* PL63, **N.** *D. anacardii* PL64, **O.** *D. infecunda* PL66, **P.** *Diaporthe* sp. PL67, **Q.** *Diaporthe* sp. PL71, **R**. *D. infecunda* PL74.

In vitro antifungal evaluation

Thorough investigations about biological active secondary metabolites produced by endophytic microorganisms supports the development of sustainable agriculture through of application of these microorganisms as an alternative method for biological control, finding out new bioactive compounds with biotechnological importance (RATNAWEERA; DILIP, 2017).

Table 2 shows the statistical groups (Scott Knott with p<0, 05) based on the inhibition rate of crude extracts obtained with acetate and hexane solvents for each strain PL01 (*D. anacardii*) and PL40 (*D. schini*) in agar diffusion test against *S. sclerotiorum*. As we can see, PL01 (*D. anacardii)* acetate crude extract was the most efficient in the control of the mycelial growth of the pathogen with an inhibition index of 31.7% (Table 2, Figure 2).

Bioactive compounds produced by endophytes have relevant ecological, biochemical, and molecular features, that enable numerous possibilities for exploring these microorganisms as sources of an infinity of known and new biologically active secondary metabolites (KUSARI et al., 2012). In the agriculture field, the biological control of phytopathogens by these eco-friendly substances are of extreme value for reducing or eliminating the adverse effects of chemical pesticides, with the advantage that pathogenic fungi are less resistant to them (TERHONEN et al., 2018; OLIVEIRA et al., 2018; TALAAT, 2019).

Kumar and Kaushik (2013) evaluated extracts in acetate and hexane against *S. sclerotiorum* produced by endophytic fungi isolated from oil-seed crop *Jatropha curcas*. These authors, as the current work, also described positive results in the antifungal assay against the pathogen, especially for acetate extracts.

They also carried out experiments with hexane extracts, however, it was less effective. Chowdhary and Kaushik (2015) have described the successfully antagonistic action of secondary metabolites of an endophyte *Diaporthe* against *S. sclerotiorum* corroborating the idea that this genus of fungi is promising source of antifungal secondary metabolites.

In 2007, Gossen and Rimmer (2007) reported the isolation of strains of *S. sclerotiorum* resistant to commercial fungicide, demonstrating the importance of the development of studies that aimed at the identification and exploration of new compounds, mainly from natural origin. It is believed that *Diaporthe* species, especially the endophytes, are a promising source of metabolites with antifungal activity, which could be used eco-friendly, in the control of diseases and pests (SINGH et al., 2011; SPECIAN et al., 2012).

Table 2. *In vitro* antifungal evaluation by agar diffusion test of crude extracts of secondary metabolites in acetate and hexane produced by *Diaporthe anacardii* and *Diaporthe schini* against *Sclerotinia sclerotiorum.*

Tratments	Growth micelial (cm²)	Inhibition index (%)**
Commercial fungicide (T1)	28.44	50.2_a
Methanol control (T2)	58.19	2.0_d
Only pathogen control (T3)	57.11	0.0_d
Culture medium control – Acetate*	55.89	2.1_d
Culture medium control – Hexane*	58.89	0.0_d
Diaporthe anacardii PL01- Acetate	38.99	31.7_b
Diaporthe anacardii PL01 – Hexane	55.80	2.3_d
Diaporthe schini PL40- Acetate	46.73	18.2_c
Diaporthe schini PL40- Hexane	53.42	6.5_d

*Extraction carried out only on the culture medium PD; **Means of five replicates. Means followed by the same letter in column do not differ by the Scott Knott test ($p < 0.05$).

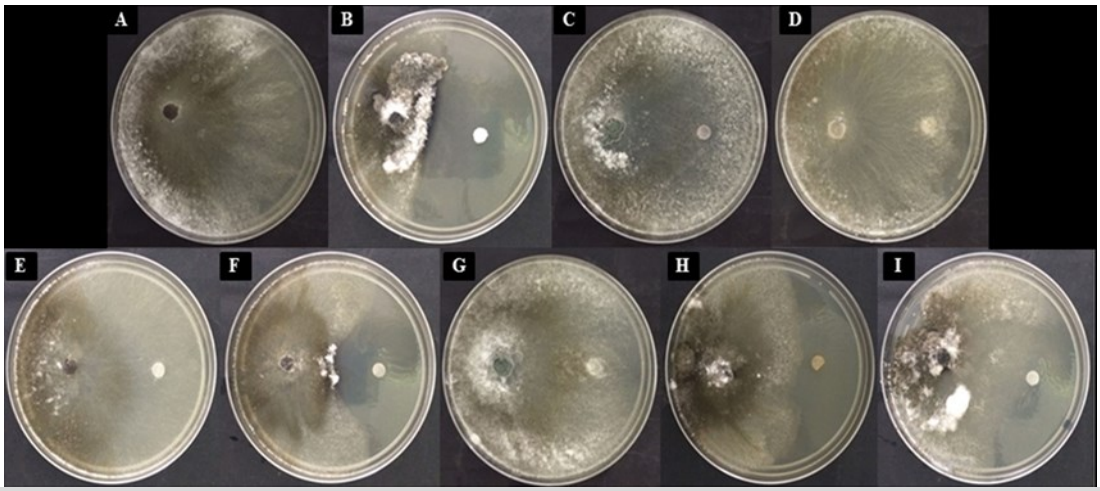

Figure 2. *In vitro* antifungal activity of crude extracts of secondary metabolites of *Diaporthe* species (right) against *Sclerotinia sclerotiorum* (left). **A.** Control only the phytopathogen *S. sclerotiorum*. **B.** Phytopathogen control (left) against commercial fungicide (right). **C.** Control of phytopathogen (left) against methanol (right). **D.** Culture medium control – Acetate. **E.** Culture medium control – Hexane. **F.** Crude extract in acetate *D. anacardii* PL01. **G.** Crude extract in hexane *D. anacardii* PL01. **H.** Crude extract in acetate *D. schini* PL40. **I.** Crude extract in hexane *D. schini* PL40.

CONCLUSIONS

There are previous studies conducted with endophytic communities to demonstrate its ability as alternative methods control for the agriculture field. Our results indicate a few *Diaporthe* species endophytic able to antagonize a *S. sclerotiorum* pathogen, especially the strains PL 03 (*Diaporthe* sp.), PL40 (*D. schini*), and PL64 (*D. anacardii*). In addition, in agar diffusion test with crude extract of secondary metabolites, we come across the same interaction between acetate extract PL01 (*D. anacardii*) against the pathogen previously observed in the paired-cultured assay, which was blocking mycelial growth of the *S. sclerotiorum* by distance, thus, a future chemical characterization of the compounds is necessary. In summary, we believe that our findings are significant because pesticides are damaging, and it is important to develop alternative control measures for phytopathogens.

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REFERENCES

ABÁN, C. L.; TABOADA, G.; SPEDALETTI, Y.; APARICIO, M.; CURTI, R. N.; CASALDERREY, N. B.; MAGGIO, M. E.; CHOCOBAR, M. O.; SALGADO, M.; GALVÁN, M. Z. Molecular, morphological and pathogenic diversity of *Sclerotinia sclerotiorum* isolates from common bean (*Phaseolus vulgaris*) fields in Argentina. **Plant Pathology**, v. 67, p. 1740-1748, 2018. DOI: 10.1111/ppa.12880

BADALYAN, S. M.; INNOCENTI, G.; GARIBYAN, N. G. Antagonistic activity of xylotrophic mushrooms against pathogenic fungi of cereals in dual culture. **Phytopathologia Mediterranea**, v. 41, p. 200-225, 2002. DOI: 10.14601/Phytopathol_Mediterr-1668

BERNARDI-WENZEL, J.; GARCIA, A.; FILHO, C. J.; PRIOLI, A. J.; PAMPHILE, J. A. Evaluation of foliar fungal endophyte diversity and colonization of medicinal plant *Luehea divaricata* (Martius et Zuccarini). **Biological Research**, v. 43, n. 4, p. 375-384, 2010. DOI: 10.4067/S0716-97602010000400001

BONGIORNO, V. A.; RHODEN, S. A.; GARCIA, A.; POLONIO, J. C.; AZEVEDO, J.L.; PEREIRA, J. O.; PAMPHILE, J. A. Genetic diversity of endophytic fungi from *Coffea arabica* cv. Iapar-59 in organic crops. **Annals of Microbiology**, v. 66, p. 855-865, 2016. DOI: 10.1007/s13213-015-1168-0

CAMPANILE, G.; RUSCELLI, A.; LUISI, N. Antagonistic activity of endophytic fungi towards *Diplodia corticola* assessed by *in vitro* and in plant tests. **European Journal of Plant Pathology**, v. 117, n. 3, p. 237-246, 2007. DOI: 10.1007/s10658-006-9089-1

CHOWDHARY, K.; KAUSHIK, N. Fungal endophyte diversity and bioactivity in the indian medicinal plant *Ocimum sanctum* Linn. **Plos One**, v. 10, n. 11, p. 01-25, 2015. DOI: 10.1371/journal.pone.0141444

FELBER, A. C.; ORLANDELLI, R. C.; RHODEN, S. A.; GARCIA, A.; COSTA, A. T.; AZEVEDO, J. L.; PAMPHILE, J. A. Bioprospecting foliar endophytic fungi of *Vitis labrusca* Linnaeus, Bordô and Concord cv. **Annals of Microbiology**, v. 66, p. 765–775, 2016. DOI: 10.1007/s13213-015-1162-6

FERREIRA, D. F. Sisvar: a computer analysis system. **Ciência e Agrotecnologia,** v. 35, p. 1039-1042, 2011. DOI: 10.1590/S1413-70542011000600001

GHORBANPOUR, M.; OMIDVANI, M.; DAHAJI-ABBASZADEH, P.; OMIDAVAR, R.; KARIMAN, K. Mechanisms underlying the protective effects of beneficial fungi against plant diseases. **Biological Control**, v. 17, p. 147-157, 2017. DOI: 10.1016/j.biocontrol.2017.11.006

GOMES, R. R.; GLIENKE, C.; VIDEIRA, S. I. R.; LOMBARD, L.; GROENEWALD, J. Z.; CROUS, P. W. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. **Persoonia-Molecular Phylogeny and Evolution of Fungi**, v. 31, p. 01-41, 2013. DOI: 10.3767/003158513X666844

GOSSEN, B. D.; RIMMER, S. R. First report of resistance to Benomyl fungicide in *Sclerotinia sclerotiorum*. **Plant Disease**, v. 85, n. 11, 2007. DOI: 10.1094/PDIS.2001.85.11.1206C

KUMAR, S.; KAUSHIK, N. Endophytic fungi isolated from oil-seed crop *Jatropha curcas* produces oil and exhibit antifungal activity. **PLos ONE**, v. 8, n. 2, e56202, 2013. DOI: 10.1371/journal.pone.0056202.

KUSARI, S.; HERTWECK, C.; SPITELLER, M. Chemical ecology of endophytic fungi: origins of secondary metabolites. **Chemistry & Biology**, v. 19, n. 7, p. 792–798, 2012. DOI: 10.1016/j.chembiol.2012.06.004

KUSARI, S.; SINGH, S.; JAYABASKARAN, C. Biotechnological potential of plant-associated endophytic fungi: hope versus hype. **Trends in Biotechnology**, v. 32, n. 6, p. 297–303, 2014. DOI: 10.1016/j.tibtech.2014.03.009

MEDEIROS, A. G.; SAVI, D. C.; MITRA, P.; SHAABAN, K. A.; JHA, A. K.; THORSON, J. S.; ROHR, J.; GLIENKE, C. Bioprospecting of *Diaporthe terebinthifolii* LGMF907 for antimicrobial compounds. **Folia Microbiologica**, v. 63, n. 4, p. 499-505, 2018. DOI: 10.1007/s12223-018-0587-2

OLIVEIRA, C. F.; MOURA, P. F.; RECH, K. S.; OLIVEIRA, C. S. P.; HIROTA, B. C. K.; OLIVEIRA, M.; SILVA, C. B.; SOUZA, A. M.; DIAS, J. F. G.; MIGUEL, O. G.; AUER, C. G.; MIGUEL, M. D. Antagonistic activity of *Diplodia pinea* against phytopathogenic fungi. **Folia Microbiologica**, v. 64, n. 3, p. 415-419, 2018. DOI: 10.1007/s12223-018-00667-y

PARK, M. S.; EOM, J. E.; FONG, J. J.; LIM, Y. W. New record and enzyme activity of four species in *Penicillium* section *Citrina* from marine environments in Korea. **Journal of Microbiology**, v. 53, n. 4, p. 219-225, 2015. DOI: 10.1007/s12275-015-4700-9

POLONIO, J. C.; ALMEIDA, T. T.; GARCIA, A.; MARIUCCI, G. E. G.; AZEVEDO, J. L.; RHODEN, A. S.; PAMPHILE, J. A. Biotechnological prospecting of foliar endophytic fungi of guaco (*Mikania glomerata* Spreng.) with antibacterial and antagonistic activity against phytopathogens. **Genetics and Molecular Research**, v. 14, n. 3, p. 7297-7309, 2015. DOI: 10.4238/2015.July.3.5

PRETO, G.; MARTINS, F.; PEREIRA, J. A.; BAPTISTA, P. Fungal community in olive fruits of cultivars with different susceptibilities to anthracnose and section of isolates to be used as biocontrol agents. **Biological Control**, v. 110, p. 01-09, 2017. DOI: 10.1016/j.biocontrol.2017.03.011

RATNAWEERA, P. B.; DILIP, E. S. Endophytic fungi: a remarkable source of biologically active secondary metabolites. In: MAHESHWARI, D. K.; ANNAPURNA, K. **Endophytes: Crop Productivity and Protection**, v. 02, p. 191- 212, 2017. DOI: 10.1007/978-3-319-66544-3_9

RIBEIRO, A.S.; POLONIO, J. C.; COSTA, A. T.; DOS SANTOS, C. M.; RHODEN, S. A.; AZEVEDO, J. L.; PAMPHILE, J. A. Bioprospection of culturable endophytic fungi associated with the ornamental plant *Pachystachys lutea*. **Current Microbiology**, v. 75, n. 5, p. 588-596, 2018. 10.1007/s00284-017-1421-9.

SINGH, S. K.; STROBEL, G. A.; KNIGHTON, B.; GEARY, B.; SEARS, J.; EZRA, D. An endophytic *Phomopsis* sp. possessing bioactivity and fuel potential with its volatile organic compounds. **Microbial Ecology**, v. 61, p. 729-739, 2011. DOI: 10.1007/s00248-011-9818-7

SPECIAN, V.; SARRAGIOTTO, M. H.; PAMPHILE, J. A.; CLEMENTE, E. Chemical characterization of bioactive compounds from the endophytic fungus Diaporthe helianthi isolated from Luehea divaricate. **Brazilian Journal of Microbiology**, v, 43, n. 3, 2012. DOI: 10.1590/S1517-83822012000300045

TALAAT, N. B. Effective microorganisms: An innovative tool for inducing common bean (*Phaseolus vulgaris* L.) salt-tolerance by regukating photosynthetic rate and endogenous phytohormones production. **Scientia Horticulturae**, v. 250, p. 254-265, 2019. DOI: 10.1016/j.scienta.2019.02.052

TANAPICHATSAKUL, C.; MONGGOOT, S.; GENTEKAKI, E.; PRIPDEEVECH, P. Antibacterial and antioxidant metabolites of *Diaporthe* spp. isolated from flowers of *Melodorum fruticosum*. **Current Microbiology**, v. 75, n. 4, p. 476-483, 2018. DOI: 10.1007/s00284-017-1405-9

TERHONEN, E.; KOVALCHUK, A.; ZARSAV, A.; ASIEGBU, F. O. Biocontrol potential of forest tree endophytes. In: PIRTTILÄ; A. M.; FRANK, A. C. (Eds.). **Endophytes of Forest Trees Biology and Applications**, v. 86, 2.Ed., p. 283-318, 2018. DOI: 1007/978-3-319-89833-9_13

WONG, C.; TAN, L. T.; MUJAHID, A.; LI-HAN, S.; WEE, J. L. S.; TING, L. F.; MÜLLER, M. Biosorption of copper by endophytic fungi isolated from *Nepenthes ampullaria*. **Letters in Applied Microbiology**, v. 67, n. 4, p. 384-391, 2018. DOI: 10.1111/lam.13049

YING, G-G. Ecological risk assessment of pesticides used in agriculture. In: MAESTRONI, B.; CANNAVAN, A. (Eds.) **Integrated Analytical Approaches for Pesticide Management**, Academic Press, p. 67-79, 2018. DOI: 10.1016/B978-0-12-816155-5.00005-1

ZHANG, Q.; JING, Z.; LONG, Y.; LEI, Z.; DAOHONG, J.; WEIDONG, C.; GUOQING, L. Diversity and biocontrol potential of endophytic fungi in *Brassica napus*. **Biological Control**, v. 72, p. 98–108, 2014. DOI: 10.1016/j.biocontrol.2014.02.018

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