

Avanços Científicos e Tecnológicos nas Ciências Agrárias 5

Júlio César Ribeiro
(Organizador)



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Ano 2020

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APRESENTAÇÃO

A obra “Avanços Científicos e Tecnológicos nas Ciências Agrárias” é composta pelos volumes 3, 4, 5 e 6, nos quais são abordados assuntos extremamente relevantes para as Ciências Agrárias.

Cada volume apresenta capítulos que foram organizados e ordenados de acordo com áreas predominantes contemplando temas voltados à produção agropecuária, processamento de alimentos, aplicação de tecnologia, e educação no campo.

Na primeira parte, são abordados estudos relacionados à qualidade do solo, germinação de sementes, controle de fitopatógenos, bem estar animal, entre outros assuntos.

Na segunda parte são apresentados trabalhos a cerca da produção de alimentos a partir de resíduos agroindustriais, e qualidade de produtos alimentícios após diferentes processamentos.

Na terceira parte são expostos estudos relacionados ao uso de diferentes tecnologias no meio agropecuário e agroindustrial.

Na quarta e última parte são contemplados trabalhos envolvendo o desenvolvimento rural sustentável, educação ambiental, cooperativismo, e produção agroecológica.

O organizador e a Atena Editora agradecem aos autores dos diversos capítulos por compartilhar seus estudos de qualidade e consistência, os quais viabilizaram a presente obra.

Por fim, desejamos uma leitura proveitosa e repleta de reflexões significativas que possam estimular e fortalecer novas pesquisas que contribuam com os avanços científicos e tecnológicos nas Ciências Agrárias.

Júlio César Ribeiro

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CAPÍTULO 7

IN VITRO ACTIVITY OF PURPUREOCILLIUM LILACINUM ISOLATES AGAINST PHYTOPATHOGENIC FUNGI OF SORGHUM

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ABSTRACT: Sorghum is the fifth cereal in importance worldwide. Its productivity is threatened by phytopathogenic fungi that reduce crop yields. Many species of *Alternaria* are natural contaminants of sorghum. The period of storage of those grains is a critical point to fungal control. Currently, there is an attempt to reduce the use of agrochemicals and to apply alternative control strategies such as biopesticides. In this context, the objective was to evaluate the *in vitro* antifungal activity of *Purpureocillium lilacinum* on the growth of *Alternaria*. Dual cultures of this specie were performed against potentially antagonistic isolates. The macro and microscopic evaluation was performed and five antagonism indicators were calculated. The interaction mechanisms involved were also determined by evaluating the effect of volatile and non-volatile

metabolites produced by antagonist on the growth of phytopathogens fungus. The three *P. lilacinum* isolates had variable inhibitory activity according to the type of assay and phytopathogen evaluated. However, the inhibition was more evident with *P. lilacinum* Ls isolate and mainly with the effect of the non-volatile compounds. This study suggests that *P. lilacinum* could be considered a promising antagonist in the control of *Alternaria*. These results motivate us to go deeper in the production, isolation and identification of the compounds produced by this specie. The studied *P. lilacinum* Ls isolate could be another tool in the different biological control strategies applied to the conservation of sorghum grains.

KEYWORDS: Sorghum, biopesticide, fungal interactionsf *Purpureocillium lilacinum*, *Alternaria*.

ATIVIDADE IN VITRO DE ISOLADOS DE PURPUREOCILLIUM LILACINUM CONTRA FUNGOS FITOPATOGÊNICOS DE SORGO

RESUMO: O sorgo é o quinto cereal em importância no mundo. Sua produtividade é ameaçada por fungos fitopatogênicos que reduzem a produtividade das culturas. Muitas espécies de *Alternaria* são contaminantes naturais do sorgo. O período de armazenamento desses grãos é um ponto crítico para o controle de fungos. Atualmente, há uma tentativa de reduzir o uso de agroquímicos e aplicar estratégias alternativas de controle, como os biopesticidas. Nesse contexto, o objetivo foi avaliar a atividade antifúngica *in vitro* de *Purpureocillium lilacinum* sobre o crescimento de *Alternaria*. Culturas

duais dessa espécie foram realizadas contra isolados potencialmente antagonísticos. A avaliação macro e microscópica foi realizada e cinco indicadores de antagonismo foram calculados. Os mecanismos de interação envolvidos também foram determinados avaliando o efeito de metabólitos voláteis e não voláteis produzidos pelo antagonista sobre o crescimento de fungos fitopatogênicos. Os três isolados de *P. lilacinum* apresentaram atividade inibitória variável de acordo com o tipo de ensaio e fitopatogêno avaliados. No entanto, a inibição foi mais evidente com o isolado Ls de *P. lilacinum* e principalmente com o efeito dos compostos não voláteis. Este estudo sugere que *P. lilacinum* pode ser considerado um antagonista promissor no controle de *Alternaria*. Esses resultados nos motivam a nos aprofundar na produção, isolamento e identificação dos compostos produzidos por esta espécie. O isolado Ls de *P. lilacinum* estudado pode ser mais uma ferramenta nas diferentes estratégias de controle biológico aplicadas à conservação de grãos de sorgo.

PALAVRAS-CHAVE: Sorgo, biopesticida, interações fúngicas *Purpureocillium lilacinum*, *Alternaria*.

1 | INTRODUCTION

Sorghum is the fifth cereal in importance worldwide, after corn, wheat, rice and barley, and the fourth most produced cereal in Argentina (MINISTERIO DE AGRICULTURA, GANADERIA Y PESCA, 2019; USDA, 2015). Historically, their destination was predominantly debated between two clearly defined uses: as human food and as fodder or animal use. Currently, sorghum is perhaps one of the most versatile crops, according to their destinations, so the total area planted with sorghum has increased markedly over the past decades. However its productivity is threatened by several fungal species that reduce yields, quality and safety of crops with significant economic losses.

Alternaria has been reported as a natural contaminant of sorghum grains throughout the world (PANCHAL and DHALE 2011; YAGO et al., 2011; CHALA et al., 2014; LAHOUAR et al., 2015; TAYE et al. 2016) and recently also in our country (EMATEGUY et al., 2018). The *Alternaria* genus is distributed throughout the world and is a pathogen with a wide range of hosts. Many species belonging to this genus are saprophytes, while most are pathogens of animals and plants causing extensive yield losses in agriculture, especially in pre and post-harvest conditions of numerous cereals (PEEVER et al., 2005). Many of these species are of special interest due to their capacity of different mycotoxins-producer on stored sorghum grains (ASTORECA et al., 2019).

The storage period is a critical point to control these fungi, which is currently based on the use of agrochemicals. The growing social pressure for access to healthier foods and for the care of the environment has focused on different national and international organizations to minimize the impact of agrochemicals on human and

environmental health. In this context, the application of alternative control strategies such as integrated pest management (IPM) as well as the development of new biopesticides is being researched (BARRA et al., 2015; SARROCCO and VANNACCI, 2018).

The advantages of the biopesticides have favored its use, the establishment of the corresponding regulatory frameworks and investments in research and development in the production area. Bacterial products are located in the first place in the biopesticides market. However, in Latin America, about 48% of the market corresponds to fungal biopesticides (PELAEZ and MIZUKAWA, 2017; BAUTISTA et al. 2018). Among the widely used and frequently studied biological control agents is *Trichoderma* spp. for their biocontrol activities and beneficial plant interaction (MARTÍNEZ-COCA et al., 2013; 2018; BHAT, 2017; RAMARAJU et al., 2017; RONNIE-GAKEGNE and MARTÍNEZ-COCA, 2018; ALFIKY, 2019; PASTIRČÁKOVÁ, 2019).

The majority of fungal biopesticides are formulated based on infectious propagules obtained from different fermentation systems. However, they can also exert their action from biomass and currently they are developing fungal biopesticides based on proteins, secondary metabolites, enzymes and RNA interference (GHORBANPOUR et al., 2017; BAUTISTA et al., 2018). The knowledge of the different mechanisms of action associated with fungi as biocontrol agents is a fundamental step in the development of new biopesticides.

Purpureocillium lilacinum (LUANGSA-ARD et al., 2011), is a natural soil fungus with a wide geographical distribution and recognized for its antagonistic activity on insects, eggs and females of plant parasitic nematodes (BARRA et al., 2015; GORTARI and HOURS, 2019; TOLEDO-HERNÁNDEZ et al., 2019). *Purpureocillium lilacinum* is also recognized as a producer of several pathogenicity factors such as enzymes, metabolic products of diverse nature and toxins (SHARMA et al., 2016; WANG et al., 2016). It is the fungi with the highest amount of nematicide formulations (WANG et al., 2016). However, there are few studies on the mycoparasitic activity of *P. lilacinum* and its incorporation in different control strategies of phytopathogenic and toxicogenic fungi (BARRA et al., 2015; SARROCCO and VANNACCI, 2018). In this context, the objective of this work was to evaluate the *in vitro* activity of three native *Purpureocillium lilacinum* isolates on the growth of two *Alternaria alternata* and three *A. tenuissima* isolates.

2 I MATERIALS AND METHODS

2.1 Antagonistic fungal isolates

Three *Purpureocillium lilacinum* isolates molecularly identified were used as

potential antagonists: *P. lilacinum* LPSC #876, Pv and Ls (GORTARI and HOURS, 2019).

2.2 Pathogenic fungal isolates

The antagonistic fungal isolates were confronted with five phytopathogenic fungi isolates obtained from sorghum grains provided by the Experimental Station of the National Institute of Agricultural Technology (INTA) of Manfredi, Córdoba. The isolates 1c and 3d were molecularly identified as *Alternaria alternata* and 6d, 14K and 17I as *A. tenuissima*.

2.3 In vitro confrontation test

To evaluate the effect of *P. lilacinum* on *Alternaria* isolates growth, the technique of dual culture described by MARTÍNEZ ÁLVAREZ et al. (2013) was used. Prior to inoculation, the reverse of the Petri dishes was marked as described in Figure 1. In the control plates the antagonist was placed in the two opposing points. A suspension of fungal spores was prepared in Eppendorf tubes with 200 μ l of semi-solid agar. The inoculation was performed centrally with a pointed loop, according to the aforementioned scheme.

Plates were incubated at $28 \pm 2^\circ\text{C}$ on dark for an incubation period of 14 days. The length of the central axis of the pathogen colony (axis C), the length of the lateral axis (axes I and D), the maximum radius of the colony (r_1) and the distance between the two colonies on the axis central (z_1) were measured according to Figure 1.

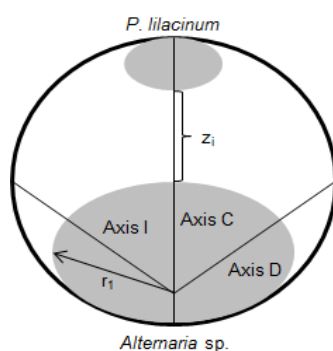


Figure 1. Inoculation scheme and detail of the variables measured.

2.4 Fungal growth rate

The growth rate was evaluated for each phytopathogenic isolate alone and with *P. lilacinum* isolate. The length of the axis C was measured at 3, 5, 7, 10 and 12 days of incubation and a growth curve was made.

2.5 Antagonism indicators

To evaluate antagonism, the following indicators were used: 1) the length of the central axis of the pathogen colony, 2) the shape coefficient (difference between the mean of the lateral axes and the axis C), 3) the percentage of inhibition calculated with the formulae $[100 \times (r_1 - \text{axis C}) / r_1]$, 4) the width of the zone of inhibition (z_i) and 5) the percentage of the width of z_i calculated with the equation $[(AB)/A] \times 100$ being A the length of the axis C of the pathogen in the control treatment and B the length of the same axis but in the dual culture with the antagonist.

2.6 Macro and microscopic evaluation

Each confrontation and their respective controls were evaluated macro and microscopically in order to evidence any alteration in their morphology and/or habitual behavior.

2.7 Effect of volatile metabolites produced by *P. lilacinum*

The effect of the volatile metabolites was evaluated according to the methodology described by MOKHTAR and DEHIMAT (2012), with some modifications. Potato dextrose agar culture medium was tipped over the base of 70 mm diameter Petri dishes, which were faced with each other according to the following scheme (Figure 2).

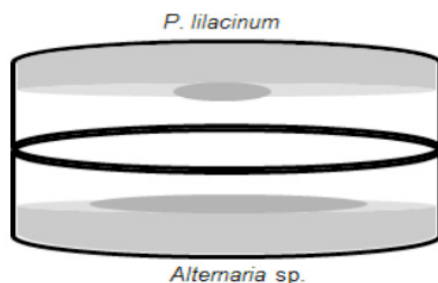


Figure 2. Representative diagram of the device used to evaluate the effect of the volatile metabolites produced by the potential antagonist on the isolates of phytopathogenic fungi.

Both plate bases were inoculated centrally with each of the isolates of *P. lilacinum* and *Alternaria* spp., respectively. As controls were inoculated each of the isolates faced to itself in order to consider the potential effects of oxygen concentration and accumulation of toxic substances inside that device. The inocula, prepared in semisolid agar, were obtained from cultures in active growth from PDA medium.

The devices were sealed with parafilm and incubated at $28 \pm 2^\circ\text{C}$ in darkness until complete development of the *Alternaria* controls. The effect of the metabolites was evaluated through the percentage of inhibition of radial growth (PICR) according to the following equation:

$$\text{PICR} = (R_1 - R_2)/R_1 \times 100$$

where R_1 is the radius of the colony of the control pathogen and R_2 is the radius of the colony of the pathogen in the confrontation.

2.8 Effect of non-volatile metabolites produced by *P. lilacinum*

To obtain the non-volatile metabolites, the isolates of *P. lilacinum* were inoculated in Erlermeyer with 100 ml of Dextrosa Potato Broth (DPB). The inoculum (8 mm diameter discs) was obtained from cultures in actively growing from PDA. They were incubated at $28 \pm 2^\circ\text{C}$ for 8 days and filtered through Millipore filters of 0.45 and 0.22 μm (Sartorius). Discs of mycelium (5 mm) of each pathogen were imbedded for 5 min in the filtrate and immediately inoculated centrally in Petri plates (90 mm diameter) containing PDA. As controls, Petri plates inoculated with disks of the pathogen imbedded for 5 min in sterile DPB were used. The Petri plates were incubated at $28 \pm 2^\circ\text{C}$ in the dark until the complete development of the *Alternaria* spp. The PICR was calculated according to the equation mentioned previously.

2.9 Statistical analysis

The statistical analysis of the assay was carried out with the statistical software Infostat (DI RIENZO et al., 2008). The analysis of the variance and Fisher's multiple comparisons test were applied. The treatments with negative results were not included in the analysis since they have no value as potential biological controllers.

3 | RESULTS

3.1 Fungal growth rate

The growth rate was evaluated for each phytopathogenic isolate alone and with *P. lilacinum* isolate using the growth curve made with the length of the C axis measured at 3, 5, 7 and 10 days of incubation (Figure 3). The evaluation of the obtained graphs indicated decrease in the growth rate of all the assayed *Alternaria* isolates confronted to *P. lilacinum* #876 isolate with respect to the growth of that isolates alone. However, the *P. lilacinum* Ls and Pv isolates modified the growth rate of the *Alternaria* isolates in a variable way (Figure 3).

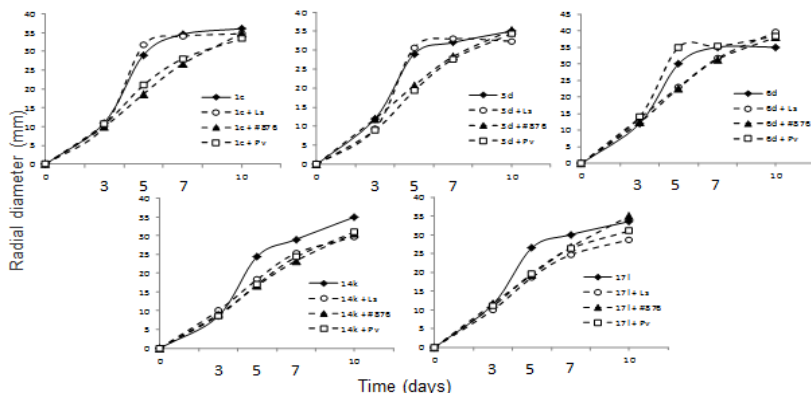


Figure 3. Radial growth in dual culture on PDA medium of the different analyzed *Alternaria* isolates: **A)** 1c, **B)** 3d, **C)** 6d, **D)** 14k y **E)** 171 against three potentially antagonistic *P. lilacinum* isolates (Ls, #876 and Pv).

3.2 Antagonism indicators

The aforementioned indicators were calculated after 10 days of incubation, moment in which both colonies come in contact and other factors such as nutrient depletion, dehydration of the culture medium, affect their growth (Table 1). Depending on the five analyzed parameters, the *P. lilacinum* Ls isolate was the one that produced the greatest effect on the growth of all the analyzed *Alternaria* isolates. The statistical analysis also showed significant differences for inhibition percentage for all the *Alternaria* isolates against *P. lilacinum* Ls and Pv isolates.

Antagonist	Parameter	<i>A. alternata</i>			<i>A. tenuissima</i>	
		1c	3d	6d	14k	171
#876	Axis C ¹	34.7 ± 1.5 ^a	35.3 ± 2.0 ^b	38.0 ± 2.0 ^a	30.6 ± 2.5 ^a	35.0 ± 2.6 ^b
	Shape coefficient	3.5 ± 0.9 ^a	2.8 ± 1.4 ^a	4.3 ± 2.8 ^a	2.1 ± 1.5 ^a	1.3 ± 0.8 ^a
	Inhibition percentage	10.3 ± 2.6 ^a	7.8 ± 2.1 ^a	11.6 ± 2.6 ^a	4.8 ± 1.7 ^a	2.7 ± 0.2 ^a
	Width of the z _i ¹	7.6 ± 0.6 ^a	7.6 ± 3.5 ^a	4.6 ± 0.6 ^a	12.0 ± 1.0 ^a	8.6 ± 2.0 ^a
	Percentage of width of the z _i	5.5 ± 3.9 ^a	-	-	12.3 ± 7.1 ^a	-
Pv	Axis C ¹	33.3 ± 2.0 ^a	34.3 ± 0.6 ^b	38.3 ± 0.6 ^a	31.0 ± 0.0 ^a	31.0 ± 1.0 ^a
	Shape coefficient	4.5 ± 0.0 ^a	4.1 ± 0.6 ^{ab}	7.5 ± 0.0 ^a	4.0 ± 1.5 ^a	2.8 ± 1.6 ^{ab}
	Inhibition percentage	18.7 ± 3.2 ^b	13.3 ± 2.5 ^b	19.5 ± 2.3 ^b	12.1 ± 3.7 ^b	12.2 ± 1.4 ^b
	Width of the z _i ¹	8.3 ± 3.0 ^a	7.0 ± 0.0 ^a	4.6 ± 1.5 ^a	11.0 ± 0.0 ^{ab}	11.0 ± 0.0 ^{ab}
	Percentage of width of the z _i	7.5 ± 5.7 ^a	1.9 ± 1.6 ^a	-	11.4 ± 0.0 ^a	7.4 ± 2.9 ^a
Ls	Axis C ¹	31.6 ± 1.5 ^a	30.6 ± 1.1 ^a	39.6 ± 4.0 ^a	29.6 ± 1.5 ^a	28.6 ± 1.5 ^a
	Shape coefficient	6.1 ± 0.7 ^b	5.0 ± 0.5 ^b	5.5 ± 3.5 ^a	4.5 ± 1.5 ^a	4.0 ± 0.5 ^b
	Inhibition percentage	21.5 ± 1.5 ^b	21.6 ± 2.6 ^c	20.0 ± 0.0 ^b	15.8 ± 4.1 ^b	11.9 ± 4.3 ^b
	Width of the z _i ¹	7.6 ± 1.1 ^a	7.0 ± 1.7 ^a	7.6 ± 0.6 ^b	12.6 ± 0.6 ^b	13.0 ± 1.0 ^b
	Percentage of width of the z _i	12.0 ± 4.2 ^a	12.3 ± 3.3 ^b	-	15.4 ± 3.4 ^a	14.4 ± 4.5 ^a

Table 1. Antagonism Indicators between *P. lilacinum* and the *Alternaria* studied isolates.

¹The values are expressed in mm. Values with a common letter are not significantly different ($p > 0.5$).

3.3 Macro and microscopic evaluation

After 10 days of incubation, closeness was observed macroscopically between both growth fronts (phytopathogenic fungi confronted to *P. lilacinum*), with a corresponding growth inhibition. Microscopy, rings of unusual hyphae, few conidia, vacuolization of the hyphae and structures compatible with hyphae disintegration in the *Alternaria* isolates were observed (Figure 4).

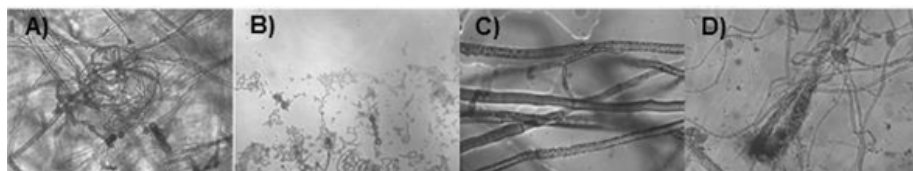


Figure 4. Morphology of *Alternaria* isolates against *P. lilacinum*. Note: **A)** Hyphae ring, **B)** Predominance of *P. lilacinum* conidia compared to those of *Alternaria* in the contact area (40x), **C)** Vacuolized hyphae and **D)** Structures compatible with hyphae disintegration (100x).

3.4 Effect of volatile metabolites

The inhibition of growth in the isolates of *Alternaria* spp., induced by the volatile metabolites produced by *P. lilacinum* #876, Pv and Ls varied between 1.7% and 16.7% after 6 days of incubation. The isolate with the highest growth inhibition was *A. alternata* 3d (16.7%) against #876, followed by 1c with values of 11.7% and 14.4% against Ls and Pv, respectively. Significant differences were found for 1c facing Pv and 3d facing #876 ($p \leq 0.05$). *Alternaria tenuissima* 6d isolate was not inhibited by any of the antagonists analyzed (Figure 5).

3.5 Effect of non-volatile metabolites

The inhibition of the growth of *Alternaria* spp. caused by the non-volatile metabolites produced by *P. lilacinum* ranged from 4.3 to 29.5% at 7 days of incubation. In this case all the treatments threw positive results, observing statistically significant differences ($p \leq 0.05$) for *A. tenuissima* 171 compared to #876, Pv and Ls (Figure 5).

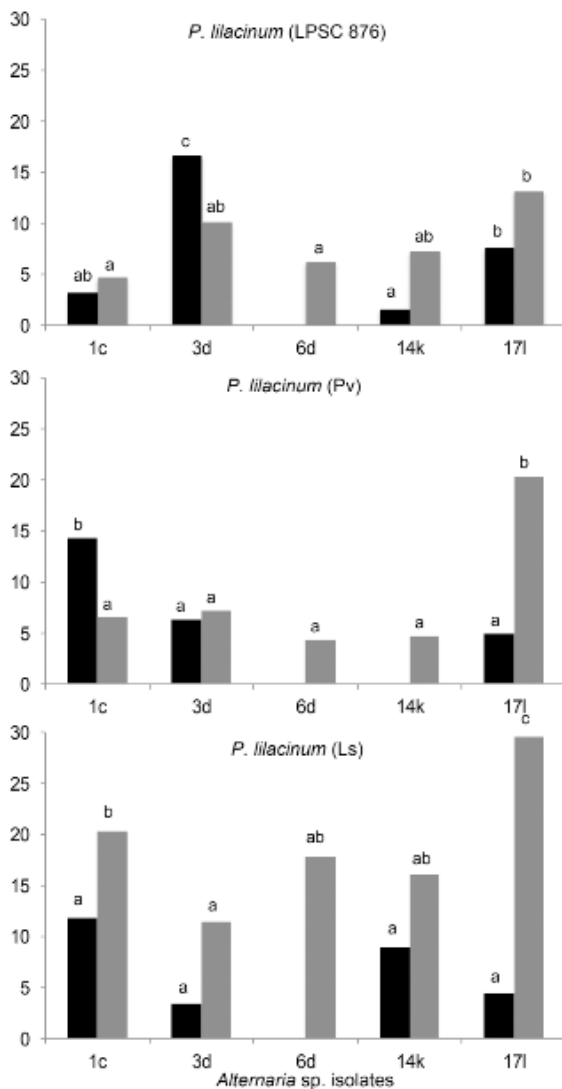


Figure 5. Inhibition percentage of radial growth of *Alternaria* spp. isolates by the effect of volatile (■) and non-volatile (▒) compounds produced by *P. lilacinum* isolates.

4 | DISCUSSION

As far as the authors are aware, the available literature does not include studies in relation to *in vitro* interaction between *P. lilacinum* and *Alternaria* isolates. Therefore, for the discussion we have chosen those results we considered as the most similar in relation to experimental conditions (culture medium, temperatures, pH, inoculum, inoculation and incubation time, growth rate of the organisms used).

The results obtained in this study showed interaction between *P. lilacinum* and

Alternaria isolates which could be verified macroscopically in the dual confrontation assay. The interaction between *P. lilacinum* and *Alternaria* may be considered as mutual inhibition according to DEMIRCI et al. (2011), RAMARAJU et al. (2017) and PASTIRČÁKOVÁ (2019) who observed a physical delimitation between the margins of each isolate without detecting invasion phenomena of the space colonized by the opposite isolate. In our study, this contact zone corresponded to a lighter area in the margin of the colony of *P. lilacinum*.

At the microscopic level only rings of unusual hyphae, few conidia, vacuolization of the hyphae and structures compatible with hyphae disintegration in the *Alternaria* isolates were found as mycoparasitism signal. No other signs were observed such as trap rings, fragmentation and/or penetration of hyphae described by other authors (BHAT, 2017; MARTÍNEZ-COCA et al., 2018).

In this study five antagonism indicators were evaluated, however the most used one to define the potential as a biological controller is the percentage of inhibition. The highest percentages of inhibition were obtained with *P. lilacinum* Ls isolate on 1c, 3d and 6d *Alternaria* isolates, reaching values of 21.5, 21.6 and 20%, respectively. MARTÍNEZ ALVAREZ et al. (2013) obtained higher values (33-47%) to the assessment potential antagonists of *Fusarium circinatum*. MARTÍNEZ-COCA et al. (2013) reported values of percentages of inhibition from 67 to 84% on dual culture of *Trichoderma* spp. and *Didymella bryoniae*. The same authors confronted *Trichoderma asperellum* with *Fusarium* spp. and observed inhibition percentages greater than 40% (MARTÍNEZ-COCA et al., 2018).

These results are in agreement with those reported by ALFIKY (2019) who studied the same species as antagonist but confronted to *Sclerotium rolfsii* and *Rhizoctonia solani*. The same year, PASTIRČÁKOVÁ (2019) showed inhibition percentages between 8 and 36% when confronting *Trichoderma harzianum* against pathogenic fungi obtained from horse-chestnut in Petri dishes with different culture media and inoculation models.

According to the percentage inhibition values obtained in this study and also considering the criteria proposed by MIS-MUT et al. (2015), who estimates as permissible a 40% of inhibition percentage, the study of the inhibitory effect of *P. lilacinum* on any of the phytopathogens tested should be deepened in future assays to be confirmed.

In the volatile metabolite assay, no growth inhibition was observed for the *A. tenuissima* 6d isolate by any of the *P. lilacinum* isolates evaluated while for the rest of the studied *Alternaria* isolates, the percentage inhibition values ranged between 1.7% to 16.7% at 6 days of incubation. Regard this same assay, MARTÍNEZ-COCA et al. (2013) found the highest inhibition percentages (17-32%) in the interaction between *Trichoderma* isolates and *Didymella bryoniae*. Recently the same authors found a high

variability in the observed results (0-50%) between *Trichoderma* and *Fusarium* isolates depending on the particular confrontation (MARTÍNEZ-COCA et al., 2018). In another study, RONNIE-GAKEGNE and MARTÍNEZ-COCA (2018) evaluated *Trichoderma* against *Alternaria solani* isolates and they reported values of the inhibition percentage from 26 to 38% after 96 hours of incubation. Similarly, ALFIKY (2019) reported inhibition percentage values between 29 and 46% depending on the evaluated condition in the cited article.

Specifically, the *P. lilacinum* isolates evaluated in this study showed a greater antagonistic potential due to the release of non-volatile metabolites. These results are consistent with those reported by MARTÍNEZ-COCA et al. (2013; 2018) and RONNIE-GAKEGNE and MARTÍNEZ-COCA (2018) who demonstrated the decrease of different pathogenic fungi growth due to the effect of segregated non-volatile metabolites by *Trichoderma* isolates on different culture medium. However, the percentages of inhibition achieved in the present study (4-30%) are lower than those reported by the research group mentioned previously (10-85%) and ALFIKY (2019) (30-70%).

The obtained results would indicate that there is no unique *P. lilacinum* isolate that produces volatile compounds with inhibitory effect on the assayed *Alternaria* isolates. In contrast, all *P. lilacinum* isolates affected the *Alternaria* growth, to a greater or lesser extent, by the non-volatile metabolite assay being the isolate *P. lilacinum* Ls the one that showed the greatest inhibitory capacity. These results suggest that *P. lilacinum* could produce compounds with antifungal ability. Currently, secondary metabolites responsible for antibiosis are the basis for the research and development of new biopesticides, which justifies the continuity of studies on the mechanisms of action of this isolate.

This study reveals that *P. lilacinum* Ls isolate would have greater potential as a biological control agent. However it is necessary to deepen the study of other factors that could significantly influence their *in vitro* antagonistic effect. These preliminary studies are the first step to understand the type of interaction between these species and to demonstrate the potentiality of this isolate of *P. lilacinum* as a biological control agent of toxigenic fungi.

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