

Antifungal activity of *Origanum vulgare* and *Rosmarinus officinalis* phenolics-containing extracts against *Colletotrichum lindemuthianum* and in the suppression of anthracnose in common beans

Atividade antifúngica de extratos contendo compostos fenólicos de *Origanum vulgare* e *Rosmarinus officinalis* contra *Colletotrichum lindemuthianum* e na supressão da antracnose em feijão comum

Actividad antifúngica de los extractos fenólicos de *Origanum vulgare* y *Rosmarinus officinalis* frente a *Colletotrichum lindemuthianum* y en la supresión de la antracnosis en frijol común

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Abstract

Oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.) may contribute to the control of anthracnose in common bean (*Phaseolus vulgaris* L.). This study aimed to investigate the chemical composition of both extracts by LC-HRMS and their *in vitro* and *in vivo* inhibitory activity against *C. lindemuthianum* isolates. We evaluated the inhibition of mycelial growth, the inhibition of the conidia germination, the phytotoxicity of the extracts in bean detached leaves and the reduction of severity of anthracnose under greenhouse conditions. Ten phenolics were identified: caffeic, p-coumaric, ferulic, gallic, gentisic, protocatechuic and vanillic acids, and quercetin, luteolin and naringenin. Gentisic acid was not reported for these plants in the consulted literature. We verified the complete inhibition of the mycelium growth of *C. lindemuthianum* by both extracts at about 2% (oregano) and 3% (rosemary), the inhibition of spore germination was for rosemary: 100% and for oregano: 85%, at a concentration of 3% of extracts and no symptoms of phytotoxicity were observed in common bean leaves treated with them. Both extracts were efficient in reducing the severity of anthracnose caused by *C. lindemuthianum* BRM 007447 in BRS *Campeiro* and *Perola* cultivars, in preventive and curative modes. These extracts have shown promise in the practice of sustainable agriculture, under the experimental conditions used.

Keywords: Alternative control; Bioproduct; *Phaseolus vulgaris*; Sustainable agriculture.

Resumo

Orégano (*Origanum vulgare* L.) e alecrim (*Rosmarinus officinalis* L.) podem contribuir para o controle da antracnose (*Colletotrichum lindemuthianum* (Sacc & Magnus) Scribner) no feijoeiro comum (*Phaseolus vulgaris* L.). Este estudo teve como objetivo investigar a composição química de ambos os extratos por CL-EMAR e suas atividades inibitórias *in vitro* e *in vivo* contra isolados de *C. lindemuthianum*. Avaliamos a inibição do crescimento micelial, a inibição da

germinação de conídios, a fitotoxicidade dos extratos em folhas destacadas de feijão e a redução da severidade da antracnose, em casa de vegetação. Foram identificados dez compostos fenólicos: ácidos caféico, p-cumárico, ferúlico, gálico, gentísico, protocatecuico, vanílico e quercetina, luteolina e naringenina. O ácido gentísico não foi relatado para estas plantas na literatura consultada. Verificamos a inibição total do crescimento micelial de *C. lindemuthianum* por ambos os extratos a 2% (orégano) e 3% (alecrim), a inibição da germinação dos esporos foi para o alecrim: 100% e para o orégano: 85%, na concentração de 3 % dos extratos e, não foram observados sintomas de fitotoxicidade nas folhas de feijão tratadas com eles. Ambos os extratos foram eficientes na redução da severidade da antracnose causada por *C. lindemuthianum* BRM 007447 nas cultivares BRS Campeiro e Pérola, nos modos preventivo e curativo. Esses extratos mostraram-se promissores na prática da agricultura sustentável, nas condições experimentais utilizadas.

Palavras-chave: Controle alternativo; Bioproduto; *Phaseolus vulgaris*; Agricultura sustentável.

Resumen

Orégano (*Origanum vulgare* L.) y romero (*Rosmarinus officinalis* L.) pueden contribuir al control de la antracnosis en frijol común (*Phaseolus vulgaris* L.). Este estudio tuvo como objetivo investigar la composición química de ambos extractos por LC-HRMS y sus actividades inhibitorias *in vitro* e *in vivo* contra los aislados de *Colletotrichum lindemuthianum*. Evaluamos la inhibición del crecimiento micelial, la inhibición de la germinación de conidios, la fitotoxicidad de los extractos en hojas desprendidas de frijol y la reducción de la severidad de la antracnosis, en condiciones de invernadero. Se identificaron diez fenoles: ácidos cafeico, p-cumárico, ferúlico, gálico, gentísico, protocatecuico, vanílico y quercetina, luteolina y naringenina. El ácido gentísico no fue reportado para estas plantas en la literatura consultada. Verificamos la inhibición completa del crecimiento del micelio de *C. lindemuthianum* por ambos extractos al 2% (orégano) y 3% (romero), la inhibición de la germinación de esporas fue para romero: 100% y para orégano: 85%, a una concentración de 3% de extractos y no se observaron síntomas de fitotoxicidad en las hojas de frijol común tratadas con ellos. Ambos extractos fueron eficientes en la reducción de la severidad de la antracnosis causada por *C. lindemuthianum* BRM 007447 en los cultivares BRS Campeiro y Perola, en modo preventivo y curativo. Estos extractos se mostraron prometedores en la práctica de la agricultura sostenible, en las condiciones experimentales utilizadas.

Palabras clave: control alternativo; bioproducto; *Phaseolus vulgaris*; Agricultura sostenible.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is the most cultivated bean species of *Phaseolus* genus, with Brazil being the top-ranked producing country in the world. It is of great importance in human food, as it contains high nutritional value. Its production in Brazil is commonly considered familiar-based activity (CONAB, 2018).

Bean cultures can be affected by several diseases, among them is the anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., often highlighted for bringing great losses in production. It causes necrotic lesions of dark brown colour in veins and on the underside of bean leaves, also observed in the aerial parts of the plant. In seeds, one may observe lesions in tissues of cotyledons, whose colour varies from yellow to dark coffee or black. Its spreading can occur through infected seeds or even cultural remains from a crop to another (Rava et al., 1993).

Controlling methods employed are the use of healthy seeds, resistant cultivars, crop rotation, elimination of cultural remains, resistance induction, biological control, chemical control of seeds and spray. Continued and excessive employment of agricultural defensive can yield in socio-environment and public health problems (Pinto et al., 2010; Bozdogan, 2014). In order to reducing it, there have been studies focusing on natural extracts as an alternative solution.

Studies based on plant extracts and their secondary metabolites with antifungal properties, have been reported emphasizing their potential in the control of phytopathogens. These extracts can display a directly fungitoxic activity, inhibition of mycelium growth, spore germination and induction of phytoalexins (Pinto et al., 2010; Sales et al., 2016; Shabana et al., 2017; Varjani et al., 2020; Itako et al., 2021).

Origanum vulgare L. (oregano) and *Rosmarinus officinalis* L. (rosemary) are important tools in the control of pathogens and plant diseases. Maia et al. (2014) reported the activity of essential oil of rosemary of reducing the severity of stains in leaves (*Pseudocercospora vitis*) and the mildew *Plasmopara viticola* of the vine. Romero et al. (2015) showed the inhibitory activity of the mycelium growth and spore germination, of essential oil of *O. vulgare* in *Corynespora cassiicola*,

Fusarium sp., *Colletotrichum gloeosporioides* and *Rhizoctonia solani*. Djordjevic et al. (2013) reported that the essential oils of oregano and rosemary are capable of inhibiting the mycelium growth of *Fusarium oxysporum f. sp. lycopersici*.

The aims of this study was to evaluate the phenolic chemical composition of *Origanum vulgare L.* and *Rosmarinus officinalis* extracts by Liquid Chromatography-Tandem High Resolution Mass Spectrometry (LC-HRMS) and investigate the antifungal activity against *Colletotrichum lindemuthianum* and in the control of anthracnose caused by this pathogen of common bean.

2. Methodology

2.1 Obtaining vegetal materials and phenolics-containing extracts

O. vulgare leaves were purchased from the Paladar company (lot: 219 9:04), from Goiânia, Goiás, Brazil. *R. officinalis* leaves were cultivated in the Agência Goiana de Assistência Técnica, Extensão Rural e Pesquisa Agropecuária - EMATER (16° 36'19" S, 49°15'48" W, 710 m altitude) situated in Goiânia-GO, in May 2016. A voucher specimen was deposited at the UFG Herbarium under number UFG-50.506.

To obtain the phenolics-containing extracts, fresh leaves of *O. vulgare L.* and *R. officinalis L.* were grounded in a blender. The extracts were obtained through maceration and exhausted percolation at about 10 % solvent-plant ratio with 80% hydroethanolic solution (v/v) and then, were concentrated in a rotary evaporator (Buchi® model R-220 SE Switzerland) at 40°C, under reduced pressure (30 rpm, 600 bar) until reach a solid content of 75.49% (w/w) for *O. vulgare* and 52.19% (w/w) for *R. officinalis*.

2.2 Chemical composition by Liquid Chromatography tandem High Resolution Mass Spectrometry (LC-HRMS)

Chromatographic separation was conducted on a liquid chromatograph Thermo Scientific®, Ultimate 3000, with a ACE® C18 column (4.6 x 100mm; 3µm), coupled to a Q-Exactive Orbitrap High Resolution Mass Spectrometry, Thermo Scientific®, with H-ESI source, operating in negative and positive mode, using spray voltage 4 kV, capillary temperature of 350° C, and scan range (m/z) 150-700. The gradient elution was done with deionized acidified water with 0.1 % formic acid (mobile phase A, v/v) and acidified methanol with 0.1 % formic acid (mobile phase B, v/v). The gradient programming realized was initiated with 93:7 (A:B %), 70:30 (A:B %) in 10 minutes, 50:50 (A:B %) in 5 minutes, 30:70 (A:B %) in 3 minutes, 20:80 (A:B %) in 2 minutes, 100 (B %) in 3 minutes, held by 3 minutes, 93:07 (A:B %) in 2 minutes, held for 2 minutes. The mobile phase flow rate was 0.3 mL min⁻¹, injection volume 10 µL and temperature of the column in 20° C. For the study of fragmentation was used the PRM experiment (Parallel Reacting Monitoring) with different energies of collision (EC).

To identify phenolic compounds, it was used a stock solution of the standards in methanol at concentration of 1 mg.mL⁻¹. Then, it was prepared the mixture solution of these standards in concentration of 50 µg mL⁻¹ which was analyzed in the same conditions of the sample. The phenolic compounds obtained from Sigma-Aldrich ® were: gallic acid (≥ 98.5%), protocatechuic acid (≥ 97%), gentisic acid (≥ 98%), caffeic acid (≥ 98%), p-coumaric acid (≥ 98%), vanillic acid (≥ 97%), ferulic acid (≥ 98%), catechin (≥ 98%), epicatechin (≥ 90%), rutin (≥ 94%), quercetin (≥ 95%), naringenin (≥ 95%), luteolin (≥ 98%), kaempferol (≥ 90%). The identification of compounds presented in the extracts was made by similarity between the data achieved from the standards. The data were processed in software Xcalibur™.

2.3 *In vitro* inhibitory activity of *O. vulgaris* and *R. officinalis* phenolics-containing extracts on the mycelium growth of *Colletotrichum lindemuthianum*

The isolates of *Colletotrichum lindemuthianum* (Table 1) were provided by the Official Collection of Multifunctional and Phytopathogenic Microorganisms of Embrapa Arroz e Feijão, Santo Antônio de Goiás, State of Goiás, Brazil.

Table 1. Plant pathogenic fungi tested against *O. vulgaris* and *R. officinalis* phenolics-containing extracts.

Fungi	Isolated (s)
<i>Colletotrichum lindemuthianum</i>	BRM 007447
<i>Colletotrichum lindemuthianum</i>	BRM 007598
<i>Colletotrichum lindemuthianum</i>	BRM 007626

Source: Authors.

In vitro inhibitory activity of the extracts on the mycelium growth were conducted in 100 mm diameter Petri dishes containing Agar Potato Dextrose (PDA). The extract was added at concentrations of 0.6 % (w/w), 1.2 % (w/w), 2 % (w/w) and 3 % (w/w). 5 mm diameter disc of fresh PDA medium containing fungi mycelium of *C. lindemuthianum* inoculated and then incubated in a temperature controlled room at 22 ° C. The evaluation of mycelial growth was carried at the 21st day after the inoculation through the measure of the diameters of colonies with the aid of a digital caliper. The assay was carried out in triplicate, for the treatments and control (Côrtes et al., 2012).

2.4 *In vitro* inhibitory activity of *O. vulgaris* and *R. officinalis* phenolics-containing extracts on conidial germination of *Colletotrichum lindemuthianum*

The spore suspension of *C. lindemuthianum* was performed by inoculation of *C. lindemuthianum* BRM 007447 previously sterilized in beans pods and then incubated for 15 days at 22 ± 1°C. The conidia were collected in distilled water and filtered in sterile cloth. The conidia counting was done using Neubauer camera and a final concentration of 1.2x10⁶ conidia/mL was obtained (Rava et al., 1993).

For the *in vitro* germination tests, solutions of extracts of *O. vulgaris* and *R. officinalis* were prepared, individually, at a concentration of 3%. The glass slides were prepared with 10 µL of conidia suspension and 10 µL of the extracts, or 10 µL of distilled water (control). The assay was carried in triplicates on hydrophobic area (cellophane paper). The glass slides were incubated at 22 ± 1° C for 24 hours, in Petri dishes with moistened filter paper (Gonçalves & Stadnik, 2012). The germinated spore count was performed under an optical microscopic.

2.5 Phytotoxicity of *O. vulgaris* and *R. officinalis* phenolics-containing extracts to detached bean leaves

It was tested the phytotoxicity of *O. vulgare* and *R. officinalis* extracts in concentrations of 2% and 3% (v/v). Detached leaves from IPA 7419 bean cultivar with approximately 14 days of germination were placed in humid chambers under artificial light (12 h light - 12 h dark). A volume of 100 µL of both extracts were sprayed, individually, on the leaf surfaces and it were kept in incubator camera for 24 and 48 h at 22 ± 1° C. The evaluation of phytotoxicity were according to the features, as esclerosis, necrosis and injury. Control containing sterile distilled water was performed. The experiments were carried out in triplicate.

2.6 Reduction of severity of anthracnose by *O. vulgare* and *R. officinalis* phenolics-containing extracts

To evaluate the effects of the extracts on anthracnose, the seeding was carried with bean seeds of susceptible cultivars, mildly susceptible and resistant (*Perola*, *BRS FC 104*, *BRS Estilo*, *BRS Campeiro*) in sterile styrofoam trays and each cell received a seed. For each treatment and control, 32 seeds were planted, each having a replicate. It was used a MAXFERTIL® substrate. After planting, the trays were kept in a greenhouse under 21° C and ambient condition of humidity, with manual irrigation once a day.

Between eight and 10 days after the seeding, it was pulverized 150 mL of *O. vulgare* or *R. officinalis* extracts at concentrations of 3 % and water (control). The treatments using the extracts occurred in preventive mode, with applications a day previous to the inoculation of *C. lindemuthianum* BRM 007447 and two hours after the inoculation. The curative mode was conducted with two applications, the first two hours after the inoculation and on the 1st day after it. Fungal spore suspensions, 1.2 x10⁶ conidia/mL, and both extracts were applied with a manual spray in both faces of the leaves and the stem of seedlings until the dripping. After, the seedlings were transferred to a nebulizer room with temperature at 21 ± 1°C. The system stayed on for 48 hours, maintaining approximately 100% relative humidity. The characteristic symptoms of anthracnose were evaluated, eight to ten days after the inoculation, using a general grade scale of 1 to 9 degrees of severity (CIAT, 1988).

3. Results and Discussion

3.1 Chemical composition by liquid chromatography tandem high resolution mass spectrometry (LC-HRMS)

Ten compounds were identified by LC-HRMS in oregano and rosemary extracts, including phenolics acids and flavonoids (Table 2). The chemical profiles found in both extracts were similar, which may be due to the plants belonging to Lamiaceae family.

Table 2. Phenolic acids and flavonoids identified in *Rosmarinus officinalis* L. and *Origanum vulgare* L. extracts by LC-HRMS, in negative mode ([M-H]⁻).

Compound	Retention time (min)	Chemical Formula	Molecular Mass Detected (m/z)	Fragments (m/z)	Error <i>Rosmarinus officinalis</i> L. (ppm)	Error <i>Origanum vulgare</i> L. (ppm)																																																																
Gallic acid	14.42	C ₇ H ₆ O ₅	169.01331	169.01331	1.30	1.54																																																																
				125.02325			Protocatechuic acid	19.26	C ₇ H ₆ O ₄	153.01840	153.01840	1.08	0.88	109.02828	Gentisic acid	22.61	C ₇ H ₆ O ₄	153.01836	153.01836	0.82	0.68	Caffeic acid	23.17	C ₉ H ₈ O ₄	179.03421	179.03421	1.81	1.70	135.04410	Vanillic acid	23.18	C ₈ H ₈ O ₄	167.03415	167.03415	1.58	0.75	152.01048	p-Coumaric acid	25.11	C ₉ H ₈ O ₃	163.03918	163.03918	1.28	4.58	119.04912	Ferulic acid	25.17	C ₁₀ H ₁₀ O ₄	193.05003	193.05003	2.56	1.10	178.02640	Quercetin	24.19	C ₁₅ H ₁₀ O ₇	301.03543	301.03543	3.82	4.58	463.08859	Naringenin	28.11	C ₁₅ H ₁₂ O ₅	271.06131	271.06131	4.46	2.72	151.00269	Luteolin
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Source: Authors.

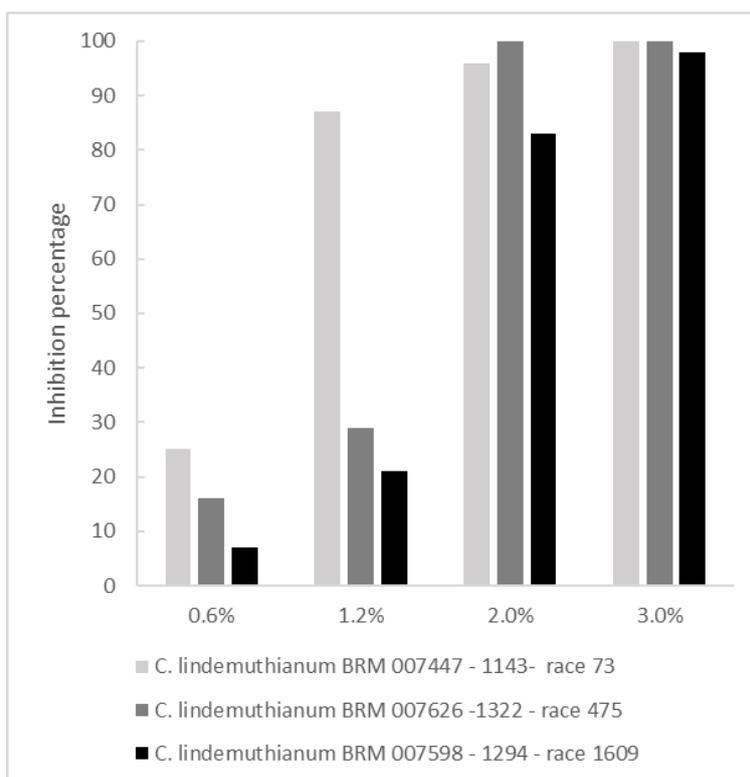
The phenolic profile of the extracts of *R. officinalis* and *O. vulgare* were similar to data reported in the literature, with the exception of gentisic acid, which was not found in the researched literature. Hossain et al. (2010) and Sakurai et al. (2016) showed the presence of p-coumaric acid, ferulic acid, caffeic acid and vanillic acid, quercetin and luteonin in *R. officinalis* and *O. vulgare* extracts. Cavero et al. (2006) reported the presence of naringenin in oregano and Baydar et al. (2009) in rosemary. Vallverdú-Queralt et al. (2014) verified gallic and protocatechuic acids, among other phenolics in rosemary and oregano.

The diverse chemical composition of both extracts studied allows us to suggest the possibility that these identified compounds may contribute to antifungal activity, based on the synergism that commonly occurs between the compounds present in the complex matrices of natural products, enhancing a biological effect (Khan, 2018) and also based on the phenolics properties (Tocci et al., 2018; Chiorcea-Paquim et al., 2020).

3.2 *In vitro* inhibitory activity of *O. vulgare* and *R. officinalis* phenolics-containing extracts on the mycelium growth of *Colletotrichum lindemuthianum*

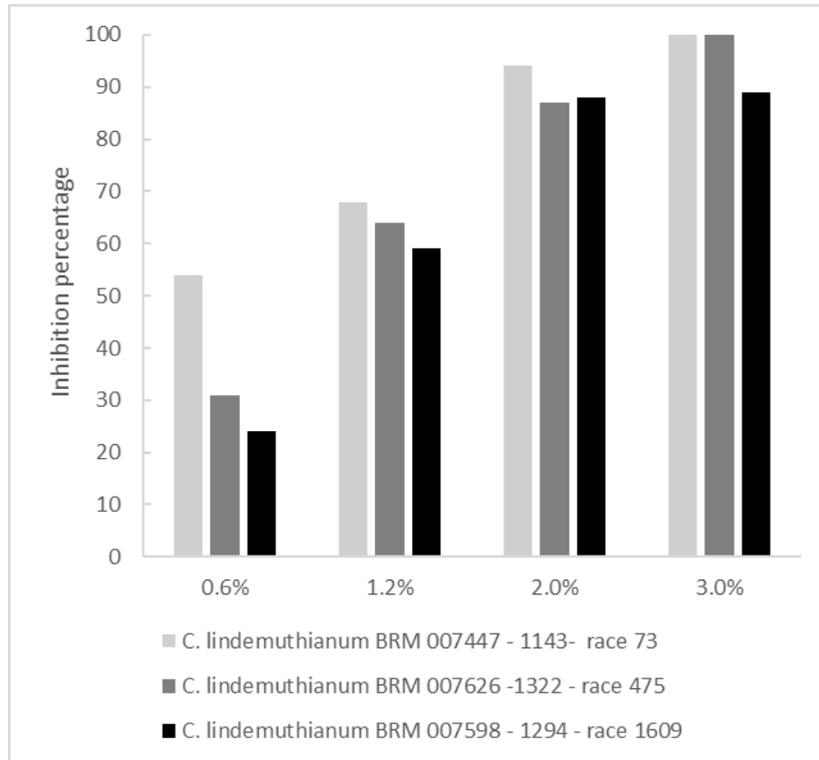
O. vulgare and *R. officinalis* phenolics-containing extracts showed variable mycelium growth inhibition according to the concentrations used. At concentration of 2%, oregano extract carried to the total suppression of *C. lindemuthianum* BRM 007626. At 3%, *C. lindemuthianum* BRM 007447 was completely inhibited by oregano (Figure 1). Rosemary extract inhibited 100% of *C. lindemuthianum* BRM 007447 and *C. lindemuthianum* BRM 007626, at 3% (Figure 2). *C. lindemuthianum* BRM 007598 was inhibited in 98% and 89% by oregano and rosemary, respectively (Figures 1 and 2). The difference in the inhibition of mycelial growth of fungal isolates may be due to the races of *C. lindemuthianum* have a great genetic variability (Santos et al., 2008; Oliveira et al., 2014).

Figure 1. Inhibition percentage of mycelium growth of *Colletotrichum lindemuthianum* at different concentrations of *Origanum vulgare* phenolics-containing extract.



Source: Authors.

Figure 2. Inhibition percentage of mycelium growth of *Colletotrichum lindemuthianum* at different concentrations of *Rosmarinus officinalis* phenolics-containing extract.



Source: Authors.

Andrade and Vieira (2016) demonstrated 22% of inhibitory activity of *R. officinalis* essential oil on the *in vitro* micelial growth of *Colletotrichum gloeosporioides*, that causes anthracnose in papaya. Brand et al. (2010) showed 18.6% of inhibition percentage of *C. lindemuthianum* by *R. officinalis* extract at concentration of 2.5%. Pinto et al. (2010) demonstrated about 30% of the micelial growth inhibitory activity of *C. lindemuthianum* pathotypes by *O. vulgare* extract.

3.3 *In vitro* inhibitory activity of *O. vulgare* and *R. officinalis* phenolics-containing extracts on conidial germination of *Colletotrichum lindemuthianum*

We selected *C. lindemuthianum* BRM 007447 for being the race with the highest incidence in our country associated with anthracnose in common bean.

The extracts of *R. officinalis* and *O. vulgare* reduced the conidial germination *in vitro* of the *C. lindemuthianum*. The percentage of inhibition was 100% and 85%, respectively, relative to the extracts of *R. officinalis* and *O. vulgare*. We attribute this due to the content of phenolics in both extracts.

Pinto et al. (2010) demonstrated 68% of *C. lindemuthianum* conidia germination inhibition by *O. vulgare* essential oil. Romero et al. (2015) observed a complete inhibition of *C. gloeosporioides* spore germination in concentrations of 500 ppm of *O. vulgare* essential oil. Andrade and Vieira (2016) showed 31.2% inhibition of germination of *C. gloeosporioides* conidia by *R. officinalis* essential oil. Although these studies have been carried out with the volatile fraction of oregano and rosemary, and not with the plant extract, it appears that the tendency towards sensitivity of *Colletotrichum* species to the secondary metabolites of these plants has been demonstrated.

3.4 Phytotoxicity of *O. vulgaris* and *R. officinalis* phenolics-containing extracts to detached bean leaves

The detached bean leaves treated with extracts of *O. vulgaris* or *R. officinalis* showed no symptom of phytotoxicity, tested at 2% and 3% (v/v) and under the experimental conditions used.

We did not find phytotoxicity data for extracts of these plants in common bean. Fialho et al. (2015) noted that 1% *R. officinalis* essential oil caused a low level of phytotoxicity on the grape rust, while the *O. vulgare* essential oil showed a higher level.

The phytotoxicity of plant extracts may be attributed to a diversity of allelochemicals presented in their composition, originated from its secondary metabolism (Brito et al., 2010) and to and the way in which the extracts are applied and the dose used (Corrêa & Salgado, 2011).

3.5 Reduction of the anthracnose severity by *O. vulgaris* and *R. officinalis* phenolics-containing extracts

We selected *C. lindemuthianum* BRM 007447 because it is the most common race of the fungus *C. lindemuthianum* in our country associated with anthracnose in common bean. We performed tests to investigate the *in vivo* performance of oregano and rosemary extracts at 3% in the reduction the severity of anthracnose.

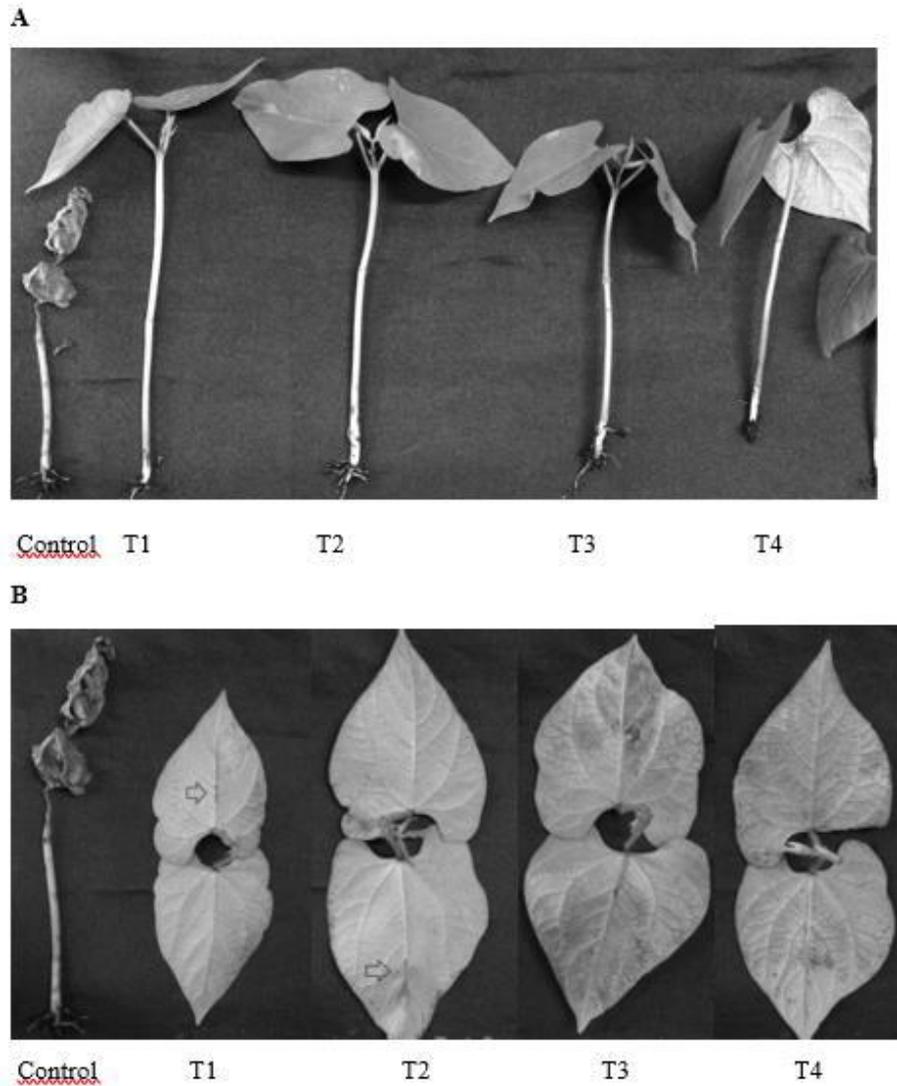
R. officinalis and *O. vulgare* phenolics-containing extract proved efficient in the control of the anthracnose when compared to the control, pulverized with water in *BRS Campeiro* and *Perola* cultivars (Table 3; Figures 3 and 4). The concentration at 3% held the percentage of foliar area affected below the areas of the control, which showed, at the end of the assay, the high test grade (9), thus leading the bean to death. The grade 2 means that up to 1% of the vessels show necrotic stains, only perceptible in the abaxial region of the leaves, and grade 1 means no presence of symptoms. The *BRS FC 104* and *Estilo* cultivars showed no symptoms in the control or in the treatments. These cultivars showed to be resistant to anthracnose under the conditions studies in this work, since they did not present any symptom (Table 3).

Table 3. Effects of *Rosmarinus officinalis* L. and *Origanum vulgare* L. phenolics-containing extracts on the severity of anthracnose (grades attributed). Treat 1 = preventive treatment with *R. officinalis* extract; Treat 2 = preventive treatment with *O. vulgare* extract; Treat 3 = curative treatment with *R. officinalis* extract; Treat 4 = curative treatment with *O. vulgare* extract.

Treatments	Severity of cultivars			
	<i>BRS Campeiro</i>	<i>BRS Perola</i>	<i>BRS FC 104</i>	<i>BRS Estilo</i>
Control	9	9	1	1
1	2	2	1	1
2	2	2	1	1
3	1	2	1	1
4	1	2	1	1

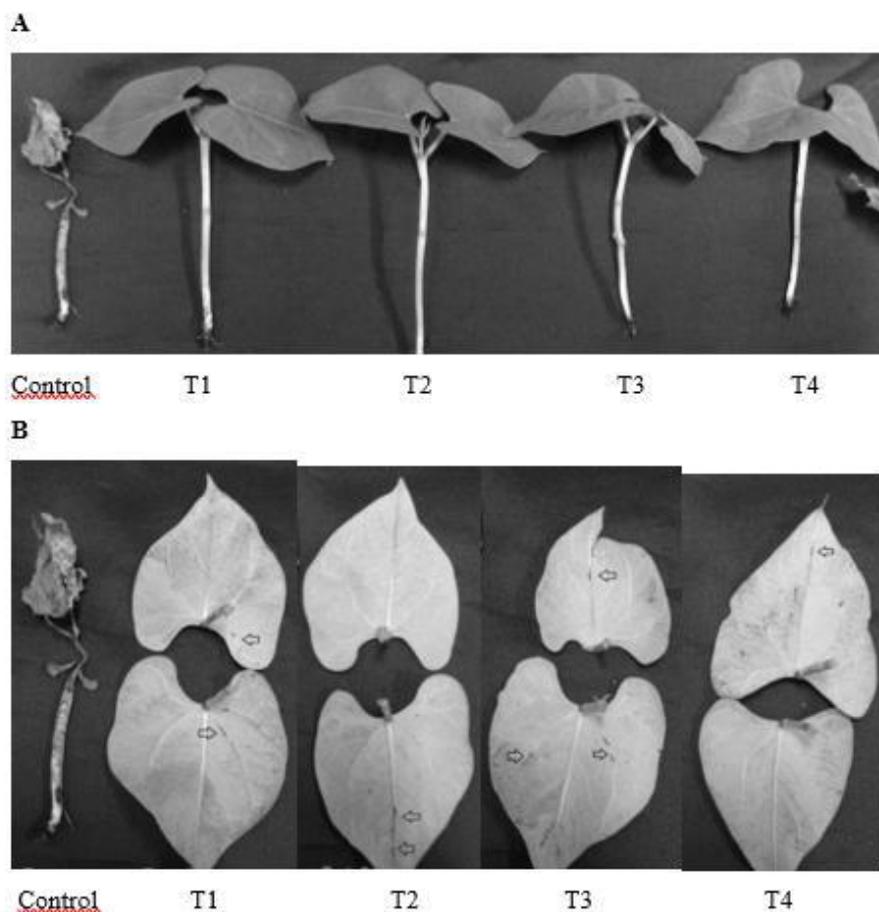
Source: Authors.

Figure 3. BRS *Campeiro* cultivar leaves (A) and its abaxial face (B) after evaluation of severity.



Treat 1 = preventive treatment with *R. officinalis* extract; Treat 2 = preventive treatment with *O. vulgare* extract; Treat 3 = curative treatment with *R. officinalis*; Treat 4 = curative treatment with *O. vulgare* extract. Source: Authors.

Figure 4. *Perola* cultivar leaves (A) and its abaxial face (B) after evaluation of severity. Treat 1 = preventive treatment with *R. officinalis* extract; Treat 2 = preventive treatment with *O. vulgare* extract; Treat 3 = curative treatment with *R. officinalis* extract; Treat 4 = curative treatment with *O. vulgare* extract.



Source: Authors.

The grades applied to the severity of anthracnose in *BRS Campeiro* cultivar, in the preventive (T1 and T2) and curative (T3 and T4) treatments with *R. officinalis* and *O. vulgare* phenolics-containing extracts were not different from each other (Table 3). However, the preventive mode resulted in a score of 2 (represents the presence of symptoms in less than 1% of the leaf area) and the curative mode, a score of 1 (no symptoms were detected). In the Pérola cultivar, there was no difference attributed to the treatments with both extracts (grade 2 was kept).

Brand et al. (2010) evaluated the anthracnose severity in bean plants sprayed with an aqueous extract of rosemary and inoculated with the fungus *C. lindemuthianum*. In the field, the extracts did not significantly reduce the severity of anthracnose. The mode of preventive treatment was used, and the pulverization of extracts occurring 48 hours before the inoculation. Andrade and Vieira (2016) demonstrated 21.6% of anthracnose reduction in papaya fruits caused by *Colletotrichum gloeosporioides* through *R. officinalis* essential oil. The fruits were inoculated with fungal mycelium and 96 hours after the measurements of the lesion diameter *in vivo* were done. Pinto et al. (2010) verified that *O. vulgare* methanolic extract caused a reduction of 37.65% in the severity of the disease caused by *C. lindemuthianum* when a local effect assay was performed. For this, the extract solutions were sprayed with a brush on the stem and leaves of both surfaces of bean plants. For the local effect test, 4 h after the treatment with the extract, a suspension of *C. lindemuthianum* at 1.2×10^6 conidia/mL was sprayed on the stem and on both leaf surfaces, followed by incubation for 48 h. Then, plants were transferred to a greenhouse, where the

symptoms of the disease were evaluated at 5 and 12 days. In the systemic effect assay, seven days after the treatment with the extract, the fungus inoculation was carried out and evaluated as described above. *O. vulgare* extract reduced the severity of the anthracnose to values 35% below the observed for the control. We emphasize that the extract used in our study is different from those used in the works cited above and contains at least 10 phenolic compounds that we identified by LC-HRMS

According to Pinto et al. (2010), to evaluate the common bean plant resistance and susceptibility to anthracnose, the extracts can be classified as adequate for the control of the disease, since they provided severities 35% below the value obtained for the control, which was observed in our study, since the assigned grade decreased from 9 to 2 or 1.

So, responding the hypothesis of this work, *R. officinalis* and *O. vulgare* phenolic-containing extracts demonstrate antifungal activity and the control of anthracnose caused by *Colletotrichum lindemuthianum* BRM 007447, representing perspectives as a natural fungicide that acts directly on the fungus that cause anthracnose.

4. Conclusion

Oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.) phenolics-containing extracts have potential to be useful in the practice of sustainable agriculture relative to *Colletotrichum lindemuthianum* BRM 007447, whose *in vitro* results were confirmed in an *in vivo* assay with the suppression of the anthracnose in the cultivars used, and were not phytotoxic to the common bean (*Phaseolus vulgaris* L.).

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