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Article

Antibacterial Activity of *Origanum vulgare* and *Rosmarinus officinalis* Standardized Extracts Against *Curtobacterium* and *Xanthomonas*

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ABSTRACT

Diseases caused by *Curtobacterium* and *Xantomonnas* species represent an agricultural problem in crops and can generate economic impacts on the commercialization of seeds and food. *Origanum vulgare* L. (oregano) and *Rosmarinus officinalis L.* (rosemary) have rosmarinic acid and others phenolics that can lead to the control of phytopathogenic bacteria in common bean (*Phaseolus vulgaris* L.). This study aimed to evaluate the *in vitro* and *in vivo* antibacterial activity of *O. vulgaris* and *R. officinalis* extracts, standardized in rosmarinic acid, against *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Xanthomonas axonopodis* pv. *phaseoli, Xanthomonas fuscans* subsp. *fuscans* and *Xanthomonas* sp. The antibacterial effect of the extracts in bean seed was also investigated. The content of rosmarinic acid was 8.55 % for *O. vulgare* and 16.30 % for *R. officinalis* extract. It was verified the complete *in vitro* inhibition of the bacteria studied by both extracts at 0.8% (w/w) with exception of *Xanthomonas axonopodis* pv. *phaseoli* BRM 025302 that was completely inhibited at 1.2% (w/w) of oregano. In addition, no symptom of phytotoxicity were noted in detached bean leaves treated with them. Under greenhouse conditions, some reduction on severity of *Curtobacterium* wilt by both extracts at 1% (w/w) was noted to bean cultivars BRS *Sublime* and BRS *Estilo.* Under the experimental conditions these extracts were not efficient to control the common bacterial blight caused by *X. axonopodis* pv. *phaseoli.* Both extracts are promising in the treatment of seeds, specially in related to contamination by *Fusarium* spp., whose percentage decreased on average an average from 94% to 10%. In addition, these bean seeds maintained the germination percentage adequate to that required by legislation. Further studies must be conducted to better investigate the potential of these standardized extracts as a bioproduct for agriculture.
Keywords: *Curtobacterium flaccumfaciens*; *Xanthomonas axonopodis*; *Xantomonas fuscans*; natural antibacterial; sustainable agriculture.

RESUMO

Doenças causadas por espécies de *Curtobacterium* e *Xantomonnas* representam um problema agrícola nas lavouras e podem gerar impactos econômicos na comercialização de sementes e alimentos. *Origanum vulgare* L. (oregano) e *Rosmarinus officinalis* L. (alecrim) possuem ácido rosmarínico e outros compostos fenólicos que podem levar ao controle de bactérias fitopatogênicas do feijoeiro (*Phaseolus vulgaris* L.). Este estudo teve como objetivo avaliar a atividade antibacteriana *in vitro* e *in vivo* dos extratos de *O. vulgaris* e *R. officinalis*, padronizados em ácido rosmarínico, frente a *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas fuscans* subsp. *fuscans* e *Xanthomonas* sp. O efeito antibacteriano dos extratos em sementes de feijão também foi investigado. O teor de ácido rosmarínico foi de 8,55 % para *O. vulgare* e 16,30 % para o extrato de *R. officinalis*. Verificou-se a completa inibição *in*

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vitro das bactérias estudadas por ambos os extratos à 0,8% (m/m), com exceção de *Xanthomonas axonopodis* pv. *phaseoli* BRM 025302 que foi completamente inibida à 1,2% (m/m) de orégano. Além disso, nenhum sintoma de fitotoxicidade foi observado em folhas de feijão destacadas tratadas com eles. Em condições de casa de vegetação, observou-se uma certa redução na severidade da murcha de *Curtobacterium* por ambos os extratos à 1% (m/m) para os cultivares de feijão BRS *Sublime* and BRS *Estilo*. Nas condições experimentais esses extratos não foram eficientes no controle do crestamento bacteriano comum causado por *X. axonopodis* pv. *phaseoli*. Ambos os extratos foram promissores no tratamento de sementes, principalmente em relação à contaminação por *Fusarium* spp., cujo percentual diminuiu, em média, de 94% para 10%. Além disso, essas sementes de feijão mantiveram a porcentagem de germinação adequada ao exigido pela legislação. Novos estudos devem ser realizados para melhor investigar o potencial desses extratos padronizados como bioproduto para a agricultura.

Palavras-chave: *Curtobacterium flaccumfaciens*; *Xanthomonas axonopodis*; *Xanthomonas axonopodis*; antibacteriano natural; agricultura sustentável.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most cultivated bean species of the *Phaseolus* genus, but can be affected by several diseases that culminate in large losses to the producer, among them *Curtobacterium* wilt and Common Bacterial Blight caused by *Xanthomonas* species (Wendland & Lobo Júnior 2018).

The bacterioses caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye and *Xanthomonas fuscans* subsp. *fuscans* has brought concern. *C. flaccumfaciens* pv. *flaccumfaciens* colonize the xylem vessels and provoke obstruction of the vessels, causing wilting, flaccidity, yellowing or burning of the leaves, vascular darkening, dwarfism and death of the bean plant, reducing its production (Valdo et al. 2016; Paiva et al. 2020). Infections by *Xanthomonas* spp. cause a wide variety of non-specific symptoms, which are water-soaked spots progressing to leaf necrosis, wilting, rot, hypertrophy, hyperplasia, rust, death and cankers (Catara et al. 2021).

We highlight that there is an abusive and uncontrolled use of agricultural pesticides in Brazil, which can lead to the emergence of socio-environmental and public health problems, despite their proven efficiency (Cassal et al. 2014). In this context, the incorporation of several productive technologies has been explored in order to reduce the use of chemicals in agriculture to control the severity of disease-causing agents and their negative impacts on the environment through bioproducts (Varjani et al. 2020).

A viable alternative practice that can be adopted for this control is the use of plant extracts (Sales et al. 2016; Shabana et al. 2017; Simas et al. 2017; Tocci et al. 2018; Carvalho et al. 2021, Itako et al. 2021). These extracts have a phytocomplex that consist of a mixture of several secondary metabolites that exerts a synergistic effect and so it may have a broad antimicrobial spectrum, in addition to reducing the risk of resistance of the pathogen (Yazdani et al. 2011). Another important strategy that seeks to favor the effectiveness of a target biological activity of plant extracts is their chemical standardization and, therefore, minimizes the variability in their composition. For this, a chemical marker is used to monitor this process (Rongai et al. 2019).

Among the plant with potential for agricultural purposes are *Origanum vulgare* L. (oregano) and *Rosmarinus officinalis* L. (rosemary), which have showed to be interesting tools to phytophatogens control (Babu et al. 2007; Vigo et al. 2009; Altundag et al. 2011; Djordjevic et al. 2013; Maia et al. 2014; Romero et al. 2015). Both plants have rosmarinic acid, a phenolic compound found in some species of *Lamiaceae* family (Hossain et al. 2010) and that have antimicrobial activity (Kostic et al. 2015; Alagawany et al. 2017).

The aims of this study was to evaluate the *in vitro* and *in vivo* potential of *O. vulgaris* and *R. officinalis* extracts, standardized in rosmarinic acid, in the control of phytopathogenic bacteria (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Xanthomonas axonopodis* pv. *phaseoli, Xanthomonas fuscans* subsp. *fuscans* and *Xanthomonas* sp) and in the control of diseases, such as the Bacterial Wilt caused by *Curtobacterium* and the Common Bacterial Blight

caused by species of *Xanthomonas*. We also evaluate its phytotoxicity in detached bean leaves and the effect of both standardized extracts on bean seeds.

2.Material and Methods

2.1. Obtaining O. vulgaris and R. officinalis standardized extracts

Oregano leaves were purchased from the *Paladar* company (lot: 219 9:04), from Goiânia, Goiás, Brazil. Rosemary 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.

The leaves were ground in a blender (Poli Metalúrgica Siemsem Ltda., Brazil). Briefly, the extracts were obtained through 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), under reduced pressure (30 rpm, 40ºC, 600 bar) until the solids content reached about 75.49% (w/w) for oregano and 52.19% (w/w) for rosemary extracts.

Both extracts were standardized in rosmarinic acid by liquid chromatography of high resolution (HPLC) according Canelas & Costa (2007). It was used a Waters® HPLC chromatograph (Massachusetts, USA) equipped with a photodiode array detector (PDA 2998) and data processing system Enpower2.0. Chromatographic separations were conducted using the Zorbax® Eclipse XDB-C18 column of Agilent® (150 mm x 4.6 mm, 5 μm) at a wavelength of 329 nm, elution mode isocratic, with the mobile phase consisting of a combination of 25 % acetronitrile (J.T. Baker®, Mexico) (v/v) (solvent A) and of 75% (v/v) of ultrapure water (H_2O Milli-Q®): acetonitrile: phosphoric acid (H_3PO_4) within a proportion 94:5:1 (solvent B) (v/v). The mobile phase flow rate was 0.5 mL min^{-1}, injection volume 10 μL and the column held at 25° C. The external standard of rosmarinic acid (purity ≥ 98 % Sigma-Aldrich®).

2.2 In vitro inhibitory activity of O. vulgaris and R. officinalis standardized extracts

The bacteria were provided by Official Collection of Multifunctional and Phytopathogenic Microorganisms of Embrapa Arroz e Feijão (Table 1).

Table 1. Pathogenic bacteria of common bean tested against *O. vulgaris* and *R. officinalis* standardized in rosmarinic acid extracts.

Curtobacterium flaccumfaciens pv. *flaccumfaciens*	BRM 014933
Xanthomonas axonopodis pv. *phaseoli*	BRM 025302
Xanthomonas fuscans subsp. *fuscans*	BRM 025304
Xanthomonas sp	BRM 025351

In vitro inhibitory activity of oregano and rosemary extracts on bacterial growth was conducted in 100 mm diameter Petri dishes containing Sucrose, Casein, Potassium phosphate, Magnesium sulfate, Yeast extract (523 medium) agar for *X. axonopodis* and Nutrient Sucrose agar for *C. flaccumfaciens*. The extracts were added individually at concentrations of 0.6 % (w/w), 0.8 % (w/w), 1.0 % (w/w) and 1.2% (w/w). 100 μl aliquots of the bacterial suspensions were used to inoculate approximately 10^4 CFU/mL of the culture medium. The plates were incubated at 25 °C for 2 days. The bacterial growth inhibitory activity was monitored by counting CFU with a magnifying glass. The control consisted only of inoculation of the bacterial suspensions in the culture

medium (Côrtes et al. 2012). The experiments were performed in triplicate of each treatment and control with two replicates.

2.3. In vitro phytotoxicity of O. vulgaris and R. officinalis standardized extracts on bean leaves

Detached leaves from IPA 7419 cultivar beans with approximately 14 days of germination were placed in humidified camera at 22 ° C under artificial light (12 h light - 12 h dark). A volume of 100 µL of both extracts at 1.2 % (w/w) were distributed, individually, in the leaf area. Afterwards, they were incubated at 22 ± 1ºC for 24 and 48 h. The evaluation of phytotoxicity was carried out considering features such as chlorosis, necrosis and injury (Varejão et al. 2013). The experiments were performed in triplicate. Witnesses containing sterile distilled water were used.

2.4. In vivo evaluation of the effect of O. vulgaris and R. officinalis standardized extracts on bean seeds

50 bean seeds of *Perola* cultivar were treated individually with 1 mL of both extracts at concentration of 1.2% (w/w). Subsequently, it was left for 15 minutes, under sporadic agitation. Controls were used, one was treated with water and the other in which the seeds did not have any type of treatment. Then the seeds were submitted to a gerbox (blotter-test**)** and paper roll tests (BRASIL, 2009) in order to see if the treatment had an effect on germination and vigor.

In the blotter test, the boxes were kept in a chamber with white fluorescent light with photoperiod of 12 h light - 12 h dark, for seven days at a temperature of 20 ± 2 ºC. Then, the macroscopic analyzes were performed in a magnifying glass and optical microscope. In paper roll, the sets were placed in germinating chambers at a temperature of 25 ± 2 ° C. The readings were performed on the 4th (germination %) and 9th (vigor %) test days. The seeds were grouped in germinated, abnormal, hard and dead. The experiments were performed in quadruplicate of each treatment.

2.5. Reduction of the severity of common bacterial blight by O. vulgaris and R. officinalis standardized extracts

Seeds of susceptible, moderately susceptible and resistant bean cultivars (BRS *Estilo*, BRS *Sublime* and BRS *FP 403*, respectively) were planted in sterile plastic cups, filled with prepared substrate (Maxfertil ® 013). Three seeds of each cultivar were planted in 5 cups, for each treatment and for the control.

Ten days after sowing, the seedlings were sprayed with 150 mL of oregano and rosemary standardized extracts, individually, at the concentration of 1.2% (w/w), to both sides of the leaves and to the stem of the seedlings until dripping The treatments performed were:

- T1 (rosemary) and T3 (oregano) in a preventive mode, with extract applications one day previously to the inoculation of the suspension of *X. axonopodis* pv. *phaseoli*, and
- T2 (rosemary) and T4 (oregano) in a curative mode, with extract applications 2 hours and 1 day after inoculation.

The inoculum was prepared by seeding the colonies of *X. axonopodis* pv. *phaseoli* (BRM 025302) in 523 culture medium and incubating at 25 ± 1 °C for 48 hours. On the day of inoculation, bacterial suspension was obtained and concentration was adjusted to 10^8 CFU/mL.

In the inoculation of the plants, the incision of the primary leaves method was used, which consists in making two cuts with the aid of a scissors previously dipped in the bacterial suspension. These cuts were perpendicular to the central vein, without reaching it, in a two centimeters distance from each other. Then, the

plants were transferred to a greenhouse with a temperature of 26 ± 1 °C and high relative humidity for ten days. Treatments were done in quadruplicate. The severity of the common bacterial blight was evaluated by observing the apparent symptoms and a diagrammatic scale with grades from 1 to 6 in which: 1 - no symptoms and 6 - advanced symptoms throughout the inoculated leaf (Rava 1984).

2.6. Reduction of the severity of Curtobacterium wilt by O. vulgaris and R. officinalis standardized extracts

Sowing was performed as previously mentioned. The inoculum preparation consisted in the multiplication of *C. flaccumfaciens* pv. *flaccumfaciens* BRM 014933 in Petri dishes containing Nutrient Sucrose agar culture medium and incubation in BOD stove at 25 ± 1 °C for 48 hours. On the day of inoculation, the suspension of *Curtobacterium* was obtained and was adjusted to 10^8 CFU/mL.

The treatments of bean plants with oregano and rosemary standardized extracts at a concentration of 1% (w/w) were carried in a preventively (treatments 1 and 3 - rosemary), with applications of 150 mL of both extracts one day before inoculation and in a curative mode (treatments 2 and 4 - oregano), with applications 2 hours and 1 day after inoculation. Control containing sterile distilled water was used.

The inoculation was performed by two perforations in the stem below the primary leaves, with a distance of 2 cm, made by a needle containing *C. flaccumfaciens* pv. *flaccumfaciens*. After inoculation of the bacterial suspension and the extracts spraying, to both sides of the leaves, the plants were transferred to a greenhouse with a temperature of 26 ± 1 °C and high relative humidity.

The severity of the *Curtobacterium* wilt was evaluated 15 days after the inoculation, by observation of the apparent symptoms scored from 1 to 9, according to the severity of the disease in which grade 1 is related to the absence of symptoms and grade 9 is related to death of plants (Wendland et al. 2009).

3. Results and Discussion

3.1.Obtaining O. vulgare and R. officinalis standardized extracts

After quantification of rosmarinic acid, its content in the oregano and rosemary extracts were 8.55 % and 16.30 %. The rosmarinic acid standard had a retention time of 6.55 min and its peak in both extracts were 6.53 min.

The chemical standardization of extracts is important to minimize the variability in the chemical composition, since plants are affected by the interaction with the environment, therefore, the concentration of secondary metabolites produced can vary (Rongai et al. 2019). We chose rosmarinic acid as marker because it is a hydroxycinnamic acid derivative well known in *Lamiaceae* species such rosemary and oregano (Hossain et al. 2010) and because it has polyphenols with antimicrobial activity (Kostic et al. 2015; Alagawany et al. 2017).

3.2. In vitro inhibitory activity of O. vulgaris and R. officinalis standardized extracts

It was observed that the bacteria studied were sensible to these extracts which completely inhibited its growth at concentration between 0.8% and 1% for rosemary and between 1.0 and 1.2% (w/w) for oregano (Table 2). *Xanthomonas axonopodis* pv. *phaseoli* BRM 025302 was the most resistant (oregano) and *Xanthomonas fuscans* subsp. *fuscans* BRM 025304 was the most sensible (to rosemary)

The *in vitro* antibacterial activity showed by oregano and rosemary standardized extracts against these phytopathogens may be due to the rosmarinic acid content and other phenolics present in these plants. This is suggested, since the oregano extract had practically half of rosmarinic acid and inhibited the bacteria studied at

a concentration close to the rosemary extract. In addition, according to Hossain et al. (2010), rosemary and oregano have several polyphenols distributed in four major categories; hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, flavonoids and phenolic terpenes. Based on the synergism that commonly occurs between the compounds present in the complex matrices of natural products, enhancing a biological effect (Khan 2018).

Table 2: Growth of *Xanthomonas axonopodis* pv. *phaseoli*, *Xanthomonas fuscans* subsp. *fuscans*, *Xanthomonas* sp. and *Curtobacterium flaccumfaciens* pv. flaccumfaciens in the presence of *O. vulgare* and R. officinalis standardized extracts.

	Rosemary extract % (w/w)				**Oregano extract % (w/w)**			
Bacteria	0.6	0.8	1.0	1.2	0.6	0.8	1.0	1.2
Xanthomonas axonopodis pv. *phaseoli* BRM 025302	+	+	-	-	+	+	+	-
Xanthomonas fuscans subsp. *fuscans* BRM 025304	+	-	-	-	+	+	-	-
Xanthomonas sp BRM 025351	+	+	-	-	+	+	-	-
Curtobacterium flaccumfaciens pv. *flaccumfaciens* BRM 014933	+	+	-	-	+	+	-	-

+ = bacterial growth; - = no bacterial growth

An important feature of some of phenolics is their lipophilic character, which may be capable of promoting damage to the integrity of the cell membrane structure and interfering with cell function (Romero et al. 2015).

Others studies involving these plants and bacteria studied by us are reported, although they did not use standardized extracts. Babu et al. (2007) observed that *O. vulgare* extract and the fraction containing phenolics led to a significant *in vitro* antibacterial activity against *Xanthomonas pathovars* viz. Vigo et al. (2009) showed an *in vitro* inhibition of the growth of *X. axonopodis* pv. *phaseolis* by the essential oil of *R. officinalis* at 1% concentration. Altundag et al. (2011) demonstrated an *in vitro* antimicrobial activity of *Origanum minutiflorum* essential oil against *C. flaccumfaciens* pv. *flaccumfaciens*, with the minimum inhibitory concentration (MIC) values in the rage of 125-400 μg/mL. Dal'maso (2014) found a low *in vitro* inhibition of *X. axonopodis* pv. *phaseolis* by the hydroalcoholic extract of rosemary.

3.3. Phytotoxicity of O. vulgaris and R. officinalis standardized extracts in detached bean leaves

Detached bean leaves after spraying both extracts at concentration of 1.2% (w/w) did not show phytotoxicity characteristics, such as chlorosis, necrosis and injury.

We did not find other studies with *O. vulgaris* and rosemary related to their phytotoxicity. Sertkaya et al. (2010) evaluated the acaricidal activity of the essential oil of *Origanum onites* L. in bean and other plants and verified the absence of phytotoxic symptoms at the different concentrations tested.

3.4. In vivo evaluation of the effect of O. vulgaris and R. officinalis standardized extracts on bean seeds

After treating the seeds with both the standardized extracts and water, blotter and paper roll tests were performed to check if there was a reduction in the incidence of microorganisms (table 3) and interference in the germination process and vigor of the infested / infected seeds.

Table 3. Blotter-test of bean seeds of cultivar *Perola* treated with *O. vulgare* and *R. officinalis* standardized extracts at 1.2% (w/w).

Detected pathogens	Treatments			
	Control (%)	Seeds treated with water (%)	Seeds treated with *O. vulgare* (%)	Seeds treated with *R. officinalis* (%)
Aspergillus sp.	0	0	9	6
Cladosporium sp.	98	97	94	96
Fusarium spp.	92	95	9	11
Penicillium sp.	0	0	1	3
Rhizopus sp.	0	1	3	4
Bacteria	1	1	0	1

It was verified the presence of *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. that are characterized as storage fungi, *Cladosporium* sp. that is considered as surface contaminant. *Cladosporium* sp. and *Fusarium* spp. were the microorganisms that most developed in the tested seed cultivars.

Comparing the results of seeds without treatment and those treated with water in relation to seeds treated with both extracts (Table 3), it was observed that the percentage of seeds contaminated with *Fusarium* spp. decreased substantially, dropping from an average percentage of 94% to 10%. *Rhizopus* sp., *Penicillium* sp. and *Aspergillus* sp. showed a slight increase in incidence in the seeds treated with the extracts, but in comparison with the seeds that did not have any type of treatment, there was no variation.

After analysis of the paper roll test, it was observed that the treatment of seeds, with extract or water, there was a decrease in germination and vigor (table 4). This demonstrated that the treatment with both extracts in the concentration of 1.25% interfere in the development of the seeds. However, the seeds showed germination percentage higher than required by BRASIL (2013), which is 70%, then they are considered as seeds of good quality.

The germinative power is the percentage of germinated seeds, that is, it verifies its viability. The vigor indicates the plant's ability to resist environmental stresses and its ability to maintain viability during storage. It can be inferred that contamination by microorganisms did not influence the quality of the tested seeds, thus being released for planting. Probably, these fungi were superficially contaminating the seeds, because by visual examination the seeds had no mechanical damage and by the results of physiological tests, they were of high quality, even after a storage.

It was observed in the germination and vigor tests (table 4) that the bean seeds treated with the extracts did not lose their viability. Thus, it is suggested that the extracts of rosemary and oregano are promising in the treatment of these seeds, especially in reducing the incidence of the pathogen *Fusarium* spp.

Table 4. Germination and vigor percentage of bean seeds of cultivar *Perola* treated with *R. officinalis* and *O. vulgare* standardized extracts at 1.2%.

	Germination (%)	**Vigor (%)**
Control	87 %	72 %
Seeds treated with water	75 %	57 %
Seeds treated with *O. vulgare* extract	75 %	66 %
Seeds treated with *R. officinalis* extract	76 %	69 %

3.5. Reduction of the severity of the common bacterial blight under controlled conditions in a greenhouse

Following the study, we performed tests to investigate the *in vivo* performance of the extracts in the reduction the severity of common bacterial blight. We tested only *Xanthomonas axonopodis* pv. *phaseoli* BRM 025302, selected according to the *in vitro* tests and in which it was the one that required the highest concentration (1.2% (w/w) of the oregano extract) to be completely inhibited.

Both extracts of oregano and rosemary at the concentration of 1.2% (w/w) were not efficient in reducing the common bacterial blight when compared to the control sprayed with water on all cultivars. The control and almost all treatments had the maximum score at the end of the trial (Table 5).

The characteristic symptons of common bacterial blight were evaluated by using a numbered grade scale of 1 up to 6 degrees of severity, in which grade 1 is related no symptoms in the leaves and grade 6 is related to advanced symptoms throughout the inoculated leaf. Grade 4 - lesion in the border area between the cuts in the leaf; grade 5 - injury in the area beyond the leaf cut (Rava 1984).

Table 5. Effect of *O. vulgare* and *R. officinalis* standardized extracts at 1.2% (w/w) on the severity of common bacterial blight caused by *X. axonopodis pv. phaseoli* in bean cultivars (Grades given after treatments)

	Cultivars severity		
	BRS *Sublime*	**BRS FP 403**	**BRS *Estilo***
Control	6	6	6
T 1	6	6	6
T 2	6	5	6
T 3	6	4	6
T 4	6	6	6

T1 = preventive treatment: *R. officinalis* extract; T2 = preventive treatment: *O. vulgare.* extract; T3 = curative treatment: *R. officinalis* extract; T4 = curative treatment: *O. vulgare* extract.

The common bacterial spot caused by *X. axonopodis* pv. *phaseoli* and *X. fuscans* subsp. *fuscans* can generate losses of 10 to 100% of the bean crops (Wendland & Lobo Jr. 2018; Paiva et al. 2020). So control measures become important.

Vigo et al. (2009) evaluated the potential of extracts and essential oils from some plants on *in vivo* suppression of common bacterial blight caused by *X. axonopodis* pv. *phaseolis* in pod bean *Bragança* cultivar. Among these plants, the essential oil from *R. officinalis* at 0.5% concentration, did not reduce the severity of the disease in relation to the control, but they had an effect on the severity.

Dal'maso (2014) evaluated the control of common bacterial blight caused by *X. axonopodis* pv. *phaseolis* in a greenhouse, against the hydroalcoholic extract of rosemary (150 $mL.L^{-1}$) and verified that the extract led to a some protection against the control of blight in common bean (IAPAR-81) with influence on bean physiology, in the treatment applied three days before the inoculation of the pathogen.

3.6. Reduction of the severity of Curtobacterium wilt under greenhouse controlled conditions

Although the *in vitro* assay reached a concentration of 0.8% (w/w) of the extracts for the complete inhibition of *C. flaccumfaciens* pv. *flaccumfaciens*, we tested in *in vivo* at a concentration of 1% (w/w), and depending on the results, we would test lower concentrations.

Both extracts of *O. vulgare* and *R. officinalis* at 1% (w/w) of concentration demonstrated the ability to reduce the severity of *Curtobacterium* wilt, when compared to the control, for cultivar BRS *Sublime* at about 50% (grade 8 compared to 4 -Table 6) to 75% (grade 8 compared to 6 - Table 6) and for cultivar BRS *Estilo* at about 40% (grade 5 compared to 2 -Table 6) to 60% (grade 5 compared to 3 - Table 6). BRS FP 403 is resistant to this disease, which was confirmed in our study (grade 1, corresponding to no symptoms of the *Curtobacterium* wilt) (Table 6).

The characteristic symptons of *Curtobacterium* wilt were evaluated by using a numbered grade scale of 1 up to 9 degrees of severity, in which grade 1 is related to the absence of symptoms and grade 9 is related to death of plants. Grade 6 for plants dwarfism; grade 5 in sagging or mosaic in the leaves associated with edge burn or curl; grade 4 in sagging and mosaic on the leaves; grade 3 in sagging in the leaves only; grade 2 in mosaic in the leaves (Wendland et al. 2009).

Table 6. Effect of *O. vulgare* and *R. officinalis* standardized extracts at 1% (w/w) on the severity of *Curtobacterium* wilt in bean cultivars (Grades given after treatments)

	Cultivars severity		
	BRS *Sublime*	**BRS FP 403**	**BRS *Estilo***
Control	8	1	5
T 1	5	1	2
T 2	4	1	3
T 3	6	1	3
T 4	5	1	3

T1 = preventive treatment: *R. officinalis* extract; T2 = preventive treatment: *O. vulgare.* extract; T3 = curative treatment: *R. officinalis* extract; T4 = curative treatment: *O. vulgare* extract.

It is noted that both preventive (T1 and T2) and curative (T3 and T4) treatments were inferior to the control for cultivars BRS *Sublime* and BRS *Estilo* (Table 6), but comparing the ways of treatment (preventive *x* curative) it was found that there was no difference between them, leading us to infer that the extracts have a direct action on *Curtobacterium*. Oregano and rosemary extracts were similar in the action against this bacterium.

According to Valentini et al. (2010), *Curtobacterium* wilt caused by *C. flaccumfaciens* pv. *flaccumfaciens* has become a threat to the cultivation of common bean. The authors emphasize that this pathogen requires measures to prevent its spread to other locations, since it is recent in Brazil and does not have an established treatment.

Our data allow us to suggest that oregano and rosemary standardized in rosmarinic acid extracts have potential as an organic antibacterial agent against *Curtobacterium* wilt in common bean cultivars BRS *Sublime* and BRS *Estilo* caused by *C. flaccumfaciens* pv. *flaccumfaciens*. In the researched literature, there are no *in vivo* studies for the alternative control of *Curtobacterium* wilt in common bean using these plant extracts.

Despite the need for additional investigations under different experimental conditions for all bacteria studied, the data obtained allow us to suggest that bioproducts based on plant extracts represent a promising alternative in the control of phytopathogens and diseases in plants. In addition, they are a way to provide environmental sustainability and minimize impacts on the environment and human and animal health.

4. Conclusion

The present study provides evidence for the *in vitro* antibacterial potential of *O. vulgare* and *R. officinalis* standardized in rosmarinic acid extracts against *Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Xanthomonas axonopodis* pv. *phaseoli, Xanthomonas fuscans* subsp. *fuscans* e *Xanthomonas* sp. Some reduction on severity of *Curtobacterium* wilt by both extracts was noted, although for the common bacterial blight caused by *X. axonopodis* pv. *phaseoli* the extracts were not efficient, in the experimental conditions used. Both extracts were not phytotoxical to the common bean (*Phaseolus vulgaris* L.) and were promising in the treatment of bean seeds.

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