

1 ***Cinnamomum cassia* essential oil and (E)-cinnamaldehyde as control agents of**  
2 **anthracnose on common bean seeds**

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21 **Highlights**

22 The essential oil *Cinnamomum cassia* used have high concentrations of (E)-  
23 cinnamaldehyde.

24 Fungicides were prepared with essential oils of *Cinnamomum cassia* essential oil and (E)-  
25 cinnamaldehyde

26 The two fungicides applied in seeds of common bean, decreases incidence of anthracnose.

27 (E)-cinnamaldehyde is the active substance against *C. lindemuthianum* causing da  
28 anthracnose.

29 **ABSTRACT**

30 Among the fungi that cause damage to the common bean and are disseminated by the  
31 seeds, *Colletotrichum lindemuthianum* (Sacc. e Magn.) Briosi e Cavara stands out. This  
32 fungus causes anthracnose in common bean (*Phaseolus vulgaris* L.). Use of natural  
33 compounds is a viable and safer option than chemicals to manage this disease. Essential  
34 oils have shown antifungal potential against phytopathogenic fungi. According to the  
35 results of the *in vitro* test, we observed complete inhibition of the growth of *C.*  
36 *lindemuthianum* with the use of cassia cinnamon essential oil (*Cinnamomum cassia*) (EO)  
37 and its major component (*E*)-Cinnamaldehyde, presenting MIC of 125 µg/mL, while the  
38 commercial fungicide presented MIC of 30.6 µg/mL. And *in vivo*, where seeds naturally  
39 infected with *C. lindemuthianum* were treated with the EO and the substance in a solution  
40 with commercial soybean oil, we observed that the treatments did not affect germination  
41 and initial seed vigor. In addition, the seed treatment with solutions formulated from EO  
42 and (*E*)-cinnamaldehyde was efficient for reducing the incidence of anthracnose over the  
43 days evaluated, as well as for the commercial fungicide used (methyl thiophanate). This  
44 is the first study to demonstrate the efficacy of *C. cassia* oil and (*E*)-cinnamaldehyde in  
45 the control of *C. lindemuthianum* through the treatment of common bean seeds.

46 **keywords:** (*E*)-Cinnamaldehyde; *Colletotrichum lindemuthianum*; *Phaseolus vulgaris*

47

48 **INTRODUCTION**

49 Common bean (*Phaseolus vulgaris*) is one of the most consumed foods worldwide  
50 (NETO and SANTOS 2018), being cultivated in Brazil in an area of 213,000.9 ha  
51 (CONAB 2015). Brazil has the potential to increase common bean yield, but one of the  
52 limiting factors is the anthracnose, caused by the fungus *Colletotrichum lindemuthianum*  
53 (Sacc. e Magn.) Briosi e Cavara (Amin et al. 2014). Production losses can reach 100%  
54 when, under favorable environmental conditions, seeds infected with the pathogen are  
55 used or susceptible cultivars are adopted (Peloso 1988; Padder et al. 2017).

56 Genetic resistance is the most effective management strategy to control plant diseases  
57 (Nelson et al. 2018). However, for anthracnose in common bean, the development of new  
58 cultivars with the desired characteristics is slow and with low durability (Assefa et al.  
59 2019), mainly due to the variability of the pathogen (Davide and de Souza 2009; Pinto et  
60 al. 2012; Costa et al. 2017). Therefore, the use of fungicides is an important component  
61 of integrated strategies to minimize losses caused by anthracnose (Conner et al. 2009;  
62 Amin et al. 2014).

63 In Brazil, a part of the harvested grains of common bean are destined for the sowing of  
64 the next crop, being one of the hypotheses for the low use of certified seeds in the common  
65 bean production (Castro and others 2017). As consequence, there is transmission of  
66 pathogens via seed and occurrence of diseases in the next crop. The treatment of common  
67 bean seeds with synthetic fungicides has been the most efficient method for the control  
68 of various fungal diseases in this crop (Oliveira et al. 2016). The use of these chemicals  
69 provides initial protection against soil pathogens, eliminates seed-associated pathogens,  
70 and prevents the spread and entry of new contaminants into the area (Lipps and Grove  
71 1985; Sharma et al. 2015). On the other hand, there are related risks, among which it is  
72 worth highlighting: inconsistent economic results, development of resistance by soil

73 pathogens to many fungicides, risks of exposure of operators to toxic substances and  
74 negative impacts on non-target organisms (Tilman et al. 2002; Lamichhane et al. 2020).  
75 In general, strategies that make use of natural substances may be more sustainable.  
76 Therefore, it is important to study such substances to ascertain whether they can also be  
77 used for the development of more efficient and less costly fungicides (Isman 2000;  
78 Hettiarachi and Dubey 2011; Bi et al. 2012; Brum et al. 2014). One of the possibilities in  
79 this area are the essential oils, whose components may present biological activities  
80 (Pavela and Benelli 2016). In several cases the control of phytopathogenic fungi occurs  
81 through the direct action of essential oil or/and its components against the fungus  
82 (Andrade Pinto et al. 2010; Hoyos et al. 2012; Ootani et al. 2013; Zabka and Pavela 2013;  
83 Bhardwaja et al. 2015; Kumar and Kudachikar 2018). An example is dill essential oil  
84 (*Anethum graveolens* Linn.), which can break through the barrier of the permeability of  
85 the plasma membrane of the fungus *Aspergillus flavus*, provoking ROS (reactive oxygen  
86 species) accumulation (Tian et al. 2012).

87 Among the plant species with fungitoxic action is *Cinnamomum cassia* Presl belonging  
88 to the *Lauraceae* family. This plant is popularly known for its medicinal properties  
89 (Ravindran et al. 2003; Barceloux 2009; Nabavi et al. 2015). The essential oil can be  
90 extracted from stem, leaves and bark of plants (Balijepalli et al. 2017) which the major  
91 component is (*E*)-cinnamaldehyde, has activity against several phytopathogenic fungi  
92 (Lee et al. 2005; Cheng et al. 2006; Xing et al. 2010; El-Mogy and Alsanius 2012; Jiang  
93 et al. 2013; Moraes et al. 2018). However, there are no reports of the use of this oil or its  
94 main component for the treatment of plant seeds. Seed treatment with essential oils has  
95 been shown to be effective in controlling plant pathogens that contaminate bean seeds  
96 (Abdel-Kader et al. 2011; Khaledi et al. 2015).

97 Thus, the objectives of the article are: a) to identify the major components of *C. cassia*  
98 essential oil; b) to evaluate the phytotoxic effects of *C. cassia* essential oil, (*E*)-  
99 cinnamaldehyde and soybean oil, on common bean seeds; c) to evaluate the fungitoxic  
100 potential of *C. cassia* oil and (*E*)-cinnamaldehyde against *C. lindemuthianum* fungus  
101 present in naturally infected seeds of common bean.

102

### 103 MATERIAL AND METHODS

104 **Essential oil:** Cassia cinnamon essential oil (*Cinnamomum cassia*) was acquired from  
105 Ferquima Indústria e comércio LTDA. The extraction was carried out by steam  
106 distillation of the leaves, bark and stem of the plant. The essential oil was analyzed by  
107 gas chromatography coupled to mass spectrometry (GC-MS, Shimadzu QP-2010,  
108 Shimadzu Corporation, Kyoto, Japan), under the conditions: fused silica capillary column  
109 (30 cm long and 0.25 mm inner diameter) with stationary phase RTX<sup>®</sup>-5MS (0.25 µm  
110 film thickness) and helium as carrier gas, with flow of 1.0 mL per minute. Injector  
111 temperature of 220°C, the initial column temperature was 60°C, with programming to  
112 increase 2°C per minute until reaching the temperature of 200°C, and 5°C per minute until  
113 reaching the maximum temperature of 250°C. The mass spectra were obtained by electron  
114 impact at 70 eV, with a scan of 29 to 400 (m/z). Being injected 1 µL of the prepared oil  
115 solution at a concentration of 10 mg/L with a split ratio of 1:20. The identification of the  
116 compounds was performed by comparing the mass spectra with those available in the  
117 NIST17 mass spectra bank, visual interpretation of the mass spectra and comparison of  
118 arithmetic indexes (AI) with those available in the literature (Adams 2007). To calculate  
119 the arithmetic indexes, a mixture of linear alkanes (C9 to C17) was injected into the  
120 chromatograph. The relative percentage of each compound was calculated by the ratio of  
121 the area of each peak to the total area of all the constituents of the sample.

122 **Reaction of common bean seeds to *C. cassia* essential oil /(*E*)-cinnamaldehyde:** To  
123 evaluate the phytotoxic effect of *C. cassia* essential oil and (*E*)-cinnamaldehyde (Sigma-  
124 Aldrich, purity  $\geq 99\%$ ), the germination test was performed according to the nacional  
125 guidelines (BRASIL 2009). Common bean seeds (*P. vulgaris* 'cultivar Pérola ') were  
126 previously sanitized with NaClO 2% (g/mL) for two minutes and rinsed under running  
127 water to remove NaClO residue. After dried on sterilized filter paper, the seeds were  
128 placed inside plastic bags to facilitate homogenization. And in each plastic bag we  
129 deposited treatments containing the *C. cassia* essential oil (10, 100, 500 and 2000  $\mu\text{g/mL}$ )  
130 or (*E*)-cinnamaldehyde (8, 80, 400 and 1600  $\mu\text{g/mL}$ ) dissolved in commercial soybean  
131 oil (Liza®, Cargill Food Service), in the proportion of 40 mL/kg of seed. Water and  
132 soybean oil were used as controls of the experiment.

133 Germination tests were performed in a Biochemical Oxygen Demand (BOD) germination  
134 chamber at 25°C, in a completely randomized design, with four replications. The plot  
135 consisted of a roll of Germitest® paper containing 50 seeds. The papers were previously  
136 sterilized at 105 °C for 2 hours, and moistened with distilled water at a ratio of 2.5 times  
137 the dry mass of the paper.

138 For seven days every 24 hours, the number of seedlings that presented root protrusion  
139 was quantified. At the end of the test, with the daily data of the number of seeds  
140 germinated, the germination speed index (GSI) was calculated, according to Maguire  
141 (Maguire 1962).

142 On the seventh day, the number of abnormal, normal seedlings and dead seeds was  
143 counted, and the results were expressed as percentage (BRASIL 2009). On the same day,  
144 the length of the shoot and the root of 10 random seedlings of each repetition was  
145 measured with a millimeter ruler. These plants were placed in a drying incubator at 70°C  
146 until they reached a constant weight (48 hours), and finally weighed in analytical

147 precision scale in order to obtain the dry mass in grams. The obtained data were submitted  
148 to the normality (Shapiro-Wilk) and homoscedasticity (Bartlett) tests (Snedecor and  
149 Cochran 1989). Then, the analysis of variance by the F test were carried out and the means  
150 grouped by the Scott Knott Test at 5% probability, with GENES software (Cruz 2016).

151

## 152 ***In vitro* and *in vivo* antifungal assays**

153 **Determination of minimal inhibitory concentration:** The fungus *C.*  
154 *lindemuthianum* (race 65, isolate LV 238) cultivated in pods was crushed in sterile water  
155 to obtain a spore suspension of  $1.2 \times 10^6$  conidia/mL after adjusting in a Neubauer  
156 chamber, using a microscope. Aliquots of 100  $\mu$ L of the suspension were transferred to 2  
157 mL microtubes, to which 100  $\mu$ L of the EOs dissolved in DMSO and M3S were added.  
158 The microtubes were kept in a B.O.D. chamber, in the dark, at 23 °C, for 5 days. After  
159 the addition of the spore suspension, the lowest concentration of the sample in the  
160 microtube that prevented fungal growth, by visual observation, was considered the  
161 minimum inhibitory concentration (MIC) (Mota et al., 2017).

162 The soybean oil, EOs, and eugenol were dissolved in 5  $\mu$ L of dimethyl sulfoxide  
163 (DMSO) and diluted with M3S medium (Tu, 1985) to a concentration of 8000  $\mu$ g/mL.  
164 These solutions were diluted sequentially to the following final concentrations: 8000,  
165 4000, 2000, 1000, 500, 250, 125, and 62.5  $\mu$ g/mL. The commercial fungicide  
166 thiophanate-methyl 70%, trade name: Cercobin 700WP (Iharabras S.A. Indústrias  
167 Químicas, Brazil) was used as a positive control. For this, Cercobin 700WP was dissolved  
168 in 5  $\mu$ L of DMSO, diluted with M3S to obtain solutions of thiophanate-methyl at  
169 concentrations of 490, 245, 122.5, and 30.63  $\mu$ g/mL. A DMSO solution in 5% M3S (v/v)  
170 was used as a negative control.

171 **Control of *C. lindemuthianum* on common bean seeds by *C. cassia* essential oil/(*E*)-**  
172 **cinnamaldehyde:** Common bean seeds (*P. vulgaris* L. 'cultivar BRSMGUai'),  
173 susceptible to anthracnose, were collected in Lavras, MG, Brazil, in plants with symptoms  
174 of anthracnose in the pods and leaves. Subsequently, the seeds were placed inside plastic  
175 bags to facilitate homogenization, and in each plastic bag were deposited the essential oil  
176 solutions of *C. cassia* (2000 µg/mL) and (*E*)-cinnamaldehyde (1600 µg/mL) in soybean  
177 oil. The commercial fungicide methyl thiophanate (dimethyl 4,4'-(*o*-phenylene)bis(3-  
178 thioallophanate); 70%; Cercobin 700WP, Iharabras S.A. Indústrias Químicas) in the  
179 dosage of 1g of the product/kg of seeds, commercial soybean oil and water. All  
180 treatments, except methyl thiophanate, were applied at a dosage of 40 mL/kg of seeds.  
181 The treated seeds were sown in 3.5 L plastic pots containing a mixture of substrate  
182 (Plantmax ®Brazil) and soil (1:2). The design was in randomized blocks with three  
183 replications. The plot consisted of 20 plants.

#### 184 **Assessed traits**

185 ***Emergence and Emergence Speed Index (ESI):*** The number of germinated seeds was  
186 evaluated daily, from the emergence of the first seedling to the stabilization of the stand  
187 for three consecutive days, when cotyledons appeared above the substrate. The ESI was  
188 calculated based on the formula proposed by Maguire (1962) (Maguire 1962).

189 ***Incidence of anthracnose:*** The incidence of anthracnose was assessed at 0, 3, 6, 9, 12,  
190 15, 18 and 21 days after the first disease symptom. We counted the number of plants with  
191 symptoms of anthracnose on leaves and stems. In percentage, the number of infected  
192 plants (with symptoms) was divided by the total number of plants assessed.

193 ***Plant Vigor (dry mass):*** After the final evaluation of the incidence of anthracnose, the  
194 aerial parts of the plants from all treatments were weighed on an analytical balance after

195 drying for 48h in a drying incubator at 70°C. The results were expressed in mass  
 196 (grams)/emerged plants.

197 **Data analysis:** Emergence, emergence speed index, and dry mass data were submitted to  
 198 the normality (Shapiro-Wilk) and homoscedasticity (Bartlett) tests (Snedecor and  
 199 Cochran 1989). Then, Analysis of variance was performed by the F test and the means  
 200 were grouped by the Scott-Knott Test at 5% probability level, using GENES software  
 201 (Cruz 2016). For incidence of anthracnose over time, Poisson regression analysis was  
 202 performed using the Generalized Linear Model in the R Software (RStudio 2019).

203

## 204 RESULTS

205 **Essential oil:** Ten chemical constituents were found in the essential oil of *C. cassia*, the  
 206 majority being (*E*)-cinnamaldehyde (82.03%), (*E*)-*o*-methoxycinnamaldehyde (9.49%)  
 207 and eugenol (3.56%) (Table 1).

208 **Table 1** – Composition of *Cinnamomum cassia* essential oil.

Calculated Index	Literature Index*	Relative Concentration (%)	Similarity (%)	Compound
955	952	0.974	94	Benzaldehyde
1110	-	0.227	-	NI
1238	1239	0.43	92	<i>o</i> -Anisaldehyde
1269	1267	82.03	97	( <i>E</i> )-Cinnamaldehyde
1353	1356	3.56	94	Eugenol
1368	1374	0.28	78	$\alpha$ -Copaene
1410	1417	0.35	93	( <i>E</i> )-Caryophyllene
1427	-	1.28	-	NI
1440	1443	1.27	95	( <i>E</i> )-cinnamyl acetate
1524	1527	9.49	97	( <i>E</i> )- <i>o</i> -methoxycinnamaldehyde

209 NI represent values not found in the literature. \* (ADAMS, 2007)

210 **Reaction of common bean seeds to *C. cassia* essential oil/(*E*)-cinnamaldehyde:** Seed  
 211 treatments with *C. cassia* essential oil and (*E*)-cinnamaldehyde, at all tested  
 212 concentrations, had no impact on seed germination, the number of dead seeds, abnormal

213 seedlings, or the dry matter of the seedlings. With the exception of *C. cassia* essential oil  
 214 at the concentration of 500 µg/ml the other treatments significantly reduced GSI when  
 215 compared with control (water). However, *C. cassia* essential oil showed an increase in  
 216 seedling root length in all tested dosages (Table 2).

217 **Table 2** – Average values of germination (G), number of abnormal seedlings (AS) and  
 218 dead seeds (DS), germination speed index (GSI), shoot length in cm (SL), root length in  
 219 cm (RL), and dry matter in grams (DM) of seedlings from common bean seeds treated  
 220 with *Cinnamomum cassia* essential oil and (*E*)-cinnamaldehyde.

Treatments	Traits*									
	G	AS	DS	GSI	SL	RL	DM			
Water	95.5	3.0	1.5	40.92	A	6.81	A	11.39	B	2.00
Soybean oil	95.0	1.5	3.5	28.13	C	5.73	B	11.74	B	1.86
( <i>E</i> )-cinnamaldehyde 8 µg/mL-1	95.5	1.5	3.0	29.75	C	7.26	A	12.18	B	1.96
( <i>E</i> )-cinnamaldehyde 80 µg/mL-1	96.0	1.0	3.0	35.69	B	7.04	A	12.52	B	1.94
( <i>E</i> )-cinnamaldehyde 400 µg/mL-1	95.5	1.5	3.0	27.71	C	6.27	B	12.28	B	1.92
( <i>E</i> )-cinnamaldehyde 1600 µg/mL-1	96.0	3.5	0.5	33.31	B	7.87	A	13.50	A	1.91
EO of <i>C. cassia</i> 10 µg/mL-1	94.5	2.0	3.5	35.21	B	6.81	A	13.46	A	1.98
EO of <i>C. cassia</i> 100 µg/mL-1	96.0	1.5	2.5	29.44	C	7.19	A	14.09	A	2.15
EO of <i>C. cassia</i> 500 µg/mL-1	95.0	3.5	1.5	38.67	A	6.90	A	15.01	A	2.07
EO of <i>C. cassia</i> 2000 µg/mL-1	95.0	3.0	2.0	28.23	C	6.71	A	13.54	A	2.00

221 \*Mean values with the same letter in the same column do not differ statistically  
 222 according to the Scott–Knott test ( $P \leq 0.05$ )

### 223 *In vitro* and *in vivo* antifungal assays

224 **Determination of minimum inhibitory concentration:** The minimum inhibitory  
 225 concentrations obtained with *C. cassia* essential oil and (*E*)-cinnamaldehyde were equal  
 226 (125 µg/mL), while the commercial fungicide methyl thiophanate presented MIC of 30.6  
 227 µg/mL. Commercial soybean oil did not cause reduction in mycelial growth at any of the  
 228 tested concentrations.

229 **Control of *C. lindemuthianum* on common bean seeds by *C. cassia* essential**  
 230 **oil/(*E*)-cinnamaldehyde** No significant effect was observed regarding emergence and  
 231 emergence speed index when seeds were treated with *C. cassia* essential oil, (*E*-  
 232 cinnamaldehyde, soybean oil and methyl thiophanate (Table 3).

233 **Table 3** – Average values of emergency (**E**), Emergence Speed Index (**ESI**) and dry  
 234 mass (**DM**) of seedlings from bean seeds treated with *Cinnamomum cassia* essential oil  
 235 (**EO**) and (*E*)-cinnamaldehyde.

<b>Treatment</b>	<b>E (%)</b>	<b>ESI</b>	<b>DM</b>
Water (control)	96.56	16.82	0.63
Soybean oil	93.39	8.13	0.56
Methyl Thiophanate- (control)	96.56	13.84	0.92
<i>Cinnamomum cassia</i> essential oil	93.65	10.67	0.64
( <i>E</i> )-cinnamaldehyde	94.97	9.63	0.81

236

237 For the incidence of anthracnose, until the 9th day of evaluation the controls did not differ  
 238 from the other treatments (Table S1). From the 12th day of evaluation, differences  
 239 between treatments and controls are already visible, and seeds treated with water and  
 240 soybean oil presented a higher anthracnose incidence in subsequent days.

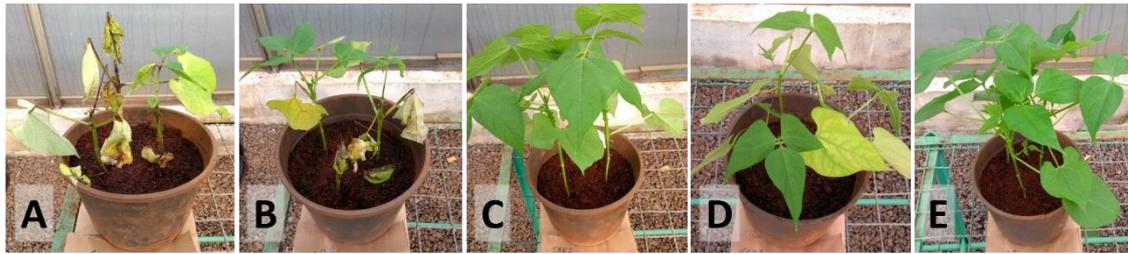
241 *C. cassia* essential oil and its major compound, (*E*)-cinnamaldehyde were allocated in the  
 242 same group of the commercial fungicide in the last four days of evaluation. In soybean  
 243 oil treatment, the incidence of anthracnose reaches 73.1%. For seeds treated only with  
 244 water, the incidence is 92.2%. The seeds treatment with solutions formulated from *C.*  
 245 *cassia* essential oil and (*E*)-cinnamaldehyde was efficient for reducing the incidence of  
 246 the disease over the days evaluated (Figure 1). The dose of 40 mL/kg formed a film  
 247 adhered to the tegument with a visually uniform coating on the seeds.

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249

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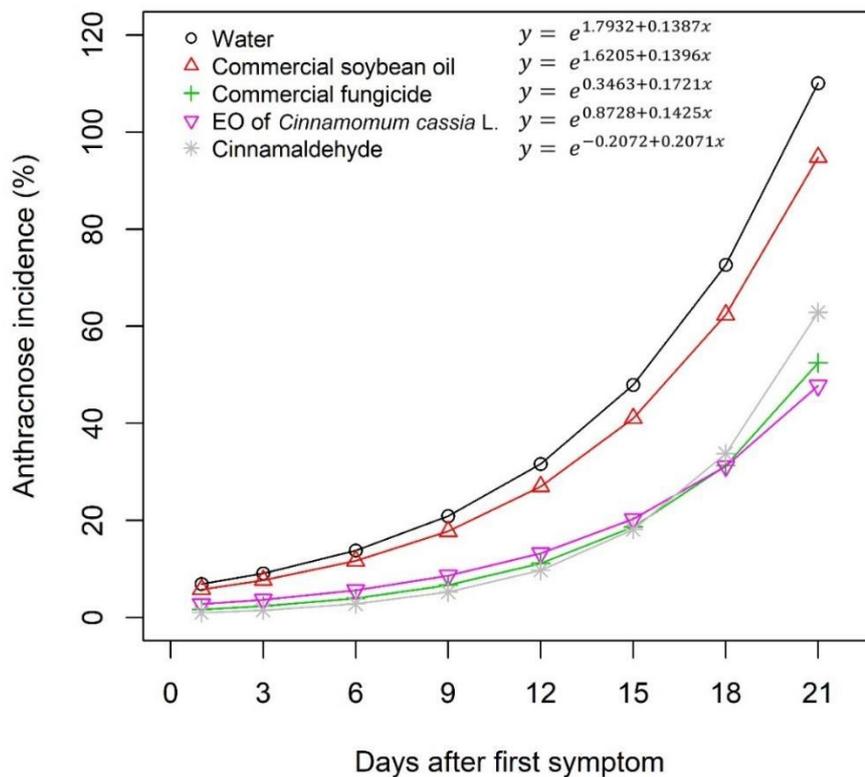


252 Figure 1: Common bean plants of the cultivar BRSMG Uai (last day of evaluation)  
253 from seeds treated with: A) Water (control); B) soybean oil (control); C) methyl  
254 thiophanate . D: *C. cassia* essential oil; E: (E)-cinnamaldehyde.

255

256 The Poisson regression model shows the type of correlation between incidence and  
257 evaluation time, summarizing the evolution of the disease over time (Figure 2).

258



259 **Figure 2.** Incidence of anthracnose (percentage) in common bean plants of the  
260 cultivar BRSMG Uai, derived from water-treated seeds, commercial soybean oil,  
261 commercial fungicide (methyl thiophanate), essential oil of *Cinnamomum cassia* and  
262 (E)-cinnamaldehyde.

263

264

265 Although there are significant differences for the incidence of anthracnose between the  
266 control treatments and the other treatments, the same was not observed for the plant vigor  
267 at 21 days (dry mass).

268

## 269 **DISCUSSION**

270 The use and application of essential oils (EOs) and their bioactive molecules with  
271 antifungal properties may represent an attractive and efficient alternative approach to  
272 inhibit the growth of fungal pathogens (Abdel-Kader et al. 2011; Stević et al. 2014), but  
273 essential oils also exhibit phytotoxicity depending on the doses applied to the seeds  
274 (Sodaeizadeh et al. 2010). Thus, the present study suggested seed treatment involves the  
275 solubilization of essential oils and their majority substances in commercial soybean  
276 vegetable oil. These strategy finds theoretical support in the high apolar substance  
277 solubilization capacity of vegetable oils, their spread capacity (Chaijuckam and Davis  
278 2010), and be easily accessible to the farmers.

279 The bioactivity of essential oil depends on the type, nature and concentration of  
280 its constituents (Batish et al. 2008). The analysis by GC-MS revealed that the amount of  
281 (*E*)-cinnamaldehyde (82.03%) is in accordance with the values found in the literature  
282 (Giordani et al. 2006; Li et al. 2013; Jardim et al. 2018). The narrow variation in the  
283 composition and percentage of compounds between oils is related to the environment in  
284 which the plant developed, the harvest stages, post-harvest storage time, as well as the  
285 extraction method of the essential oil (Yildirim et al. 2004; de Souza et al. 2017).

286 In the conditions of the present work there is no phytotoxic effect on seed germination,  
287 because in all treatments the germination percentages of the seeds were higher than 85%,  
288 which is above the national standards of production and commercialization of *P. vulgaris*  
289 seeds, which is 80% for certified seeds (MAPA 2015). In addition, seeds treated with

290 essential oil of *C. cassia* have significantly increased the length of the initial roots of the  
291 seedling, which can favor the establishment of the plant in the field, the water-capturing  
292 capacity of the root system, as well as a better utilization of nutrients (Lynch and van  
293 Beem 1993). The *in vivo* test with the seeds treated with the solutions of essential oils and  
294 (*E*)-cinnamaldehyde in the highest concentrations used *in vitro* showed no significant  
295 difference in the emergence, emergence speed and dry mass, evidencing that the essential  
296 oil of *C. cassia* and (*E*)-cinnamaldehyde are not phytotoxic to common bean seeds, in the  
297 evaluated conditions.

298 *In vitro* antifungal activity of *C. cassia* essential oil and (*E*)-cinnamaldehyde against *C.*  
299 *lindemuthianum* were similar, with MIC value (125 µg/mL). Since (*E*)-cinnamaldehyde  
300 corresponds to approximately 82% of the essential oil (Table 1), if this substance is the  
301 only component of the oil with antifungal activity, it would be expected that the MIC  
302 value of the oil would correspond to approximately 102 µg/mL (125 µg/mL x 0.82).  
303 However, the concentrations used were always multiplied at base 2, which means that  
304 there is no differentiation between these values in the experiment performed. Therefore,  
305 although it is clear that the majority component of *C. cassia* essential oil has activity  
306 against the fungus *C. lindemuthianum*, it is not possible to state that other components are  
307 inert. In any case, the antifungal activity observed in the present work for (*E*)-  
308 cinnamaldehyde is in accordance with the work of Neri et al. 2006. The authors  
309 investigated the effect of some substances on the fungus *Penicillium expansum*, and  
310 observed that (*E*)-cinnamaldehyde showed significant antifungal activity on mycelial  
311 growth, germination and sporulation of the fungus, presenting MIC value of 147 µL/L.  
312 Still on the *in vitro* test, we observed that soybean oil did not show antifungal activity,  
313 and this oil can be used as a neutral solvent for essential oils in seed treatment.

314 ***In vivo* applicability of *C. cassia* oil/(*E*)-cinnamaldehyde in control of *C.***  
315 ***lindemuthianum* on common bean seeds**

316 The pathogen did not affect the emergence of the seedling which suggests that the fungus  
317 did not infect the whole seed, because when the infection in the field is high, there is not  
318 even the seed formation. When to the seed developed completely, consequently it carry  
319 the inoculum (Yesuf and Sangchote 2007). In general, the plants presented only typical  
320 symptoms of anthracnose (Figure 3), which confirms the presence of the fungus in the  
321 seed.

322



323 **Figure 3** – Typical symptom of anthracnose on leaves of young (15 days) common bean  
324 plants (cultivar BRS Carioca MG) treated only with water.  
325

326 The cinnamon known as 'true cinnamon' (*Cinnamomum zeylanicum*), which contains (*E*)-  
327 cinnamaldehyde, has been studied. Teixeira et al. 2013 demonstrated that the incidence  
328 of *Stenocarpella maydis* fungus was reduced from 57% to 28% with the use of *C.*  
329 *zeylanicum* oil in the treatment of corn seeds at a concentration of 0.1  $\mu\text{L}/\text{mL}$ . Leite et al.  
330 2018 observed that this oil was also efficient in decreasing the incidence of the fungus

331 *Penicillium spp.* in common bean seeds during storage, using the dose of 2.3 mL/Kg of  
332 seeds.

333 In conclusion, the use of essential oil of *C. cassia* and (E)-cinnamaldehyde dissolved in  
334 soybean oil for the treatment of common bean seeds was effective to reduce the symptoms  
335 caused by anthracnose, and could replace conventional fungicides recommended for seed  
336 treatments. In addition these products, did not presented phytotoxicity. Our study is the  
337 first in demonstrating the efficacy of *C. cassia* essential oil and (E)-cinnamaldehyde  
338 against *C. lindemuthianum* present in common bean seed, using seed treatment.

### 339 **Conflicts of Interest**

340 The authors declare that the research was conducted in the absence of any commercial or  
341 financial relationships that could be construed as a potential conflict of interest.

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518 **Supplementary material**

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520 **Table S1** – Incidence of anthracnose in bean plants of the carioca type, cultivar BRSMG

521 Uai, from seeds treated with water, commercial soybean oil, positive control (methyl

522 thiophanate), essential oil (EO) of *Cinnamomum cassia* and (*E*)-cinnamaldehyde.

Treatments	Anthracnose incidence (%)*							
	Days after first symptom							
	0°	3°	6°	9°	12°	15°	18°	21°
Water	1.6 <b>A</b>	6.9 <b>A</b>	14.1 <b>A</b>	18.0 <b>A</b>	40.4 <b>A</b>	61.5 <b>A</b>	78.5 <b>A</b>	92.2 <b>A</b>
Commercial Soybean oil	0.0 <b>A</b>	5.4 <b>A</b>	12.4 <b>A</b>	16.1 <b>A</b>	28.4 <b>A</b>	63.1 <b>A</b>	69.7 <b>A</b>	73.1 <b>B</b>
Methyl thiophanate	1.6 <b>A</b>	1.6 <b>A</b>	1.6 <b>A</b>	1.7 <b>A</b>	16.2 <b>B</b>	26.3 <b>B</b>	38.1 <b>B</b>	41.3 <b>C</b>
EO of <i>C. cassia</i>	1.9 <b>A</b>	1.9 <b>A</b>	3.5 <b>A</b>	14.3 <b>A</b>	14.3 <b>B</b>	19.6 <b>B</b>	34.4 <b>B</b>	43.3 <b>C</b>
( <i>E</i> )-Cinnamaldehyde	0.0 <b>A</b>	0.0 <b>A</b>	2.0 <b>A</b>	5.9 <b>A</b>	9.4 <b>B</b>	20.4 <b>B</b>	47.8 <b>B</b>	49.7 <b>C</b>

523 \*Mean values with the same letter in a column do not differ statistically according to the  
 524 Scott–Knott test ( $P \leq 0.05$ )