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1 **Biological control of tomato gray mold caused by *Botrytis cinerea* by using**
2 ***Streptomyces* spp.**

3

4 Sawai Boukaew*, Poonsuk Prasertsan, Claire Troulet, Marc Bardin*

5

6 S. Boukaew, College of Innovation and Management, Songkhla Rajabhat University,

7 Songkhla 90000, Thailand; e-mail: sawai.bo@skru.ac.th

8 P. Prasertsan, Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of

9 Songkla University, Songkhla 90112, Thailand

10 S. Boukaew, C. Troulet, M. Bardin, Pathologie Végétale, INRA, 84140 Montfavet, France ; e-

11 mail: marc.bardin@inra.fr

12 * corresponding authors

13

14 **Abstract**

15 *Streptomyces* is a genus known for its ability to protect plants against many pathogens
16 and various strains of this bacteria have been used as biological control agents. In this study,
17 the efficacy of *Streptomyces philanthi* RM-1-138, *S. philanthi* RL-1-178, and *S. mycarofaciens*
18 SS-2-243 to control various strains of *Botrytis cinerea* was evaluated both *in vitro* and *in vivo*.
19 *In vitro* studies using confrontation tests on PDA plates indicated that the three strains of
20 *Streptomyces* spp. inhibited the growth of 41 strains of *B. cinerea*. Volatile compounds
21 produced by *Streptomyces* spp. had an influence on the growth of 10 strains of *B. cinerea* while
22 its culture filtrate at low concentration (diluted at 10^{-3}) showed a complete inhibition (100%) of
23 spore germination of *B. cinerea* strain BC1. A significant protection efficacy of tomato against
24 *B. cinerea* was observed on both whole-plant test (57.4%) and detached leaf test (60.1%) with
25 *S. philanti* RM-1-138. Moreover, this antagonistic strain had a preventive and a curative effect.

26 These results indicated that *S. philanthi* RM-1-138 may have the potential to control gray mold
27 caused by *B. cinerea* on tomato but further work is required to enhance its efficacy and its
28 survival *in planta*.

29

30 **Keywords:** *Botrytis cinerea* * Biological control * Durability * Sensitivity * *Streptomyces* spp.

31

32

33 **Introduction**

34 Gray mold disease caused by the fungus *Botrytis cinerea* Pers.: (teleomorph *Botryotinia*
35 *fuckeliana* (de Bary) Whetzel) is an economically important disease on numerous vegetable
36 including tomato (Jarvis 1980; Helbig 2002; Elad et al. 2015). *Botrytis cinerea* can infect the
37 plants either by direct penetration or through wounds caused by cultivation practices. Infection
38 is promoted by high humidity, free moisture on the plant surface, and low temperatures (Lee et
39 al. 2006). In general, disease management strategy largely relies on the use of chemical
40 fungicides that are generally considered harmful to the environment and can result in fungicide
41 resistance as previously reported (Locke and Fletcher 1988; Yourman and Jeffers 1999; Chung
42 et al. 2006). The development of efficient antifungal microbial agents could be an alternative
43 method to control the disease.

44 Species of *Streptomyces* are potential biocontrol agents since they are ubiquitous in the
45 environment and many of them produce secondary metabolites such as enzyme inhibitors and
46 antibiotics with diverse biological activities, including the ability to inhibit plant pathogenic
47 fungi (Omura 1992; Lange and Sanchez 1996; Vaz Jauri et al. 2016). Several species of
48 *Streptomyces* have been isolated and used to control plant pathogens on various crops, such as
49 *Pyricularia oryzae* and *Rhizoctonia solani* on rice (Prabavathy et al. 2006), *R. solani* and
50 *Sclerotium rolfsii* on sugar beet (Sadeghi et al. 2006; Errakhi et al. 2007) and *Sclerotinia*

51 *sclerotiorum* on sunflower (Baniyadi et al. 2009). In particular, strain RM-1-138 and RL-1-
52 178 of *S. philanthi* and strain SS-2-243 of *S. mycarofaciens*, isolated from the rhizosphere of
53 chili pepper in southern Thailand (Boukaew et al. 2011), have shown a good efficacy to control
54 *S. rolfsii*, *Ralstonia solanacearum* on chili (Boukaew et al. 2011), and *R. solani* on rice
55 (Boukaew et al. 2013, 2014a; 2014b). However, few studies explored the effect of *Streptomyces*
56 sp. on *B. cinerea*. For instance, the strain A01 of *Streptomyces lydicus* produces natamycin and
57 chitinase that have the potential to control gray mold caused by *B. cinerea* (Wu et al. 2013).

58 In all the studies cited above, the effect of the biocontrol agent *Streptomyces* spp. was
59 tested against a single strain of the plant pathogen considered. However, some studies highlight
60 differences in the sensitivity of various isolates of plant pathogens to biocontrol agents, with
61 the existence of less sensitive isolates in natural populations of plant pathogens and even the
62 capacity of plant pathogens to adapt to biocontrol agents (Bardin et al. 2015). Diversity of
63 sensitivity of plant pathogens to biocontrol agents may result in a reduction of efficacy of the
64 biocontrol agent in the field and if selection pressure would increase, *i.e.* intensive use of
65 biocontrol agents by growers, we can expect a selection of the less sensitive isolates and
66 consequently a reduced efficacy of biocontrol agents against plant pathogens in the field.

67 The first objective of the present study was to evaluate the ability of three strains of
68 *Streptomyces* spp. to inhibit the growth of *B. cinerea in vitro* and on tomato plants. The three
69 tested strains, RM-1-138 and RL-1-178 of *Streptomyces philanthi* and SS-2-243 of *S.*
70 *mycarofaciens*, are known for their antifungal effect on various plant pathogens (Boukaew et
71 al. 2011, 2013, 2014a; 2014b), but they have never been tested against *B. cinerea*. The second
72 objective of the study was to assess the possible variability in susceptibility of various strains
73 of *B. cinerea* to these antagonistic strains. To this end, 41 strains of *B. cinerea* differing in their
74 date of isolation, geographic and host or substrate of origin were used.

75

76

77 **Materials and Methods**

78 Microorganisms and inoculum preparation

79 Strains RM-1-138 and RL-1-178 of *S. philanthi* and strain SS-2-243 of *S. mycarofaciens*
80 were isolated in a previous study from the rhizosphere of chili pepper in southern Thailand
81 (Boukaew et al. 2011). Spores of *Streptomyces* were collected in 10 ml water from 10-day-old
82 culture on glucose yeast-malt agar at 30°C and counted using a hemacytometer. Inoculum
83 was prepared by dilution in distilled sterilized water to achieve the required concentration.

84 Forty-one strains of *Botrytis cinerea* differing in their date of isolation, geographic and
85 plant hosts or substrate of origin were used in this study. All isolates were single-spored and
86 conserved at -20°C before use. In all tests, the *B. cinerea* strain BC1, known to be very
87 aggressive on tomato (Decognet et al. 2009), was used. An inoculum of *B. cinerea* was prepared
88 by cultivating the strain on potato dextrose agar (PDA) plates under cool white fluorescent light
89 (14-h photoperiod at 65 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in a growth chamber at 21°C. Spores were collected in 5
90 ml water from 14-day-old culture and counted using a hemacytometer. Inoculum was
91 prepared by dilution in distilled sterilized water to achieve the required concentration.

92

93 Dual culture

94 The three strains of *Streptomyces* spp. were evaluated for their antagonistic properties
95 against forty-one strains of *B. cinerea* using a dual culture technique (Islam et al. 2009). For
96 each strain of *Streptomyces* spp., a streak of spore suspension at 10^7 spores.ml⁻¹ was deposited
97 on one side of a PDA medium in Petri dishes. In previous study, Boukaew et al. (2011) have
98 shown that the antagonistic activity of the 3 strains of *Streptomyces* by dual culture on agar was
99 observed after 7 days of incubation at 28°C. For RM-1-138 the optimal activity of antifungal
100 metabolites was observed for culture filtrate obtained after 9 to 11 days of incubation at 28°C

101 (Boukaew and Prasertsan 2014b). Plates were then incubated in a growth chamber for 10 days
102 at 21°C in the dark.

103 A 5-mm-diameter mycelial plug, excised from a 3-day-old *B. cinerea* colony, was
104 transferred to the center of each plate. As a control, a mycelial plug of *B. cinerea* was placed
105 on a PDA plate without any *Streptomyces* strain. The dual culture plates were further incubated
106 in a growth chamber (21°C, dark) for 2 days, after which the radial mycelial growth of *B.*
107 *cinerea* was measured and compared to that of the control. Three replicates were conducted for
108 each *Streptomyces–B. cinerea* strain combination. The colony size in each treatment was
109 recorded and the percentage inhibition of hyphal growth was calculated.

110

111 Volatile effect

112 *Streptomyces philanthi* RM-1-138 and RL-1-178, and *S. mycarofaciens* SS-2-243 were
113 evaluated for the effect of their volatile substances on the mycelial development of ten strains
114 of *B. cinerea*. Strains of *B. cinerea* tested were selected based on their susceptibility to
115 *Streptomyces* spp. in the dual culture assay: the 5 most sensitive strains and the 5 less sensitive
116 strains to *Streptomyces* spp. were tested. For each strain of *Streptomyces* spp., a streak of a
117 spore suspension at 10^7 spores.ml⁻¹ was deposited on PDA medium in a Petri dish. After a ten
118 days incubation in a growth chamber (21°C, dark) a Petri dish containing *Streptomyces* spp.
119 was covered with another Petri dish containing PDA medium inoculated with a 5 mm-diameter
120 mycelial plug of *B. cinerea*. The two plates were then sealed using double layers of Parafilm to
121 obtain a double-plate chamber (Raza et al. 2015). Double plates were incubated in a growth
122 chamber at 21°C (14 h day and 10 h night). After 2 days of incubation, the diameters of the
123 colonies of *B. cinerea* were recorded, and the percentage inhibition of hyphal growth was
124 calculated. For each treatment, three replicates were realized.

125

126 Culture filtrate effect

127 *Streptomyces philanthi* RM-1-138 and RL-1-178, and *S. mycarofaciens* SS-2-243 were
128 each grown at 30°C in a 250 ml flask containing 100 ml liquid GYM medium (glucose yeast-
129 malt extract broth, pH adjusted at 7.0 before autoclaving) on a rotary shaker at 150 rpm. After
130 3 days of incubation, 5 ml aliquots of this culture was transferred into 100 ml fresh GYM
131 medium, and incubated for 10 days under the same conditions. The culture broth was
132 centrifuged (8880 g for 20 min), then filtered through a 0.45 mm Millipore membrane to recover
133 the culture filtrate.

134 Serial dilutions of the culture filtrate (1/10, 1/100 and 1/1000) were prepared in distilled
135 sterile water. Ten µl of the different dilutions were then mixed with 2 µl of a spore suspension
136 of the strain BC1 of *B. cinerea* dosed at 10⁵ spores.ml⁻¹ and dropped on a glass-slide. Sterile
137 Potato Dextrose Broth (PDB) was used instead of the culture filtrate as a positive control. The
138 slides were further placed in Petri dishes with humid absorbent paper to maintain high relative
139 humidity and incubated under cool white fluorescent light (14-h photoperiod-65 µmol m⁻² s⁻¹)
140 in a growth chamber at 21°C for 24 h. Spores that germinate were counted under the microscope
141 and the inhibition of spore germination (in percentage) was calculated.

142

143 Efficacy of *Streptomyces* spp. against *B. cinerea* on tomato

144 Seed of tomato cv. Monalbo (INRA Plant Pathology, Avignon, France) were sown in
145 compost and transplanted after 1 week in an individual pot containing a horticulture compost
146 mix (De Baat, Coevorden, The Netherlands). Plants were grown in a glasshouse for 7 to 8 weeks
147 where they received a standard commercial nutrient solution once or twice a day, depending on
148 needs. They had at least 8 fully expended leaves when used.

149 Two types of test were performed to evaluate the efficacy of *Streptomyces* spp. to
150 control *B. cinerea* on tomato plants: a detached-leaf assay and a whole-plant assay. Tests were

151 first carried out with the three strains of *Streptomyces* spp. (RM-1-138, RL-1-178 and SS-2-
152 243) against the strain BC1 of *B. cinerea*. Additional tests with RM-1-138 were realized with
153 3 strains of *B. cinerea* (BC1, C10 and CEN12-13) having different level of sensitivity to RM-
154 1-138 in dual culture tests.

155 For the detached-leaf assay, leaves were sprayed with a spore suspension of
156 *Streptomyces* spp. at the concentration of 10^6 or 10^7 spores.ml⁻¹. When the leaves were dried
157 (between 30 and 60 minutes after treatment), they were removed, and two leaflets were placed
158 in a transparent plastic box with humid absorbent paper to maintain high relative humidity
159 (close to 100%). Mycelial plugs (5-mm in diameter) of *B. cinerea* excised from the growing
160 margin of a 3-day-old PDA cultures were deposited onto the leaflets of tomato. For each
161 treatment, 6 leaflets of tomato (3 plastic boxes) were inoculated. Following inoculation, the
162 detached-leaves were incubated in a growth chamber in conditions conducive to disease
163 development (21°C, 14 h-photoperiod). Leaves removed from non-treated plants were
164 inoculated as a control. The lesion area was determined with ImageJ software 2 days after
165 inoculation. Three independent repetitions of the test were realized.

166 For the whole-plant test, three leaves per plant were removed leaving 5-10 mm petiole
167 stubs on the stems and each pruning wound was inoculated with 10 µl of a spore suspension of
168 *B. cinerea* dosed at 10^6 spores.ml⁻¹, in order to place *Botrytis* in the most favorable conditions
169 for infestation of the stem. *Streptomyces* spp. was then applied to pruning wounds with 10 µl
170 of a spore suspension dosed at 10^6 or 10^7 spores.ml⁻¹, 5 min after the inoculation of *B. cinerea*,
171 the time that the drop was absorbed by the petiole. The pathogen was inoculated alone as a
172 control. All plants were incubated in a growth chamber with a photoperiod of 14 h and
173 maintained at 21°C with a relative humidity above 80% to favor disease development. The
174 experiment was repeated three times independently, each with three replicates per treatment.

175 Lesion expansion on tomato stem was recorded daily for 7 days after inoculation (DAI).
 176 To take into account the kinetics of disease development, we computed the area under the
 177 disease progress curves (AUDPC) as described by Decognet et al. (2009), as:

$$178 \quad AUDPC = \left[\frac{Y_1}{2} + \sum_2^{n-1} Y_j + \frac{Y_n}{2} \right] \cdot [I]$$

179 where Y_j was the observed lesion length (in mm) at the j^{th} observation time, n was the total
 180 number of observations, and I the interval between each observation (in days). These values
 181 were computed for individual pruning lesions for $n=5$ observations at daily intervals during the
 182 period from the 3rd to the 7th day after inoculation. For both tests, a percentage of protection of
 183 tomato generated by the bacteria, compared to the control, was computed.

184

185 Preventive and curative effect of *Streptomyces* against *B. cinerea* on tomato plants

186 To test a potential preventive effect of the 3 strains of *Streptomyces* against *B. cinerea*
 187 on tomato plants, three leaves per plant were removed, leaving 5-10 mm petiole stubs on the
 188 stems. Each pruning wound was treated with 10 μl of a spore suspension at 10^7 spores. ml^{-1} of a
 189 strain of *Streptomyces* sp. and then inoculated with 10 μl of a spore suspension at 10^6 spores. ml^{-1}
 190 ¹ of the strain BC1 of *B. cinerea*. Inoculation of BC1 was realized 0, 2, 4 or 6 h after treatment
 191 with the selected strain of *Streptomyces*.

192 To test a potential curative effect of the 3 strains of *Streptomyces* against *B. cinerea*,
 193 the same method was used except that inoculation of *B. cinerea* at 10^6 spores. ml^{-1} was realized
 194 before the treatment with a spore suspension at 10^7 spores. ml^{-1} of a strain of *Streptomyces* sp.
 195 In this case, treatment with *Streptomyces* was realized 0, 2, 4 or 6 h after the inoculation with
 196 the strains BC1 of *B. cinerea*.

197 All plants were incubated in a growth chamber with a photoperiod of 14 h and
 198 maintained at 21°C with a relative humidity above 80% to favor disease development. The

199 experiment was repeated three times independently, each with three replicates (3 plants each
200 with 3 pruning wounds) per treatment. Lesion expansion on tomato stem was recorded from the
201 3rd to the 7th day after inoculation (DAI) and AUDPC and protection index were computed as
202 described above.

203

204 Statistical analysis

205 The data were subjected to one- or two-factor analysis of variance (ANOVA) using
206 Statistical Package for the Social Sciences (SPSS) version 15 for Windows. When appropriate,
207 the means were compared with the Duncan's Multiple Range Test (DMRT) and a $P < 0.05$ was
208 considered as being significantly different.

209

210

211 Results

212 *In vitro* antagonism of *Streptomyces* spp. against different strains of *B. cinerea*

213 The *in vitro* dual culture assay shows that the three strains of *Streptomyces* spp. inhibited
214 the mycelial growth of the forty-one strains of *B. cinerea* in the range of 73% to 100% inhibition
215 on PDA medium after two days of incubation at 21°C, compared with the control treatment
216 without *Streptomyces* (Fig. 1). Significant differences in sensitivity to the 3 strains of
217 *Streptomyces* sp. were observed between strains of *B. cinerea* (*S. philanthi* RM-1-138; $F = 4.36$,
218 $df = 40, 82$, $P < 0.0001$; *S. philanthi* RL-1-178; $F = 3.45$, $df = 40, 82$, $P < 0.0001$; *S.*
219 *mycarofaciens* SS-2-243; $F = 3.03$, $df = 40, 82$, $P < 0.0001$). The strains RM-1-138 and RL-1-
220 178 of *S. philanthi* completely inhibit the mycelial growth of 31 out of the 41 strains of *B.*
221 *cinerea* tested and the strain SS-2-243 of *S. mycarofaciens* completely inhibited the mycelial
222 growth of 25 strains of *B. cinerea*. Culture filtrates of each strain of *Streptomyces* have a strong
223 inhibitory effect on the spore germination of the strains BC1 of *B. cinerea* (Fig. S1;

224 Supplementary data). This inhibitory effect is very effective (100% inhibition of spore
225 germination) even when the culture filtrate is diluted at 10^{-3} , suggesting a strong antifungal
226 effect of the 3 strains of *Streptomyces* spp. tested.

227 To test the effect of volatile compounds produced by *Streptomyces* spp. on the mycelial
228 growth of *B. cinerea*, the *in vitro* inhibitory action of volatiles was tested on ten isolates of *B.*
229 *cinerea* having various level of sensitivity to the bacteria based on dual culture assay (Fig. 2).
230 Significant differences between strains of *Streptomyces* spp. was observed (*S. philanthi* RM-1-
231 138; $F = 63.64$, $df = 9, 20$, $P < 0.0001$; *S. philanthi* RL-1-178; $F = 92.53$, $df = 9, 20$, $P < 0.0001$;
232 *S. mycarofaciens* SS-2-243; $F = 51.28$, $df = 9, 20$, $P < 0.0001$) with an inhibition ranging from
233 6 to 30% depending both on the strain of *B. cinerea* and on the strain of *Streptomyces* spp. Some
234 strains of *B. cinerea* are significantly less sensitive to *Streptomyces* than others. The strain
235 E258 of *B. cinerea* is for instance among the least sensitive strain against the three strains of
236 *Streptomyces*.

237

238 Efficacy of *Streptomyces* spp. against *B. cinerea* on tomato

239 The protective efficacy of the three strains of *Streptomyces* spp. against the strain BC1
240 of *B. cinerea* was evaluated on tomato plants, both on a detached-leaf test and on a whole-plant
241 test. Two spore concentrations of *Streptomyces* spp. (10^6 or 10^7 spores.ml⁻¹) were evaluated.
242 Globally, the highest is the spore concentration of *Streptomyces* spp. the most effective is the
243 control of *B. cinerea* on plant organs (Fig. 3). A 2-way analysis of variance (strain of
244 *Streptomyces* spp. x dose of bacteria done on the protection of the biological control agent) was
245 realized independently on detached-leaf and on whole-plant bioassays. It reveals a *Streptomyces*
246 strain effect ($F = 15.62$, $df = 2$, $P < 0.0001$) and a dose effect ($F = 49.04$, $df = 1$, $P < 0.0001$)
247 on detached-leaf bioassay and a *Streptomyces* strain effect ($F = 9.47$, $df = 2$, $P = 0.003$) and a
248 dose effect ($F = 7.95$, $df = 1$, $P = 0.015$) on whole-plant bioassay, with no interaction effect (F

249 = 0.82, $df = 2$, $P = 0.464$ and $F = 1.58$, $df = 2$, $P = 0.246$ for detached-leaf and whole plant
250 bioassays, respectively). The strain RM-1-138 of *S. philanthi* at 10^7 spores.ml⁻¹ exhibits the
251 strongest protection effect against *B. cinerea* on tomato plants with 57% protection on whole
252 plants and 60% protection on detached leaves. The two other strains of *Streptomyces* sp. provide
253 a lower protective effect against *B. cinerea* on tomato.

254

255 Efficacy of the strain RM-1-138 of *S. philanthi* against 3 different strains of *B. cinerea* on
256 tomato

257 Three isolates of *B. cinerea* having various levels of *in vitro* sensitivity to the strain RM-
258 1-138 were tested on tomato plants to estimate the efficacy of protection generated by this strain
259 (Fig. 4). On both bioassays (whole plant test and detached leaf test) and whatever the dose of
260 RM-1-138 applied (10^6 or 10^7 spores.ml⁻¹), lesions caused by *B. cinerea* were significantly
261 reduced compared to the control without the biocontrol agent. A 2-way analysis of variance
262 (strain of *B. cinerea* X dose of RM-1-138 on the protection of the biological control agent) was
263 realized for each bioassay. On detached-leaf bioassay, analysis reveals a *B. cinerea* strain effect
264 ($F = 5.08$, $df = 1$, $P = 0.025$) and a dose effect ($F = 17.02$, $df = 2$, $P = 0.001$) with no interaction
265 ($F = 0.36$, $df = 2$, $P = 0.703$). On whole-plant bioassay, an absence of *B. cinerea* strain effect
266 ($F = 1.40$, $df = 2$, $P = 0.284$), a dose effect ($F = 4.22$, $df = 2$, $P = 0.05$) and no interaction effect
267 ($F = 0.28$, $df = 2$, $P = 0.764$) were observed. *S. philanti* RM-1-138 at 10^7 spores.ml⁻¹ displays
268 a protection of tomato plants comprised between 45% and 52% on whole plant tests and
269 between 44% and 60% on detached-leaf tests, depending on the strain of *B. cinerea* tested.

270

271

272

273 Preventive and curative effect of *S. philanthi* RM-1-138 against *B. cinerea* on tomato plant

274 Treatment of pruning wounds of tomato with a spore suspension of *S. philanthi* RM-1-
275 138 concomitantly with an inoculation of a spore suspension of *B. cinerea* gives a protection
276 index of approximately 50% (Fig. 5). When the plants are treated with *S. philanthi* few hours
277 before the inoculation with *B. cinerea* the protective effect decreases significantly (ANOVA on
278 the protection index; $F = 342.06$, $df = 3, 8$, $P < 0.0001$). A treatment realized on pruning wounds
279 with *S. philanthi*, 6 hours before the inoculation with *B. cinerea* tends to favor the development
280 of *B. cinerea* (protection index = -23%). When the plants are treated with *S. philanthi* few hours
281 after the inoculation with *B. cinerea*, the protective effect also decreases significantly (ANOVA
282 on the protection index; $F = 172.14$, $df = 3, 8$, $P < 0.0001$). The protection index attained less
283 than 5% when *S. philanthi* was inoculated 6 hours after the fungal pathogen *B. cinerea*.

284

285 Discussion

286 The three strains of *Streptomyces* spp. tested in this study had a significant effect on the
287 mycelial growth of all strains of *B. cinerea* tested on PDA agar plates, suggesting a strong direct
288 antifungal effect. Inhibition of germination of *B. cinerea* conidia is also achieved by the culture
289 filtrate of the three strains of *Streptomyces* spp. tested. This result was also observed for other
290 strains of *Streptomyces* such as the culture filtrate of *S. globisporus* JK-1 that inhibit the spore
291 germination of *Magnaporthe oryzae* and reduced its appressorial formation on rice leaves (Li
292 et al. 2011). Moreover, volatile compounds produced by the strains *Streptomyces* spp. have also
293 an inhibitory effect against *B. cinerea* mycelial growth. The genus *Streptomyces* is known to
294 produce volatile antifungal compounds (Vaz Jauri et al. 2016). Volatile compounds produced
295 by *S. globisporus* JK-1 was for instance able to control *Penicillium italicum* on *Citrus*
296 *microcarpa* and *B. cinerea* on tomato fruit (Li et al. 2010; Li et al. 2012). *Streptomyces albulus*
297 NJZJSA2 also produced volatile compounds effective against *Fusarium oxysporum* and
298 *Sclerotinia sclerotiorum* (Wu et al. 2015).

299 Testing 41 strains of *B. cinerea* for their degree of sensitivity to the three strains of
300 *Streptomyces* spp. revealed that some strains of *B. cinerea* are less sensitive to the inhibitory
301 effect of antimicrobial compounds produced by these bacteria. Studies highlighting the
302 diversity in sensitivity of plant pathogens to antimicrobial compounds produced by biocontrol
303 agents have been listed by Bardin et al. (2015). However to our knowledge, this study is the
304 first one revealing the diversity of sensitivity of a plant pathogen to volatile compounds
305 produced by a biocontrol agent. Detection of less sensitive isolates to these compounds may
306 indicate a risk of developing a resistance in case of increased selection pressure exerted by the
307 given biocontrol agents. Even if a limited diversity in the sensitivity of the different strains of
308 *B. cinerea* was observed, further studies are needed to evaluate the capacity of *B. cinerea* to
309 become resistant to these compounds. Indeed, previous studies have demonstrated that *B.*
310 *cinerea* can evolve and become resistant to antibiotic produced by biocontrol agents (Ajouz et
311 al. 2010; Fillinger et al. 2012).

312 In this study, we demonstrated that spore suspension of the three isolates of *S. philanthy*
313 and *S. mycarofaciens* tested in this study can significantly control *B. cinerea* on leaves and stem
314 of tomato plants. Among the three strains tested, *S. philanthy* RM-1-138 exhibit the strongest
315 protective efficacy against *B. cinerea* with 57% protection on whole-plant and 60% protection
316 on the detached leaf, suggesting that this strain may have the best potential to be used for
317 protection of tomato grown in greenhouses against gray mold. These results confirm the
318 potential of strains belonging to the genus *Streptomyces* to control *B. cinerea* (Ge et al. 2015;
319 Jiang et al. 2016). The best protection of tomato against *B. cinerea* was observed when spore
320 suspension of *S. philanthy* were inoculated concomitantly with spores of *B. cinerea*, given a
321 protection index superior to 50% both on leaves and on the stem. However, when the plants
322 were treated with *S. philanthy* before or after the inoculation of *B. cinerea*, the protective effect
323 decreased dramatically. The absence of a preventive effect by *S. philanthy* suggested that this

324 bacteria is not able to induce a strong systemic resistance in tomato plants and also that the
325 strain may not be able to survive in the plant. This low capacity to survive within the plant could
326 be due to its sensitivity to molecules produced by the plant. It could also be due to the
327 temperature of the tests (21°C) which is not favorable to the growth of *S. philanthi* (Boukaew
328 and Prasertsan 2014b). Experiments will be done to test these hypothesis and to measure the
329 ability of this strain to survive in tomato tissues. The absence of curative effect suggests that
330 the bacteria is not able to produce enough amount of antifungal compounds to limit the
331 development of *B. cinerea* on the plant. It reinforces the hypothesis that this bacteria is not able
332 to survive and to develop on the plant. More work is therefore needed to develop a formulation
333 to consider using this strain preventively or curatively.

334 Our results demonstrated that the 3 strains of *Streptomyces* spp. produce very effective
335 anti-*Botrytis* substances. The antagonistic mechanism of *Streptomyces* spp. is therefore
336 probably related to the production of inhibitory antifungal volatile and non-volatile compounds.
337 These results confirm that species of *Streptomyces* spp. can produce a wide spectrum of
338 antifungal compounds such as antibiotics and volatile compounds (Vaz Jauri et al. 2016). The
339 isolation and the characterization of these substances could lead to the development of effective
340 and stable molecule-based product. Other modes of action could be tested, and a variety of
341 fungal cell wall-degrading enzymes associated to biocontrol activity, such as chitinases and β -
342 1,3-glucanase are known to be synthesized by *Streptomyces* (de Boer et al. 1998; Mahadevan
343 and Crawford 1997; Mukherjee and Sen 2006; Vaz Jauri et al. 2016).

344 In conclusion, the strains of *Streptomyces* tested in this study may have the potential to
345 control gray mold caused by *B. cinerea* on tomato but further work is definitely required to
346 enhance its efficacy *in planta*. For instance improvement in the production process and
347 development of a suitable formulation of the bacteria may result in a more effective product.

348

349

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463 **Figure Captions**

464 **Fig. 1** Mycelial growth inhibition of the forty-one strains of *Botrytis cinerea* caused by the
465 strains RM-1-138 (a) and RL-1-178 (b) of *S. philanthi* and the strain SS-2-243 (c) of *S.*
466 *mycarofaciens* revealed by dual culture technique on PDA agar plates and incubated at 21°C
467 for 2 days. Values are mean of three replications (\pm SE). Values with the same letter are not
468 significantly different (ANOVA, $P < 0.05$; Duncan multiple range test).

469

470 **Fig. 2** Mycelial growth inhibition of ten strains of *B. cinerea* caused by volatile substances
471 produced by each of the strains RM-1-138 (a) and RL-1-178 (b) of *S. philanthi* and the strain
472 SS-2-243 (c) of *S. mycarofaciens* on PDA agar plates and incubated at 21°C for 2 days. Values
473 are mean of three replications (\pm SE). Values with the same letter are not significantly different
474 (ANOVA, $P < 0.05$; Duncan multiple range test).

475

476 **Fig. 3** Protection of tomato plants (in percentage) on whole-plant assay (a) and detached-leaf
477 assay (b) against the strain BC1 of *B. cinerea* with the strains RM-1-138 and RL-1-178 of *S.*
478 *philanthi* and the strain SS-2-243 of *S. mycarofaciens* at two spore concentrations (10^6 and 10^7
479 spores.ml⁻¹). Values are mean of three repetitions (\pm SE). To test the hypothesis that the efficacy
480 of a biocontrol agent was not dependent on the strain of *Streptomyces* spp. and on the dose of
481 bacteria used, a 2-way analysis of variance (strain of *Streptomyces* spp. X dose of bacteria) was
482 realized for each bioassay and the Duncan's Multiple Range Test was used to compare the
483 percentage of protection of tomato against *B. cinerea*. Values with the same letter are not
484 significantly different for each bioassay independently (2-way ANOVA done on protection, P
485 < 0.05 ; Duncan multiple range test).

486

487

488 **Fig. 4** Protection of tomato plants (in percentage) on whole-plant assay (a) and detached-leaf
489 assay (b) against 3 different strains of *B. cinerea* with the strain RM-1-138 of *S. philanthi* at
490 two spore concentrations (10^6 and 10^7 spores.ml⁻¹). Values are mean of three repetitions (\pm SE).
491 To test the hypothesis that the efficacy of a biocontrol agent was not dependent on the strain of
492 *B. cinerea* and on the dose of the strain of RM-1-138 of *S. philanthi*, a 2-way analysis of
493 variance was realized and the Duncan's Multiple Range Test was used to compare the means
494 of the percentage of protection of tomato against *B. cinerea*. Values with the same letter are
495 not significantly different at $P < 0.05$.

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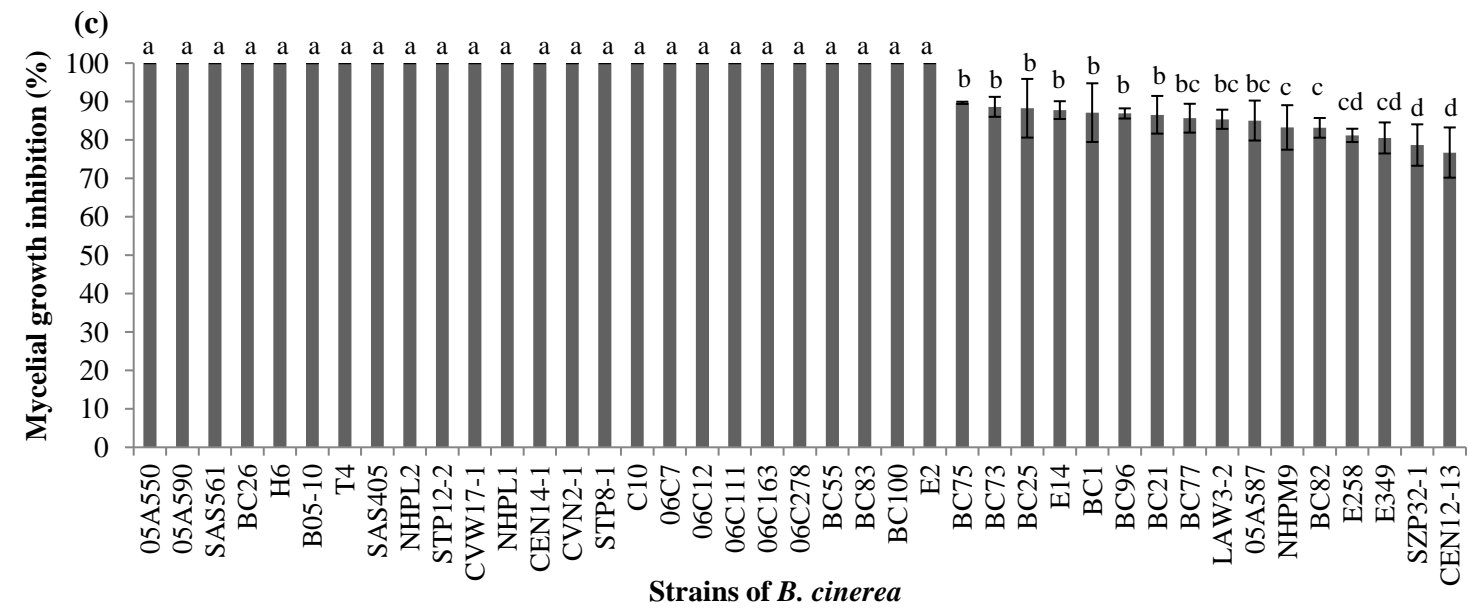
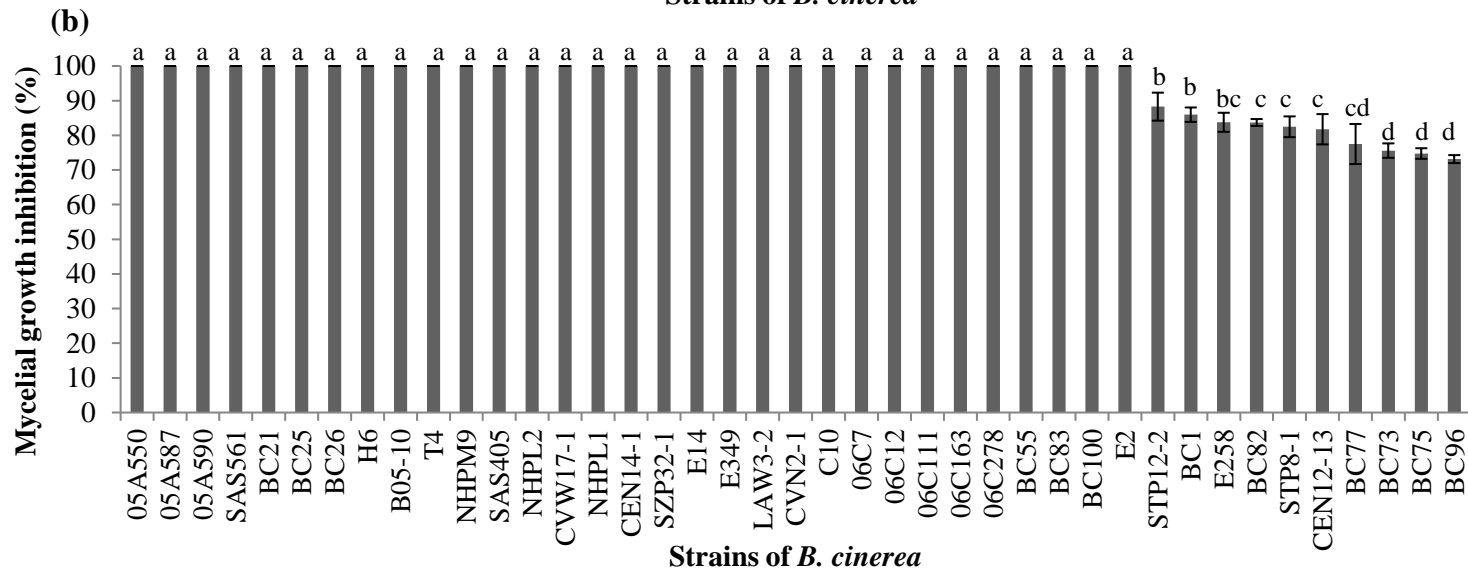
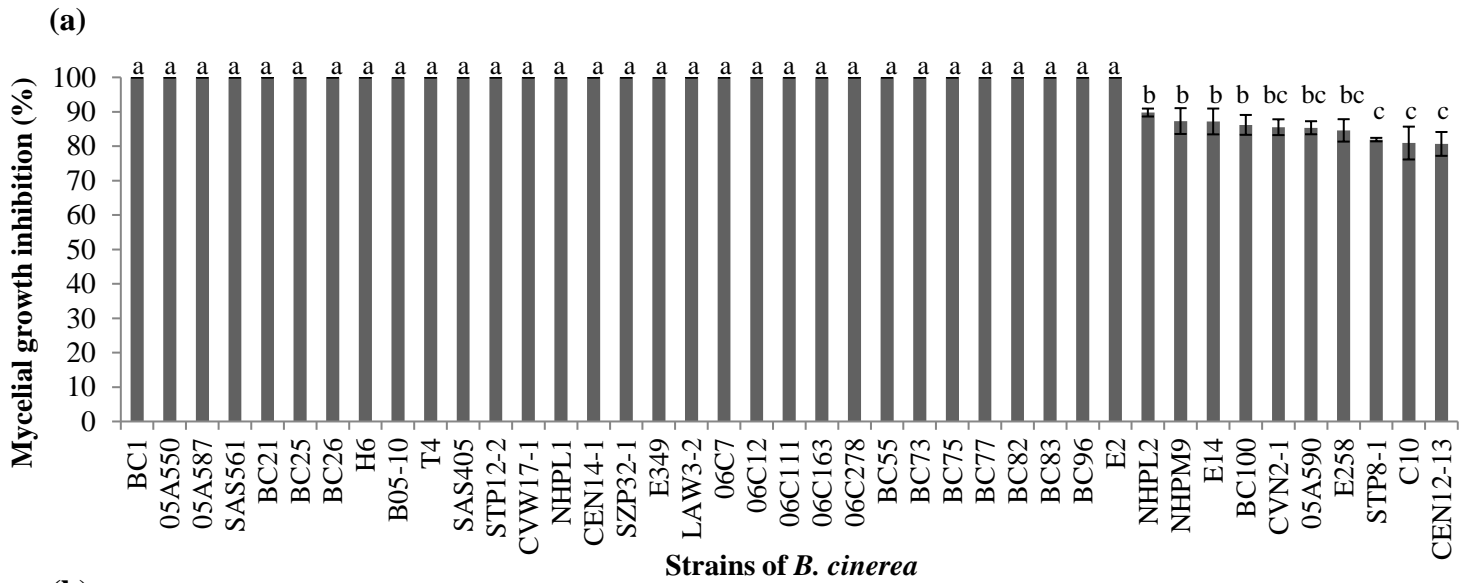
498 **Fig. 5** Timing of preventive (a) or curative (b) measures by the strain RM-1-138 of *S.*
499 *philanthi* on the protection of tomato plants inoculated with *B. cinerea*. Values are mean of
500 three replications (\pm SE). Values with the same letter are not significantly different (ANOVA,
501 $P < 0.05$; Duncan multiple range test).

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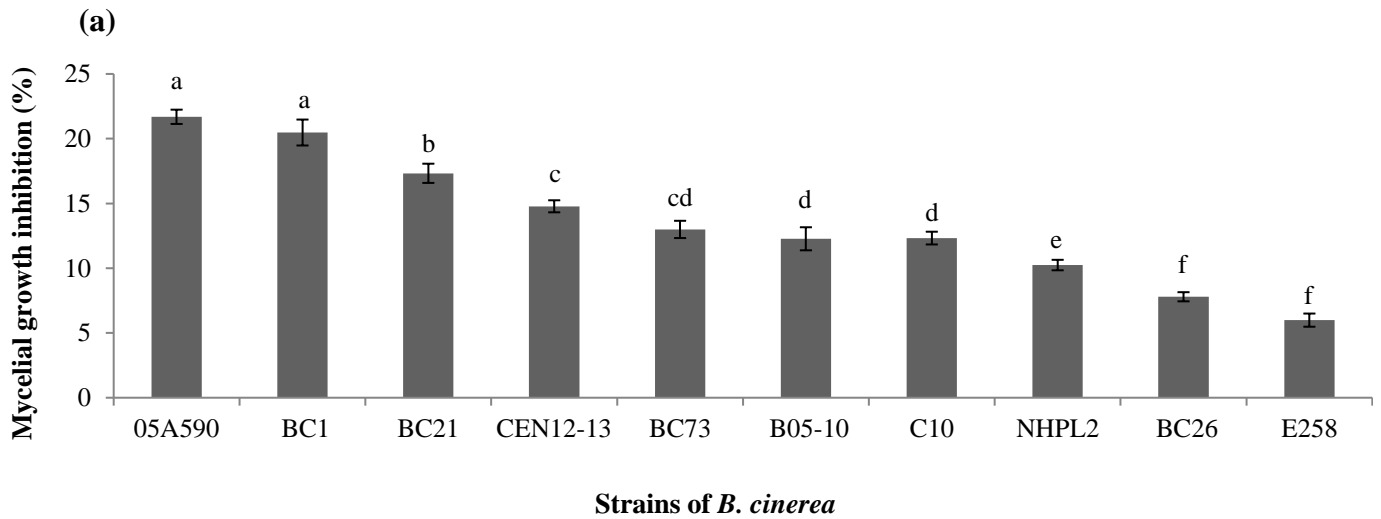
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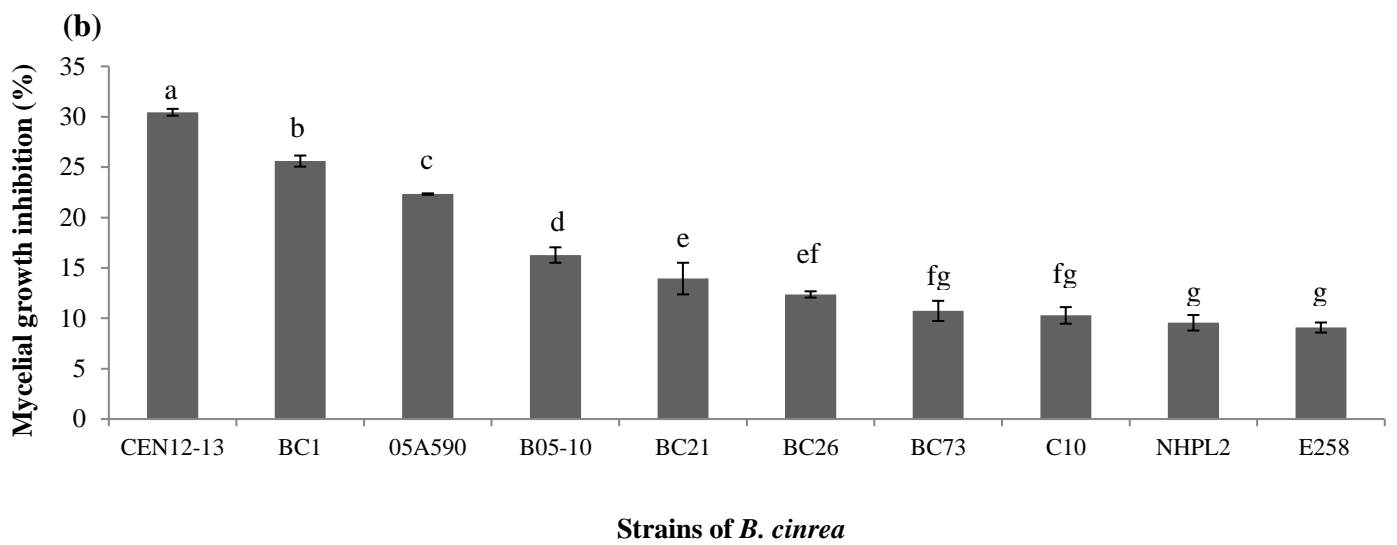


509 **Fig. 1**

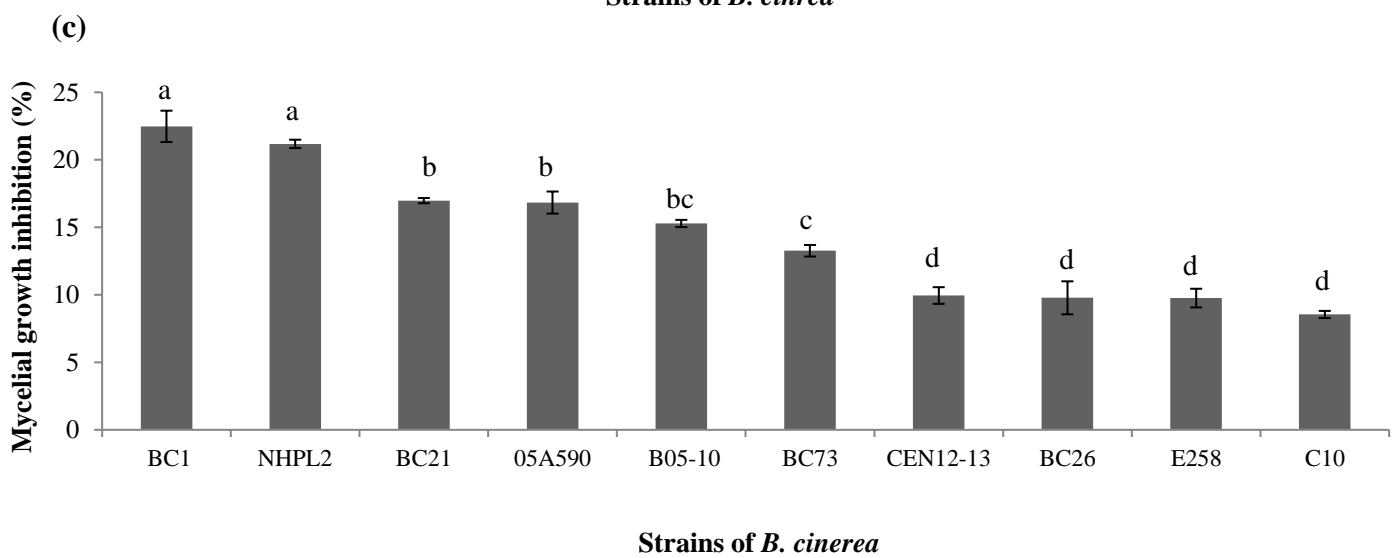
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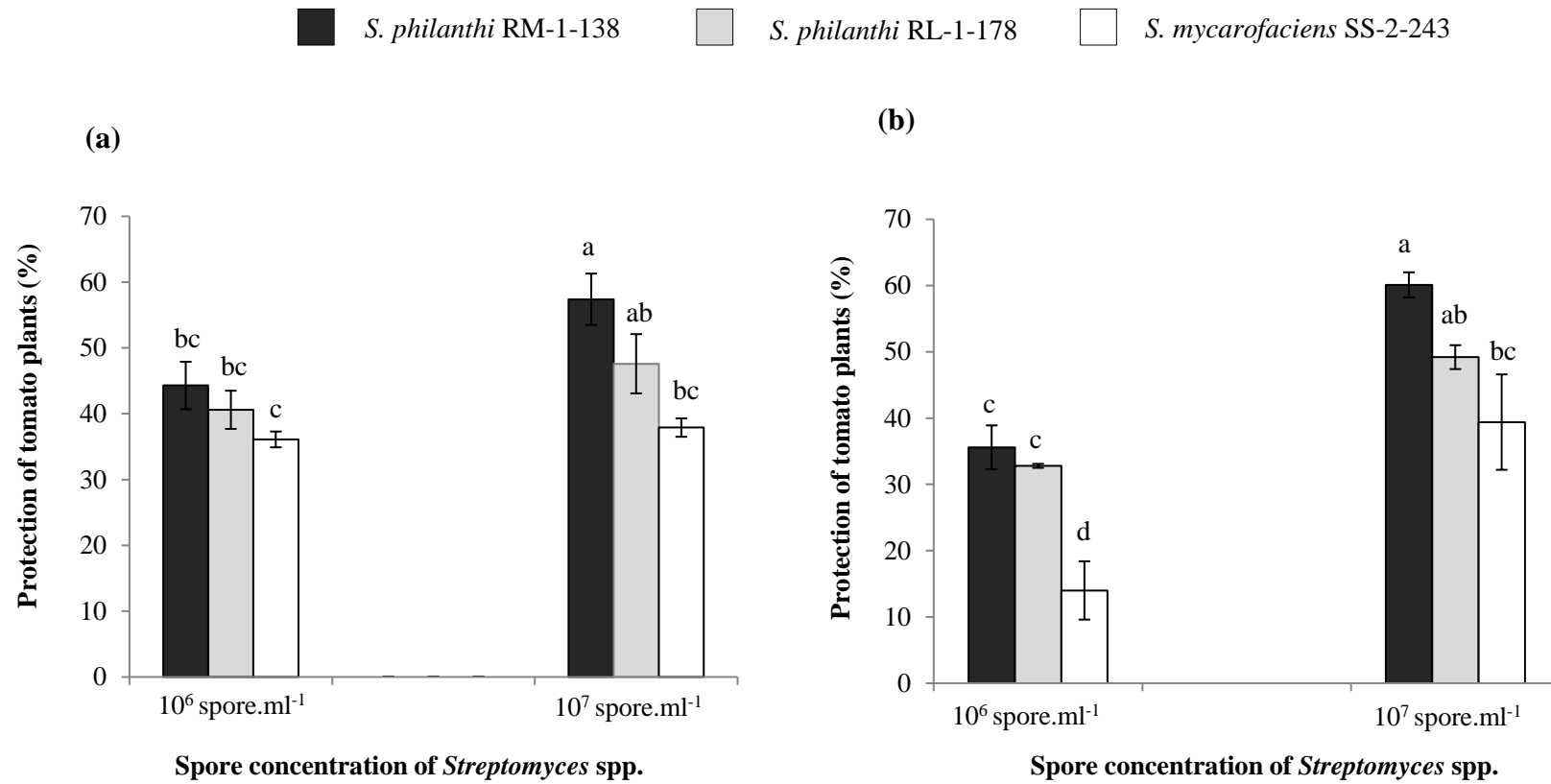
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Fig. 2

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519 **Fig. 3**

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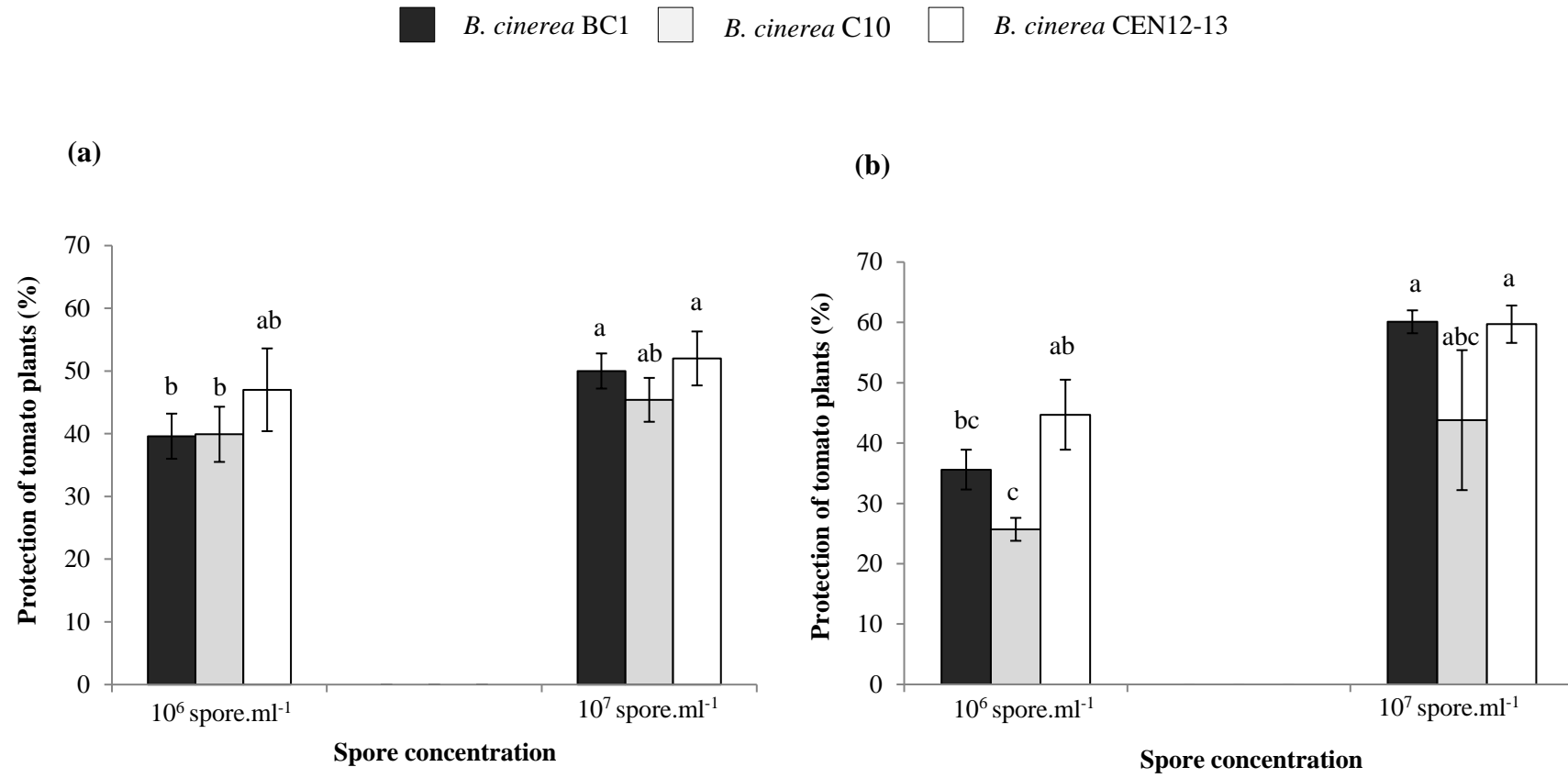
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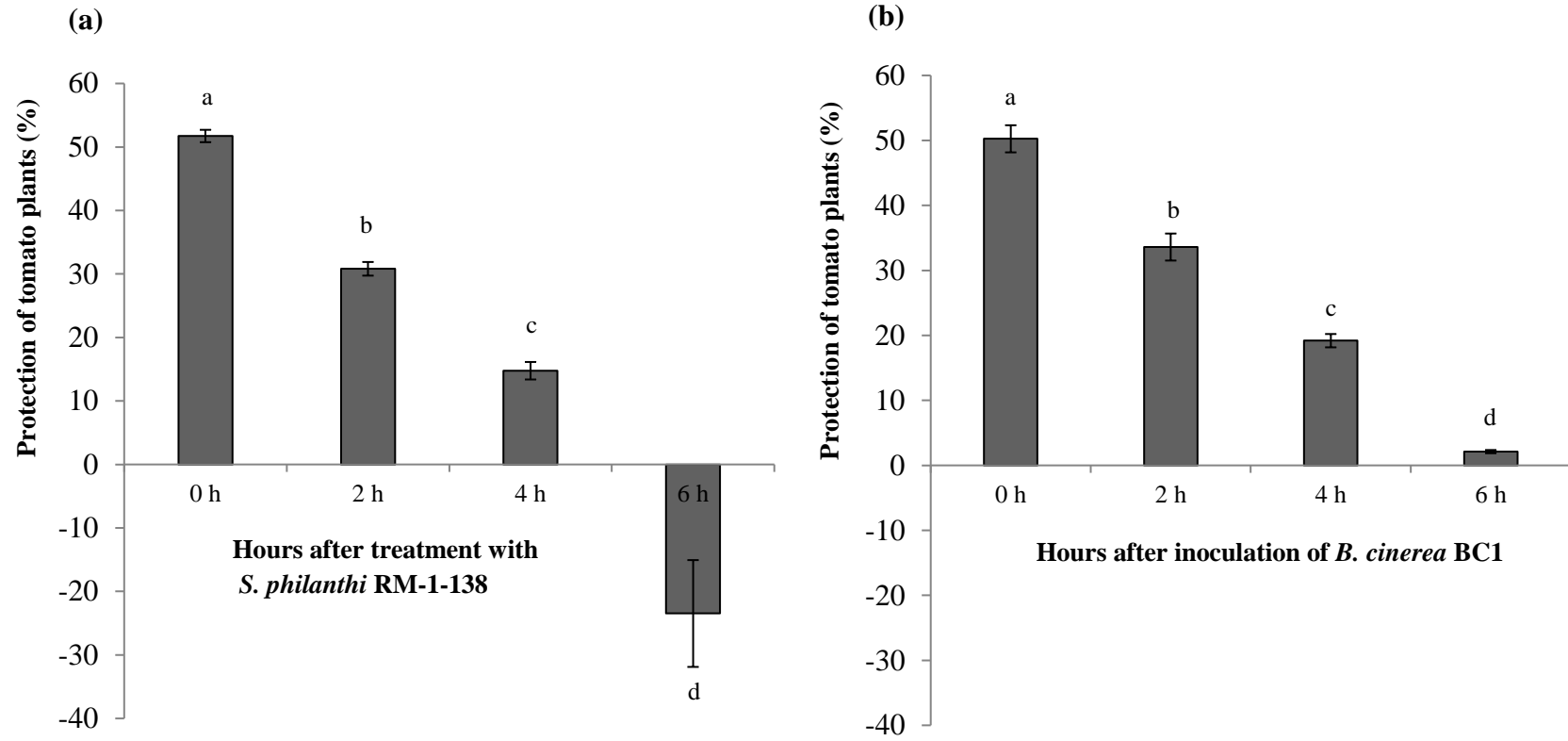
529 **Fig. 4**

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535 **Fig. 5**

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