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Evaluation of *in vitro* inhibition of mycelial growth of *Fusarium solani* f. sp. *piperis* by different products in Brazil

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The black pepper (*Piper nigrum* L.) is one of the most popular peppers in the world. Nonetheless, there are several limitations on cultivation, resulting in reduced production or a complete loss of the crop. The major disease affecting this crop is fusarium root rot caused by *Fusarium solani* f. sp. *piperis*, which is responsible for decimating whole crops in Brazil, with losses reaching millions of dollars per year. So far, there is no effective control measure against this fungus and no cultivars resistant to it. In this study, *in vitro* effects of different products on colony growth was evaluated. Carbendazim, chitosan, silicon, and phosphate were tested against *F.solani* f. sp. *piperis* isolates CML-2466, CML-2353, E-637, and E-596. Chitosan and silicon did not inhibit mycelial growth of any of the isolates, while phosphite inhibited mycelial growth by 100%. Carbendazim was found to be fungitoxic for isolates CML-2353 and E-596 and fungistatic for CML-2466 and E-637, inhibiting the mycelial growth of these isolates by 60 and 80%, respectively. There were no dose effects of the products tested.

Key words: *Fusarium solani*, Black pepper, chitosan, silicon, phosphite, carbendazim

INTRODUCTION

Among the major factors limiting agricultural production are infections with fungi, bacteria, viruses, and nematodes, insects, mites, and weeds (Kreyci and Menten, 2013). It is estimated that, agricultural losses due to pest attacks reach US $1.4 trillion, or almost 5% of global gross domestic product (GDP) worldwide. According to Nojosa et al. (2015), the losses for the Brazilian agribusiness can be as high as $55 billion annually due to diseases of crops, which is equivalent to the average annual loss of 7.7% or 25 million tons of agricultural produce. According to the Food and Agriculture Organization(FAO) of the United Nations (UN)

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pathogens are responsible for 13.3% of such damages.

The black pepper (*Piper nigrum* L.), which accounts for ~57,800 tons of national agricultural produce (IBGE, 2016), has been affected by root rot, also known as fusariosis (Kimati et al., 1997). This severe disease can cause a lot of damage to the crop, with an annual reduction of 3% in the cultivated area and production. A healthy pepper crop has a productive cycle potential of 12 years on average. This disease reduces the cycle by 5 to 6 years (Tremacoldi, 2014). The causal agent is *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans.f. sp. *piperis*, Albuquerque (Teleomorphs *Nectria haematococca* Berk. & Br. f. sp. *piperis* Albuq.). The *Fusarium* species are widely distributed in the soil, and in an adverse environment, form a resistant structure called *chlamydospore*, which can remain viable for more than 20 years (Pfenning and Lima, 2007). After infection, the fungi settlesdown in the vascular system of the plant, hindering the absorption of water and nutrients (Bedendo, 1995). Once inside the root system, the fungi are initially limited to the root or the plant base and, at some point, begin to spread to the vascular system. The damage is due to colonization of xylem vessels by hyphae and microconidia; hypertrophy and hyperplasia of the cambium, xylem, and phloem; destruction of xylem fibers and amyloplasts in parenchymatous cells; and production of gels by the plant (Ortiz et al., 2014). Eventually, the combination of the fungal growth in the vascular system, fungal toxins such as naphthoquinones and fusaric acid (Rocha et al., 2016), and defense structures produced by the plant hamper the absorption and transport of water, causing wilt and death of the plant (Wheeler and Rush, 2001).

Strategies for *F. solani* sp. *piperis* control are limited because there is still no information on resistant cultivars and an effective fungicide does not exist (or is not officially approved in Brazil). *In vitro*, studies are needed to identify the products with the possible ability to control the fungus. To this end, various compounds are being tested. Benzimidazole fungicides are used extensively in agriculture due to strong systemic activity against a great number of fungal species (Reis et al., 2001). Carbendazim, a systemic fungicide with a benzimidazole chemical group, exerts both preventive and curative action (Kus and Altanlar, 2003). Among the products with great potential antifungal stand out chitosan, silicon and phosphite. Such products have not been tested effectively in black pepper as an alternative method of controlling fusarium or were used on a small scale without scientific evidence. Thus, various control methods are used to minimize the severity of the disease. Chitosan, a high-molecular-weight polysaccharide, has many physicochemical and biological properties (El-Ghaouth et al., 1994), e.g., antimicrobial activity against some fungi (yeasts) and bacteria (Allan and Hadwiger, 1979; Roller and Covill, 1999). Among mineral nutrients used in pest management, silicon (Si) stands out as an element that reduces severity of major diseases in several crops (Epstein, 1999). Silicon can act on the constitution of the physical barrier to prevent penetration of fungi and affects the signals between the host and the pathogen, resulting in more rapid and extensive activation of pre-and post-formed defense mechanisms in the plant (Chérif et al., 1994; Epstein, 1999), e.g., by increasing the synthesis of phenolic compounds and peroxidase, polyphenoloxidase, chitinase, and β-glucosidase (Fauteux et al., 2005). Phosphites are characterized by their effectiveness in controlling downy mildew and various diseases caused by genus *Phytophthora* (Ouimette and Coffey, 1989), exert acropetal and basipetal systemic action and suppress foliar and root diseases (Guest and Grant, 1991). Furthermore, they have high stability in plants and may remain active for substantial periods (Smillie et al., 1989). Regarding the mechanism of action of phosphites, some authors discuss direct action on the pathogen (Fenn and Coffey, 1984; Fenn and Coffey, 1985; McGrath, 2004). Others suggest that the mechanism is indirect, via activation of plant defense mechanisms (Nemestothy and Guest, 1990; Saindrenan et al., 1990) or a combination of direct and indirect effects (Smillie et al., 1989; Jackson et al., 2000).

The objective of the present study was to evaluate the effects of alternative antifungal agents such as chitosan, silicon, and phosphite as well as the known fungicide carbendazim on mycelial growth of *F. solani* f. sp. *piperis in vitro*.

MATERIALS AND METHODS

F. solani f. sp. *piperis* isolates

The isolates that we tested were CML-2466 and CML-2353 (Coleção Micológica de Lavras, Universidade Federal de Lavras - Minas Gerais State) and E-637 and E-596 (Incaper– Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural - Espírito Santo State). The isolates were maintained in Petri dishes containing potato dextrose agar (PDA) in refrigerator at 4°C. Every month, an agar disk (5mm) from a pure culture of *F. solan i* was placed in the center of a PDA plate containing the same medium. The plates were incubated at 25°C in biochemical oxygen demand (B.O.D.), with the photoperiod of 12 h.

Preparation of chitosan, silicon, phosphite, and carbendazim concentrations

Chitosan was added to the PDA medium at concentrations of 0.5, 1.0, 1.5, 2.0%, 2.5, and 3.0%. Chitosan (Fagron®) was extracted in acetic acid and diluted in water to a concentration of 2% at pH 4.4. This substance has high viscosity and was diluted with sterilized distilled water to obtain the desired concentrations. The other products added to the culture medium at the following concentrations were: silicon (SiO_2) at 0.25, 0.50, 1.0, 1.5, 2.0, or 3.0 g L^{-1}; phosphite (Phosethyl Al) at 1.0, 2.0, 3.0, 4.0, 5.0, or 6.0 g L^{-1}; and carbendazim (Carbomax 500®) at 0.83, 1.67, 2.50, 3.34, 4.16, or 5.0 ml L^{-1}. As controls, we used Petri dishes containing PDA medium supplemented with 2% of acetic acid for the treatment with

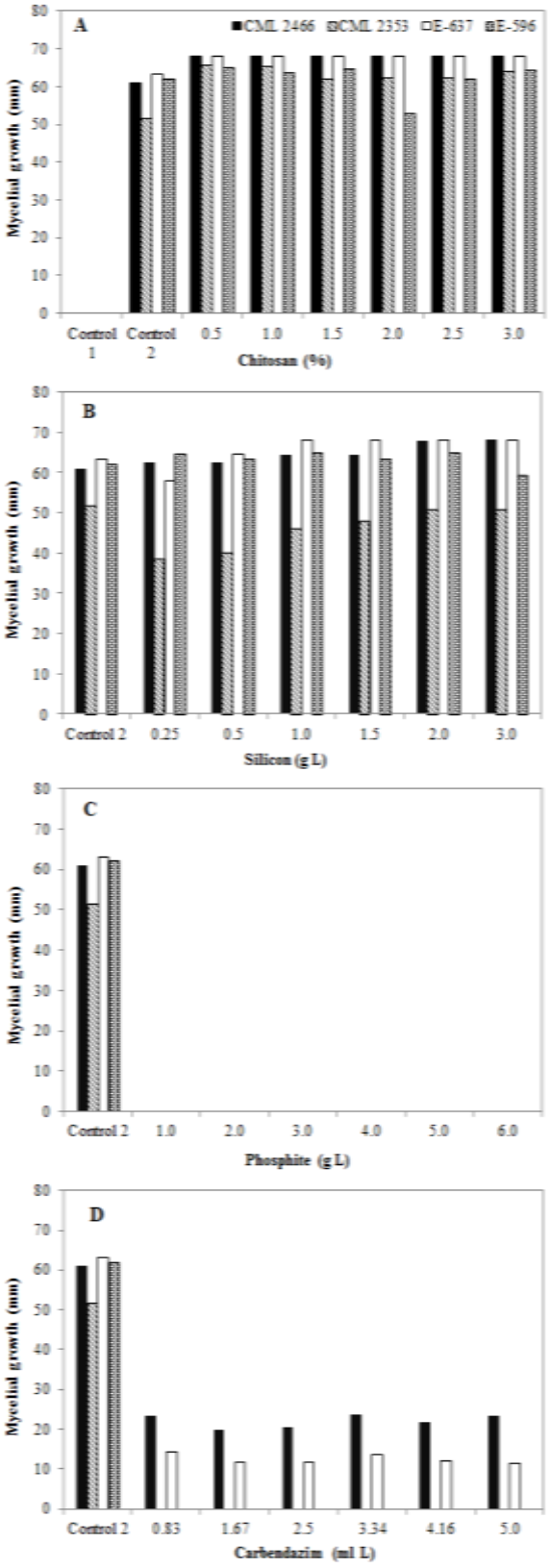

Figure 1. Effect of chitosan (A), silicon (B), phosphite (C) and carbendazim (D) on the mycelial growth of *F. solani* isolates. Control 1: PDA medium supplemented with 2% of acetic acid; Control 2: only PDA medium.

chitosan (Control 1) and/or only the PDA medium (Control 2). After solidification, a fungal mycelial disc 5mm in diameter, 15 days old, was transferred to the center of each Petri dish (68-mm diameter). This procedure was performed for each *F.solani* isolate. The plates were sealed with parafilm and maintained in B.O.D at 25°C, with a photoperiod of 12 h.

Effect of different products on mycelial growth of *F. solani* isolates

The mycelial growth of *F. solani* isolates was assessed daily by measuring the diameter of the colonies in orthogonal directions by means of a pachymeter, until the colonies in control treatments reached the edge of the board. The percent growth inhibition was calculated according to Guo et al. (2006), using the following formula: antifungal index (%) = (1 – Da/Db) × 100, where Da was the diameter of the zone of growth in the test plates, and Db was the diameter of growth zone in the control plate.

Statistical analysis

The experiment was performed using randomized block design (RBD) with 28 treatments and five repetitions for each isolate of *F. solani*. Each repetition consisted of a Petri dish. Each experiment was repeated three times. The significance of treatment effects on radial growth among isolates was tested with analysis of variance (ANOVA). Where significant F values were obtained, Tukey's all pairwise comparison test, which includes a correction for multiple comparisons, was used to assess the significance of differences between meansin the statistical software ASSISTAT 7.1 beta (Silva and Azevedo, 2009).

RESULTS

The mycelial growth of *F. solani* was not inhibited by any chitosan concentrations tested except for the test plate PDA with added acetic acid. The presence of chitosan favored the growth of the four fungal isolates: CML-2466 and E-637 reaching the edge of the plate after 9 days of incubation, and CML-2353 and E-596 showed maximal growth after 11 days (Figure 1A). The fungi were also seeded on agar-agar with added chitosan (same concentrations) or agar-agar only and all reached the edge of the plate after 9 days of growth (data not shown). Silicon, at the concentrations tested, did not inhibit fungal growth (Figure 1B). For CML-2353, at concentrations of 0.25 and 0.5 gL^{-1}, the inhibition rate was 25.28 and 22.07%, respectively. At other concentrations, the inhibition rate was below 11%. The other strains grew normally at all concentrations of silicon tested (Table 1B). Phosphite proved to be effective in inhibiting the mycelial growth of fungi under all our experimental conditions (Figure 1C). None of the plates showed mycelial growth at the tested doses of phosphite.

Carbendazim was 100% effective against two of the four isolates tested. The mycelial growth of CML-2353 and E-596 were completely inhibited at the various concentrations of carbendazim. Carbendazim exerted a fungistatic effect on the isolates CML-2466 and E-637,

Table 1. Colony diameter (C.D.) and percent growth inhibition (P.I.) of F. solani isolates in chitosan (A), silicon (B), phosphite (C) and carbendazim (D).

A

Chitosan (%)	CML 2466**		CML 2353**		E-637**		E-596**	
	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)
Control 1	0[c]	100	0[c]	100	0[b]	100	0[b]	100
Control 2	60.95[b]	0	51.58[b]	0	63.16[a]	0	62.03[a]	0
0.5	68.00[a]	-11.57	65.56[a]	-27.10	68.00[a]	-7.66	65.04[a]	-4.86
1.0	68.00[a]	-11.57	65.53[a]	-27.05	68.00[a]	-7.66	63.76[a]	-2.79
1.5	68.00[a]	-11.57	62.16[a]	-20.51	68.00[a]	-7.66	64.64[a]	-4.21
2.0	68.00[a]	-11.57	62.49[a]	-21.15	68.00[a]	-7.66	52.95[a]	14.63
2.5	68.00[a]	-11.57	62.21[a]	-20.61	67.85[a]	-7.43	61.96[a]	0.12
3.0	68.00[a]	-11.57	64.17[a]	-24.41	68.00[a]	-7.66	64.49[a]	4.30
V.C. (%) =	4.72		7.96		4.12		12.71	

B

Silicon (g L^{-1})	CML 2466 n.s.		CML 2353*		E-637**		E-596 n.s.	
	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)
Control 2	60.95[a]	0	51.58[a]	0	63.16[ab]	0	62.03[a]	0
0.25	62.48[a]	-2.51	38.54[b]	25.28	58.00[b]	8.17	64.63[a]	-24.22
0.5	62.50[a]	-2.54	40.20[ab]	22.07	64.57[ab]	-2.24	63.43[a]	-21.92
1.0	64.33[a]	-5.54	46.15[ab]	10.53	68.00[a]	-7.66	64.90[a]	-24.73
1.5	64.43[a]	-5.72	47.82[ab]	7.29	68.00[a]	-7.66	63.38[a]	-21.81
2.0	67.78[a]	-11.20	50.78[ab]	1.56	68.00[a]	-7.66	65.13[a]	-25.18
3.0	68.00[a]	-11.57	50.66[ab]	1.78	68.00[a]	-7.66	59.33[a]	-14.03
V.C. (%) =	9.82		12.47		5.98		12.05	

C

Phosphite (g L^{-1})	CML 2466**		CML 2353**		E-637**		E-596**	
	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)
Control	60.95[a]	0	51.58[a]	0	63.16[a]	0	62.03[a]	0
1.0	0[b]	100	0[b]	100	0[b]	100	0[b]	100
2.0	0[b]	100	0[b]	100	0[b]	100	0[b]	100
3.0	0[b]	100	0[b]	100	0[b]	100	0[b]	100
4.0	0[b]	100	0[b]	100	0[b]	100	0[b]	100
5.0	0[b]	100	0[b]	100	0[b]	100	0[b]	100
6.0	0[b]	100	0[b]	100	0[b]	100	0[b]	100
V.C. (%) =	34.0		13.35		28.70		10.34	

D

Carbendazim (ml L^{-1})	CML 2466**		CML 2353**		E-637**		E-596**	
	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)	C.D. (mm)	P.I. (%)
Control 2	60.95[a]	0	51.58[a]	0	63.16[a]	0	62.03[a]	0
0.83	23.45[b]	61.52	0[b]	100	14.18[b]	77.55	0[b]	100
1.67	19.77[b]	67.57	0[b]	100	11.81[b]	81.30	0[b]	100
2.50	20.61[b]	66.19	0[b]	100	11.65[b]	81.55	0[b]	100
3.34	23.67[b]	61.16	0[b]	100	13.50[b]	78.63	0[b]	100
4.16	21.89[b]	64.09	0[b]	100	12.09[b]	80.86	0[b]	100
5.0	23.49[b]	61.46	0[b]	100	11.40[b]	81.96	0[b]	100
V.C. (%) =	16.56		13.35		14.73		10.34	

Averages followed by the same letter are not statistically different among themselves, by Tukey test. V.C. = Variation coeficient; ** significant at 1% probability ($p < 0.01$); * significant at 5% probability ($0.01 = < p < 0.05$); [ns] not significant ($p > = 0.05$).

and the effect was not dosedependent. The inhibition of growth of these isolates was 60 and 80%, respectively (Figure 1D, Table 1D).

DISCUSSION

The absence of inhibition of mycelial growth by chitosan suggested that the *F .solani* f. sp. *piperis* isolates can use this substance as anadditional carbon source. This is possibly because chitosan is a polysaccharide, and probably, the fungus uses it as a source of nutrients for its growth. Nascimento et al. (2007), studying fungi causing grapevine trunk diseases, found that chitosan inhibited the growth of all fungi tested except *Neonectria liriodendri*, which grew at all the concentrations analyzed. According to Baños et al. (2004) and Bhaskara-Reddy et al. (1998), mycelial growth and sporulation of *Penicillium digitatum* and *Alternaria alternata*, respectively, were stimulated by the presence of chitosan. These authors believed that such behavior may be a response to stress caused by the chitosan. Several studies have shown that the biological activity of chitosan is significantly dependent upon its molecular weight, acetylation degree (Alfredsen et al., 2004; Wu et al., 2004; Torr et al., 2005), pH of the medium (Devlieghere et al., 2004), and the microorganism membrane characteristics (Qi et al., 2004). In general, the lower the molecular weight and degree of acetylation of chitosan, the greater the efficacy atreducing the growth and multiplication of microorganisms (Goy et al., 2009). The other possibility is the unusual pH of the culture medium, which remained acidic (about 4.0). The ability of fungi to grow in wider pH ranges is associated with the presence of pH-regulatory systems. These regulatory systems are mediated by differential production of extracellular enzymes and metabolites as a function of pH of the medium (Denison, 2000). It is likely that this pH adjustment mechanism also exists in *F. solani*. This phenomenon may be associated with fungal survivability for long periods in the soil, even under adverse conditions. In control plates where we added acetic acid, there was no growth for any of the isolates tested. Sholberg et al. (2000) reported that the inhibitory effect of acetic acid on microorganisms is due to the reduction in pH as well as the ability of the coupled molecules of acetic acid to pass easily through the membrane of conidia, exerting its toxic effect by reducing the cellular protoplasm.This mechanism may explain the inhibition of mycelial growth of *F. solani* in control plates containing only PDA with added acetic acid. Chitosan's effects on growth of microorganisms are well known, but the mechanisms underlying its antifungal action have not been fully elucidated. The response to this possible antifungal agent may vary depending on the pathogen (El-Ghaouth et al. 1992).

Our results suggest that silicon does not have direct action on the *F. solani* isolates tested because it induced mycelial growth at all concentrations. Silicon probably acts as a resistance inducer in the plant. Similar results were reported by Carré-Missio et al. (2010), who studied the effect of silicon on Pestalotia leaf spotin cultivated strawberry. *In vitro* results showed that silicon at the dose of 8 g L^{-1}does not inhibit mycelial growth of*Pestalotia longisetula*. In another study, the growth of *Fusarium* spp. and *Verticillium* spp. were enhanced at silicon concentrations of 5 and 10 ml L^{-1}, respectively (Kaiser et al. 2005). Generally, silicates do not act directly on microorganisms that cause diseases in plants, but have alternative mechanisms of action, which in some cases—because of their beneficial effect on the plant—may reduce abiotic and biotic types of stress (Zambolim et al. 2012). In the literature, there are reports of reduced and increased intensity of diseases in plants after treatment with silicon (Zambolim and Ventura, 1996). Silicon can act locally by inducing defensive reactions in cells and can also contribute to systemic resistance by increasing the production of stress hormones. Nonetheless, the exact mechanism by which silicon modulates signaling in plants remains unclear. Evidence suggests that silicon can act as an enhancer of plant defense responses or as a strategic signaling proteins. Silicon can therefore interact with several key components of the plant stress response-related signaling pathways, leading to effective resistance to pathogenic fungi.

In agreement with the results of our study, Araújo et al. (2008), while studying *Colletotrichum gloeosporioides*, showed that potassium phosphite (Fitofós K®) has a direct effect on this fungus, almost completely inhibiting the mycelial growth *in vitro*. Potassium phosphite was tested against *Penicillium expansum*, which causes postharvest blue mold infections on apple fruits; this compound completely inhibited the mycelial growth (Amiri and Bompeix, 2011). In a study made by Lobato et al. (2010), phosphate exerted a fungicidal effect on pathogens of potatoes: *F. solani*, *Rhizoctonia solani*, and *Streptomyces scabies*. According to Guest and Grant (1991), phosphites inhibit the growth of pathogens in plants via a complex mechanism of action. The first stage is a direct fungistatic effect, which is dependent on the concentration of phosphite that accumulates in the fungus. This, in turn, is influenced by the concentration of phosphate, and the effectiveness of the phosphite oxidation system. The second step is a change in the metabolism of the pathogen, such that a faster and more effective defensive response by the plant can develop. These alterations imply a reduction in the amount of suppressor molecules on the pathogen's surface or an increase in the number of receptors exposed to agonists in host cells, or both, suggesting that phosphites may have multiple modes of action. As for the direct action on the pathogen, it is known that phosphorous acid and its derivatives act by inhibiting the process of oxidative phosphorylation in Oomycetes (McGrath, 2004). In general, the effects of phosphites on the phytopathogens are mediated by the formation of membrane pores due to

damage to the plasma membrane and cell wall of the hyphae, probably because of transcription changes in genes that encode proteins involved in the biosynthesis of their components and other parts of the overall cellular metabolism. These changes compromise the morphology, physiology, and sporulation of the fungus, interfering with the parasitism (Smillie et al., 1989; King et al., 2010). The indirect action of phosphate involves activation of plant defense mechanisms such as stimulation of the production of phytoalexins (Guest and Grant, 1991; Daniel and Guest, 2006) or lignification and production of phenols (Nojosa et al., 2005).

The biological activity of benzimidazoles (such as carbendazim) is mediated by interference with the formation and functioning of microtubules in eukaryotic cells. The affinity of benzimidazole for tubulin is the main factor determining its fungicidal activity. The stronger the binding affinity of the compound for tubulin, the more sensitive is the organism to the fungicide. Also, resistance to carbendazim is described as a change in the□□-tubulin protein, which reduces the binding of the fungicide to this protein (Osmani and Oakley, 1991). In our study, carbendazim showed different effects when administered to the fungus. In two *F. solani* isolates, CML-2466 and E-637, this compound had a fungistatic effect at all the doses analyzed, whereas for CML-2353 and E-596 isolates, this compound showed a fungitoxic effect. According to Sultana and Ghaffar (2013), carbendazim completely inhibits colony growth of *F. oxysporum*. When tested on mycelial growth of *Rhizoctonia solani*, carbendazim reduced it by 86% *in vitro* (Schurt et al., 2013). In addition to *in vitro* results, chitosan (El-Ghaouth et al., 1994), silicon (Epstein, 1999) and phosphites (Ouimette and Coffey, 1989) due to resistance induction characteristics are being field tested by us in Black pepper plants, to evaluate the behavior of such plants inoculated with *F. solani*.

In summary, chitosan and silicon did not inhibit the growth of *F. solani* and instead promoted the growth of most isolates. Carbendazim exerted growth control in 50% of the isolates and in the other 50%, had a fungistatic effect, and these effects were not dosedependent. Among the products tested for possible inhibition of the mycelial growth of *F. solani* f. sp. *piperis in vitro*, the action of phosphite stands out: 100% inhibition in isolates CML-2466, CML-2353, E-637, and E-596.

Conflict of Interests

There is no conflict of interest of any kind related to this work.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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REFERENCES

Alfredsen G, Eikens M, Militz H, Solheim H (2004). Screening of chitosan against wood-deteriorating fungi. Scand J. Forest Res. 19(5):4-13

Allan CR, Hadwiger LA (1979). The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp. Mycol. 3:285-287

Amiri A, Bompeix G (2011). Control of *Penicillium expansum* with potassium phosphite and heat treatment. Crop Protect. 30:222-227

Araújo L, Stadnik MJ, Borsato LC, Valdebenito-Sanhueza RM (2008). Fosfito de potássio e ulvana no controle da mancha foliar da gala em macieira. Trop. Plant Pathol. 33:148-152.

Baños SB, López MH, Molina EB (2004). Growth Inhibition of Selected Fungi by Chitosan and Plant Extracts. Revista Mexicana de Fitopatología. 22:178-186.

Bedendo IP (1995). Oídios. In: Bergamin Filho A, Kimati H, Amorim L (eds) Manual de Fitopatologia 3rd edn. Ceres. pp. 866-871

Bhaskara-Reddy MV, Essaid AB, Castaigne F, Arul J (1998). Effect of chitosan on growth and toxin production by *Alternaria alternata* f sp *lycopersici*. Biocontrol Sci. Technol. 8:33-43

Carré-Missio V, Rodrigues FÁ, Schurt DA, Rezende DC, Ribeiro NB, Zambolim L (2010). Aplicação foliar de silicato de potássio, acibenzolar-S-metil e fungicidas na redução da mancha de Pestalotia em morango. Trop. Plant Pathol. 35:182-185

Chérif M, Asselin A, Bélanger RR (1994). Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. Phytopathology. 84:236-242

Daniel R, Guest D (2006). Defence responses induced by potassium phosphonate in *Phytophthora palmivora*-challenged *Arabidopsis thaliana*. Physiol. Mol. Plant Pathol. 67:194-201

Denison SH (2000). pH Regulation of gene expression in fungi. Fungal Genet Biol. 29(2):61-71

Devlieghere F, Vermeulen A, Debevere J (2004). Chitosan: antimicrobial activity interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 21:703-714

El-Ghaouth A, Arul J, Grenier J, Asselin A (1992). Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathology. 82:398-402

El-Ghaouth A, Arul J, Wilson C, Benhamou N (1994). Ultrastructural and cytochemical aspects of the effect of chitosan on decay of bell pepper fruit. Physiol. Mol. Plant Pathol. 44:417-432.

Epstein E (1999). Silicon. Annu Rev Plant Phys. 50:641-664

Fauteux F, Rémus-Borel W, Menzies JG, Belanger R (2005). Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiol Lett. 249:1-6

Fenn ME, Coffey MD (1985). Further evidence for direct mode of action of Fosethyl-Al and Phosphorous acid. Phytopathology. 75:1064-1068

Fenn ME, Coffey MD (1984). Studies on the in vitro and in vivo antifungal activity of Fosetyl-Al and Phosphorous acid. Phytopathology. 74:606-611

Goy RC, Britto D, Assis OBG (2009). A review of the antimicrobial activity of chitosan. Polímeros: Ciência e Tecnologia. 19(3):241–247

Guest D, Grant B (1991). The complex action of phosphonates as

antifungal agentes. Biol Rev. 66(2):159-187
Guo Z, Chen R, Xing R, Liu S, Yu H, Wang ,P Lia C, Lia P (2006). Novel derivatives of chitosan and their antifungal activities *in vitro*. Carbohyd Res. 341(3):351-354
IBGE Instituto Brasileiro de Geografia e Estatística (2016). Levantamento Sistemático da Produção Agrícola Dados de Previsão de Safra. Available at: http://w w w .sidra.ibge.gov.br/bda/prevsaf/default.asp
Kaiser C, Merw e R Van Der, Bekker TF, Labuschagne N (2005). In-vitro inhibition of mycelial grow th of several phytopathogenic fungi, including *Phytophthora cinnamomi* by soluble silicon. South African Avocado Grow ers' Association Yearbook. 70-74.
Kimati H, Gimenez-Fernandes N, Soave J, Kurozaw a C, Brignani Neto F, Bettiol W (1997). Guia de fungicidas agrícolas - Recomendações por cultura. Grupo Paulista de Fitopatologia Jaboticabal, São Paulo, Brazil
King M, Reeve W, Van Der Hoek MB, Williams N, McComb J, O'Brien PA, Hardy GE (2010). Defining the phosphite-regulated transcriptome of the plant pathogen *Phytophthora cinnamomi*. Mol. Genet. Genomics. 284:425-435
Kreyci PF, Menten JO (2013). Limitadoras de produtividade. Cultiv Cad Técnico. 167:1-12.
Kus C, Altanlar N (2003). Synthesis of some new benzimidazole carbamate derivatives for evaluation of antifungal activity. Turk. J. Chem. 27:35-39
Lobato MC, Olivieri FP, Daleo GR, Andreu AB (2010). Antimicrobial activity of phosphites against different potato pathogens. J. Plant Dis. Protect. 117(3):102-109
McGrath MT (2004). What are fungicides? The Plant Health Instructor.
Nascimento T, Rego C, Oliveira H (2007). Potential use of chitosan in the control of grapevine trunk diseases. Phytopathol. Mediterr. 46(2):218-224
Nemestothy GS, Guest DI (1990). Phytoalexin accumulation phenylalamine ammonia lyase activity and ethylene biosynthesis in Fosetyl-Al treated resistant and susceptible tobacco cultivars infected w ith *Phytophthora nicotiana* var *nicotianae*. Physiol. Mol. Plant 37:207-219
Nojosa GBA, Henz GP, Sathler FGL (2015). A Introdução de Pragas e seu Impacto sobre o Acesso a Mercados In: Sugayama RL, Silva ML, Silva SXB, Ribeiro LC, Rangel LEP (eds) Defesa Vegetal: Fundamentos Ferramentas Políticas e Perspectivas, 1st edn. Sociedade Brasileira de Defesa Agropecuária, Belo Horizonte
Nojosa GBA, Resende MLV, Resende AV (2005). Uso de fosfitos e silicatos na indução de resistência. In: Cavalcanti LS, Di Piero RMP Cia P, Pascholati SF, Resende MLV, Romeiro RS (eds) Indução de Resistência em Plantas a Patógenos e Insetos. FEALQ, Piracicaba, pp. 139-153
Ortiz E, Cruz M, Melgarejo LM, Marquínez X, Hoyos-Carvajal L (2014). Histopathological features of infections caused by *Fusarium oxysporum* and *F. solani* in purple passion fruit plants (*Passiflora edulis* Sims). Summa Phytopathol. 40(2):134-140
Osmani AS, Oakley BR (1991). Cell cycle and tubulin mutations in filamentous fungi. In: Bennett JW, Lasure LL (eds) More gene manipulations in fungi. Academic Press, San Diego, pp 107-125
Ouimette DG, Coffey MD (1989). Comparative antifungal activity of four phosphonate compounds against isolates of nine *Phytophthora* species. Phytopathology 79:761-767.
Pfenning LH, Lima CS (2007). Descrição das espécies do w orkshop. In: Tropical *Fusarium* Workshop Anais Uiversidade Federal de Lavras, Minas Gerais, Brazil.
Qi L, Xu Z, Jiang X, Hu C, Zou X (2004). Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr. Res. 339:2693-2700.
Reis EM, Forcelini CA, Reis AC (2001). Manual de Fungicidas – Guia para o Controle Químico de Doenças de Plantas. Florianópolis, Santa Catarina, Brazil
Rocha FS, Ferreira GHS, Silva TCSR, Amaral FL, Muniz MFS, Pereira EA (2016). Caracterização de *Fusarium solani* f sp *piperis*: produção de fitotoxina e incidência da fusariose no norte de Minas Gerais. Summa Phytopathol. 42(1):67-72
Roller S, Covill N (1999). The antifungal properties of chitosan in laboratory media and apple juice. Int J Food Microbiol. 47:67-77
Saindrenan P, Barchietto T, Bompeix G (1990). Effect of phosphonate on the elicitor activity of culture filtrates of *Phytophthora cryptogea* in *Vigna unguiculata*. Plant Sci. 67:245–251
Schurt DA, Rodrigues FA, Souza NFA, Reis RD (2013). Eficiência de diferentes moléculas na redução dos sintomas da queima das bainhas em arroz e no crescimento de *Rhizoctonia solani in vitro*. Rev. Ceres. 60(2):221-225
Sholberg P, Haag P, Hocking R, Bedford K (2000). The use of vinegar vapor to reduce postharvest decay of harvested fruit. HortSci. 35(5):898-903
Silva FAZ, Azevedo CAV (2009). Principal Components Analysis in the Softw are Assistat-Statistical Attendance In: World Congress on Computers in Agriculture 7 Reno-NV-USA: American Society of Agricultural and Biological Engineers.
Smillie R, Grant BR, Guest D (1989). The Mode of Action of Phosphite: Evidence for Both Direct and Indirect Modes of Action on Three *Phytophthora* spp Plants. Phytopathology 79(9):921-926
Sultana N, Ghaffar A (2013). Effect of fungicides microbial antagonists and oil cakes in the control of *Fusarium oxysporum* the cause of seed rot and root infection of bottle gourd and cucumber. Pak. J. Bot. 45(6):2149-2156
Torr KM, Chittenden C, Franich RA, Kreber B (2005). Advances in understanding bioactivity of chitosan and chitosan oligomers against selected w ood-inhabiting fungi. Holzforschung 59(5):559-567
Tremacoldi CR (2014). Doenças causadas por fungos. In: Lemos OF, Tremacoldi CR, Poltronieri MC (eds) Boas práticas agrícolas para aumento da produtividade e qualidade da pimenta-do-reino no Estado do Pará. Embrapa Brasília, Brazil. pp. 29-34
Wheeler T, Rush CM (2001). Soilborne diseases. In: Maloy OC, Murray TD (eds) Encyclopedia of Plant Pathology. John Wiley and Sons, New York. pp. 935-947
Wu T, Zivanovic S, Draughon FA, Sams CE (2004). Chitin and chitosan-value-added products from mushroom w aste. J. Agric. Food Chem. 52:7905-7910
Zambolim L, Zanão Júnior LA, Ventura JA (2012). Mecanismos de ação do silício na redução de doenças de plantas. In: Zambolim L, Ventura JA, Zanão Júnior LA (eds) Efeito da nutrição mineral no controle de doenças de plantas. Universidade Federal de Viçosa, Minas Gerais, Brazil. pp. 253-285
Zambolim L, Ventura JA (1996). Resistência a doenças induzidas pela nutrição mineral das plantas. Informações Agronômicas 75:1-16