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In vitro evaluation of drying supports and adhesive polymers as adjuvants for biocontrol of *Diplodia seriata* by *Trichoderma harzianum* and *Clonostachys rosea*

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Summary. Grapevine trunk diseases (GTDs) can cause large losses in vineyards. *Diplodia seriata* is an important GTD pathogen in Chile. Development and use of bioprotectors is a complementary alternative to the use of agrochemicals for disease management. To produce bioformulations for management of *D. seriata*, additives could be used to maintain viability and survival of biocontrol agents, such as *Trichoderma harzianum* and *Clonostachys rosea*. Effects of drying supports (inulin, maltodextrin, lactose, or talc) and adhesive polymers (carboxymethylcellulose, Aloe vera gel, or chitosan) were assessed on *D. seriata* conidium viability and mycelium development of *T. harzianum* and *C. rosea*, and for their biocontrol capacity against *D. seriata*. *T. harzianum* and *C. rosea* cultured in Potato Dextrose Agar containing inulin (at 10% w/v) maltodextrin (10% w/v), lactose (6% w/v), or talc (4% w/v), or the adhesive polymers carboxymethylcellulose (0.5% w/v), Aloe vera gel (0.5% w/v), or chitosan (1.5% w/v), maintained their biocontrol activity against *D. seriata*. These additives did not enhance *D. seriata* development. Therefore, these preparations, at the respective indicated concentrations, can be included in bioformulations for management of disease caused by this pathogen.

Keywords. Adjuvants, bioformulation, *Botryosphaeria*, drying supports, sticky polymers.

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INTRODUCTION

Grapevine trunk diseases (GTDs) can cause severe damage to grapevine productivity (OIV, 2016). These diseases are caused by a complex of pathogenic fungi, and there are no satisfactory methods for their management (Matei *et al*., 2017; Besoain, 2018). Botryosphaeria dieback, an important GTD, causes yield losses between 36 to 48% in Chilean 'Cabernet Sauvignon' vineyards located in O'Higgins and Maule regions, with average yield losses estimated to be 5,800 kg ha^{-1} (Torres *et al*., 2017; Larach *et al*., 2020). *Dip-*

lodia seriata has the greatest prevalence as the cause of Botryosphaeria dieback in Chile (Morales *et al*., 2012; Díaz *et al*., 2013; Torres *et al*., 2017).

Botryosphaeria dieback pathogens enter grapevine plants mainly through pruning wounds, in the absence of any chemical and/or biological control organisms (Gramaje *et al*., 2018; Mondello *et al*., 2018). Chemical fungicides may induce occurrence of resistant pathogen strains and may cause environmental damage. Biological controls can be alternatives to prevent plant infections by these pathogens, due to the different mechanisms of the biocontrol agents (BCAs). In addition, BCAs could be included in rotations with chemical pesticides (Fravel, 2005; Khamna and Yokota, 2008; Gramaje *et al*., 2018).

Trichoderma spp. and *Clonostachys rosea* (Link: Fr.) could be BCAs against several pathogens, such as *Rhizoctonia solani* (Kühn) (Kakvan *et al.,* 2013), *Phytophthora* spp. (Bae *et al.,* 2016), *Alternaria* spp. (Jensen *et al*., 2004), *Sclerotinia sclerotiorum* (Lib.) de Bary (Rodríguez *et al*., 2011), and others (Jensen *et al*., 2000; Morandi *et al*., 2003; Morandi *et al*., 2007). *Trichoderma harzianum* Rifai and *C rosea* have also been tested individually and together, as conidium suspensions, for the control of *Diplodia seriata* and *Neofusiccocum australe* in greenhouse and field assays (Arriagada, 2015).

To overcome instability of conidium suspensions, it is probably important to incorporate BCAs into appropriate formulations, that could be used alone or combined with chemical pesticides to achieve effective pathogen control (Papavizas, 1985; Arriagada, 2015). However, formulations can additionally contain adjuvants that are used to formulate, facilitate application, and maintain microbial viability in harmful field and/or storage conditions (Gašić and Tanović, 2013). These adjuvants may modify the biocontrol capacity of the active ingredients or favor development of target pathogens (Bernhard *et al*., 1998). Thus, it is important that candidate adjuvants are assessed for effects on biocontrol capacity of the active principals before they are incorporated into formulations. It is also important to consider formulation types and methods of field application (Chammem *et al*., 2022). Potential adjuvants include drying supports for the preparing powder formulations, and polymers to ensure adherence to host plants.

Drying supports are adjuvants used for microencapsulation of microorganisms, when spray drying technology is used. These include compounds that do not affect the environment due to their short or null persistence, and include lactose, inulin, talc, or maltodextrin, among others (Wilkins, 1990). Use of lactose can improve survival of *T. harzianum* during storage (Kumar *et al*., 2016), and inulin protects *T. harzianum* viability against freezing and freeze drying (Mensink *et al*., 2015; Nunes *et al.,* 2018). Maltodextrin has been used to extend bioformulation shelf-life after production using the spray drying technology (Leslie *et al*., 1995; Agudelo *et al.,* 2017), and talc has been used as a carrier additive for solid formulations containing *Trichoderma* spp. (Kakvan *et al.,* 2013).

Polymers have been used to promote adherence of bioformulations to plant tissues (Gašić and Tanović, 2013). Carboxymethylcellulose (CMC) is commonly used for this purpose because in addition to its function as adherent (Bernhard *et al*., 1998), it provides a carbon source for microbial development. This compound has been tested in formulations containing *C. rosea* (Musiet, 2015) and *T. harzianum* (Samolski, 2014). Chitosan could also trigger host defense mechanisms, and has been tested for control of *D. seriata* (Meng and Tian, 2009; Cobos *et al*., 2015). Extracts from *Aloe vera* that contain a viscous gel with antimicrobial activity have also been tested against bacteria (Pereira *et al*., 2013) and fungi (Sitara *et al*., 2011; Navarro, 2013).

The mixture of *T. harzianum* and *C. rosea* that controls *D. seriata* (UChile, 2021) could be used in a formulation to which adjuvants could be added to aid biocontrol activity. The objective of the present study was to assess drying supports and polymers for efficacy as biocontrol formulation additives to *T. harzianum* and *C. rosea* for control of disease caused by *D. seriata*.

MATERIALS AND METHODS

Fungus strains and culturing

Trichoderma harzianum (strain RGM2218) and *C. rosea* (strain RGM2217) were used, from the laboratory fungal collection at Laboratorio de Fitopatología y Control Biológico de Enfermedades, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile. These strains had previously shown good biocontrol activity against *D. seriata* (strain 1009), whether alone or in mixture (Arriagada, 2015). The isolates were activated on potato dextrose agar (PDA; Difco), and were grown on PDA plates in darkness at 30°C. *Diplodia seriata* was activated and grown on PDA in darkness at 25°C. Cultures were stored at 4°C and sub cultured weekly. All microbiological procedures were carried out under sterile conditions.

Chemicals

All reagents were technical grade, and were obtained from the following providers: a) drying supports; inu-

lin (Reutter S.A.), maltodextrin (Quimatic S.A.), lactose (Reutter S.A) and talc (Reutter S.A); b) polymers carboxymethyl cellulose (CMC, Winkler Ltd.), Aloe vera gel (liquid extract; Proaltec) and chitosan (Reutter SA); c) Culture media; potato dextrose agar and agar (DIFCO), glucose (Merck).

Biocontrol agent conidium viability

Conidium suspensions of *T. harzianum* and *C. rosea* were prepared, respectively, from 7- or 14-d-old cultures, in 9 g·L^{-1} sterile NaCl solution, and were then filtered through two layers of sterile gauze to remove mycelia. Conidium suspension (100 mL containing 1 × 10^5 conidia·mL^{-1} (*T. harzianum* or *C. rosea*) was placed in a Petri dish containing glucose agar (GA; 10 g L^{-1} glucose and 5 g L^{-1} agar). The inoculum was spread over the entire agar surface in the dish using a Drigalski's spatula. All plates were incubated for 24 h at 30°C. One hundred conidia were assessed for germination, with conidia classified as germinated when germ tubes were ≥ twice the diameter of the conidia (Latorre and Rioja, 2002).

To test effects of different compounds on conidium viability, the compounds were added to culture medium before autoclaving. Drying supports were added at 0, 2.0, 4.0, 6.0, 8.0, or 10.0% (w/v) and polymers were added at 0, 0.5, 1.0, 1.5 or 2.0 % (w/v).

Mycelium growth from biocontrol agents

Mycelium discs (0.5 cm diam.) were taken from a 5-d-old PDA culture of *T. harzianum* or a 10-d-old culture of *C. rosea,* and were each placed in the middle of a Petri dish containing PDA. Plates were then incubated at 30°C for 2 d for *T. harzianum*, and for 6 d for *C. rosea*. Colony growth in each dish was then measured two perpendicular axes, then averaged, and expressed as diameter in mm.

To test the effects of the different compounds on mycelium growth, the compounds were added to culture medium (PDA) before autoclaving. Drying supports were added at 0, 2.0, 4.0, 6.0, 8.0 or 10.0% (w/v), and polymers were added at 0, 0.5, 1.0, 1.5 or 2.0 % (w/v).

Antagonistic activity against Diplodia seriata

The antagonistic activities of *T. harzianum* or *C. rosea* against *D. seriata* were assessed in the absence or presence of each adjuvant that did not affect growth of *T. harzianum* or *C. rosea* growth, in dual cultures. Ten µL of conidium suspension (1 × 10^5 conidia mL^{-1}) of each biocontrol agent was applied to a 0.5 cm diam. mycelium disc from a 5-d-old *D. seriata* culture (Figure 1). Conidium suspensions and PDA medium containing specific adjuvants were prepared as described above. Experimental controls were included, replacing conidium suspensions with sterile water. All plates were incubated for 5 d at 25°C. Radii of *D. seriata* colonies were measured for each treatment (Figure 1), and percent inhibition was determined.

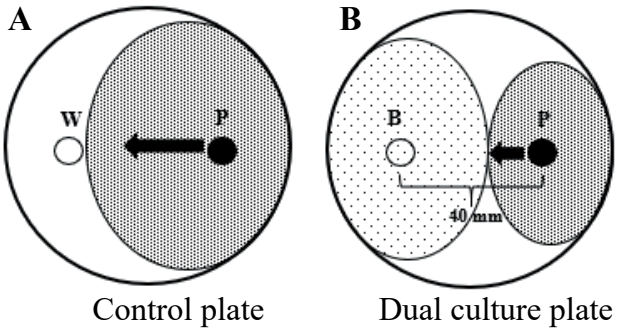

Figure 1. Measurement of radial colony growth of *Diplodia seriata* in dual cultures A: W, sterile water and P, pathogen (*D. seriata*); B: B, biocontrol agent (*Trichoderma harzianum* or *Clonostachys rosea* conidium suspension) + adjuvant and P, pathogen (*D. seriata*). The culture medium was PDA.

Experimental design and statistical analyses

Completely randomized designs (CRDs) with unifactorial structures were used to determine effects of the different concentrations of the drying supports and the polymers, and to establish the greatest concentration of these where no negative effects were detected on viability of conidia or mycelium growth of the of the antagonists. The factors assessed were type of drying support (inulin, maltodextrin, lactose, or talc) or polymers (CMC, Aloe vera gel, or chitosan), each independently, at their different % w/v concentrations.

The experimental units were four Petri dishes (each as a subsample), with three repetitions for each treatment. Infostat software was used for the statistical analyses with interface of R, and Wald Tests were applied, through a general and mixed linear model. If statistically significant differences ($P \leq 0.05$) were determined, LSD Fisher LSD Multiple Comparison Tests was carried out.

A CRD with a factorial structure was used to determine the biocontrol capacity of the antagonists on *D. seriata* (antagonism), using type of drying support (inulin, maltodextrin, lactose, or talc) or polymer (CMC, Aloe vera gel, or chitosan), and type of antagonist (*T. harzianum*, or *C. rosea*). Drying supports and polymers were analyzed independently.

RESULTS

Viability of conidia in the presence of different adjuvants: drying supports or polymers

The drying supports inulin and maltodextrin did not affect viability of *T. harzianum* conidia at any of the concentrations tested, while lactose decreased mean viability by 3.02%, and talc by 15.67% (Table 1A). Viability of *C. rosea* conidia was not affected by inulin, lactose or talc (Table 1B), but maltodextrin at 8% (w/v) in the culture medium increased viability of *C. rosea*.

The sticky polymers Aloe vera gel and chitosan did not affect viability of *T. harzianum* conidia, while CMC decreased viability by 5% at concentrations of 1% (w/v) and greater (Table 2A). Viability of *C. rosea* conidia was not affected by CMC or by chitosan, while concentrations of Aloe vera gel of 1.5% (w/v) and greater decreased viability (Table 2B).

Growth of biocontrol agents in the presence of different adjuvants

Mycelium growth of *T. harzianum* was not modified by the presence of the drying supports inulin, maltodextrin, or talc in the culture media (Table 3A). Lactose concentrations of 8% w/v or greater decreased growth of this fungus. Growth of *C. rosea* was not affected by inulin, maltodextrin, or talc, but lactose at 6% w/v or greater significantly increased growth of this BCA (Table 3B).

Growth of *T. harzianum* was not modified by the sticky polymer CMC in culture medium at the different concentrations tested (Table 4A). Aloe vera gel concentrations of 1% w/v and greater and chitosan at 2% w/v, significantly decreased growth of this BCA. None of the adjuvants at any of the assessed concentrations modified growth of *C. rosea* (Table 4B).

Antagonistic activity against Diplodia seriata

The antagonistic activity of the BCAs against *D. seriata* was tested with the highest concentrations of the drying support and sticky polymers adjuvants that did not modify either their viability or growth. These were 10% inulin, 10% maltodextrin, 6% lactose and 4% talc for the drying supports; and 0.5% CMC, 0.5% Aloe vera and 1.5% chitosan for the sticky polymers.

Results showed that none of the drying supports tested (Table 5A) or the sticky polymers tested (Table 5B), at the concentrations already mentioned, modified the ability of *T. harzianum* or of *C. rosea* to inhibit *D. seriata* growth, although significant differences were observed between the two BCAs in % inhibition of the pathogen. Controls performed in the absence of the BCAs showed that none of the adjuvants modified the growth of *D. seriata*.

Table 1. Mean conidium viability (%) of *Trichoderma harzianum* (A) and *Clonostachys rosea* (B) after culture in Petri dishes containing glucose agar amended with different drying supports adjuvants (inulin, maltodextrin, lactose, or talc) at different concentrations (0, 2.0, 4.0, 6.0 ,8.0 or 10.0 % w/v).

A. *Trichoderma harzianum*

Drying supports	Concentrations (% w/v)						Wald Test *P* value
	0	2.0	4.0	6.0	8.0	10.0	
Inulin	91.17a	94.83a	96.25a	94.00a	95.17a	96.08a	0.3502
Maltodextrin	95.25a	94.92a	96.83a	96.00a	95.42a	97.42a	0.4895
Lactose	97.67a	93.75b	95.58b	95.83b	94.33b	93.75b	0.0154
Talc[a]	97.00a	81.67b	81.00b	-	-	-	0.0047

B. *Clonostachys rosea*

Drying supports	Concentrations (% w/v)						Wald Test *P* value
	0	2.0	4.0	6.0	8.0	10.0	
Inulin	92.33a	95.92a	95.08a	96.58a	95.92a	91.58a	0.4760
Maltodextrin	94.25c	96.42bc	98.17ab	96.58bc	99.00a	97.92ab	0.0002
Lactose	87.42a	87.67a	85.58a	89.00a	89.42a	86.42a	0.6214
Talc[a]	83.33a	86.25a	86.58a	-	-	-	0.8973

[a]Concentrations of talc greater than 4% (w/v) interfered with viability analyses.
Means for each treatment followed by different letters indicate differences ($P \leq 0.05$), according to Fisher's LSD tests.

Table 2. Mean conidium viability (%) of *Trichoderma harzianum* (A) and *Clonostachys rosea* (B) in Petri dishes containing glucose agar amended with polymers adjuvants (carboxymethylcellulose (CMC), Aloe vera gel, or chitosan), each at different concentrations (0, 0.5, 1.0, 1.5 or 2.0 % w/v).

A. *Trichoderma harzianum*

Polymers	Concentration (% w/v)					Wald Test *P* value
	0	0.5	1.0	1.5	2.0	
CMC	97.25a	96.62a	91.92b	92.58b	92.25b	0.0001
Aloe vera gel	96.25a	95.08a	93.83b	94.25b	93.75b	0.0001
Chitosan	92.83a	82.42b	83.25b	86.00b	84.08b	<0.0001

B. *Clonostachys rosea*

Polymers	Concentration (% w/v)					Wald Test *P* value
	0	0.5	1.0	1.5	2.0	
CMC	84.00a	84.00a	83.58a	84.58a	86.50a	0.9943
Aloe vera gel	88.75a	81.83ab	82.17a	75.17b	58.25c	0.0003
Chitosan	91.08a	85.25b	88.58ab	88.92ab	89.63ab	0.0036

Means for each treatment followed by different letters indicate differences ($P \leq 0.05$), according to Fisher's LSD tests. CMC= carboxymethylcellulose.

DISCUSSION

Bioformulation development requires assessment of included adjuvants for viability modification, growth and the biocontrol effects on the BCAs that are the active ingredients of the formulations. Also, each bioformulation will focus on biocontrol of specific or groups of pathogens, so it is important to consider pathogen characteristics, routes of entry to plant host, and the diseases produced. As fungi belonging to the *Botryosphaeriaceae* mainly enter hosts through pruning wounds, the biocontrol formulations should be applied directly to the damaged zone of the grapevines. The formulations should remain on damaged host surfaces to prevent infections by these fungi. Solid formulations (powders), or semi-solids (pastes), could be appropriate. Therefore, adjuvants such as the drying supports and polymers tested in the present study are likely to be these types of formulation.

Presence of the drying supports inulin, maltodextrin, lactose, or talc, or of the polymers CMC, chitosan, or Aloe vera gel, at the concentrations assessed (Table 5) did not modify the biocontrol properties of *T. harzianum* or of *C. rosea*. It has been previously shown that some components may decrease or increase the antagonistic capacity of BCAs towards the pathogen targets (Bernhard *et al.*, 1998). This was not observed with the selected adjuvants in the present study. On the other hand, assessments of viability and growth of the BCAs in the presence of different adjuvants (Tables 1 to 4) allowed selection of the adjuvants that could be used in bioformulations, including their appropriate concentrations, to ensure that the adjuvants do not harm biocontrol agent conidium viability, reproductive structures, or

Table 3. Mean colony diameters (mm) of *Trichoderma harzianum* (A) and *Clonostachus rosea* (B) after culture in Petri dishes containing potato dextrose agar amended with different drying support adjuvants (inulin, maltodextrin, lactose, or talc) at different concentration (0, 2.0, 4.0, 6.0, 8.0, and 10.0 % w/v)

A. *Trichoderma harzianum*

Drying supports	Concentrations (% w/v)						Wald Test *P* value
	0	2.0	4.0	6.0	8.0	10.0	
Inulin	80.76a	84.59a	85.00a	84.14a	85.00a	84.44a	0.0818
Maltodextrin	80.71a	81.23a	81.46a	83.72a	84.18a	85.00a	0.8702
Lactose	85.00a	84.72a	85.00a	83.38a	78.77b	75.58c	<0.0001
Talc	82.29a	79.22a	83.43a	83.70a	83.24a	80.86a	0.7307

B. *Clonostachys rosea*

Drying supports	Concentrations (% w/v)						Wald Test *P* value
	0	2.0	4.0	6.0	8.0	10.0	
Inulin	36.78a	37.03a	37.56a	36.83a	36.55a	37.26a	0.0501
Maltodextrin	45.04a	44.99a	44.67a	45.93a	45.49a	45.76a	0.6139
Lactose	44.02b	45.09b	45.33b	46.01a	46.23a	46.16a	0.0004
Talc	44.69a	46.67a	47.61a	47.33a	48.95a	47.73a	0.1755

Means for each treatment followed by the different letters indicate differences ($P \leq 0.05$), according to Fisher's LSD tests.

Table 4. Mean colony diameters (mm) of *Trichoderma harzianum* (A) and *Clonostashys rosea* (B) after culture in Petri dishes containing potato dextrose agar amended with polymer adjuvants (carboxymethylcellulose, Aloe vera gel, or chitosan) at different concentration (0, 0.5, 1.0, 1.5 and 2.0 % w/v).

A. *Trichoderma harzianum*

Polymers	Concentration (% w/v)					Wald Test *P* value
	0	0.5	1.0	1.5	2.0	
CMC	85.00a	76.26a	64.79a	54.33a	67.96a	0.3003
Aloe vera gel	85.00a	81.81a	74.67b	70.18bc	66.04c	0.0026
Chitosan	76.41a	68.87ab	65.84ab	62.40ab	56.45b	0.0179

B. *Clonostachys rosea*

Polymers	Concentration (% w/v)					Wald Test *P* value
	0	0.5	1.0	1.5	2.0	
CMC	44.34a	42.22a	41.85a	41.17a	42.02a	0.5045
Aloe vera gel	45.59a	44.51a	45.38a	44.33a	42.93a	0.5557
Chitosan	45.49a	46.25a	44.05a	45.40a	44.38a	0.2733

Means for each treatment followed by the different letters indicate differences (P≤ 0.05), according to Fisher's LSD statistical. CMC = carboxymethylcellulose.

Table 5. Mean percent inhibition of *Diplodia seriata* growth caused by *Trichoderma harzianum* or *Clonostachys rosea* in the presence of: (A) drying supports (inulin, maltodextrin, lactose, or talc); or (B) sticky polymers (carboxymethyl cellulose, Aloe vera gel, or chitosan), in dual cultures in Petri dishes containing potato dextrose agar.

A. Drying supports

Drying supports	% (w/v)	% Inhibition of *D. seriata* growth			Wald Test *P* value
		T. harzianum	*C. rosea*	None	
Inulin	10.0	61.39a	26.85b	0.00c	<0.0001
Maltodextrin	10.0	61.07a	23.70b	2.26c	<0.0001
Lactose	6.0	61.16a	27.40b	0.00c	<0.0001
Talc	4.0	59.93a	34.43b	2.73c	<0.0001
H_2O (control)	-	60.40a	29.57b	0.00c	<0.0001

B. Polymers

Polymers	% (w/v)	% Inhibition of *D. seriata* growth			Wald Test *P* value
		T. harzianum	*C. rosea*	None	
CMC	0.5	61.00a	26.53b	0.06c	<0.0001
Aloe vera gel	0.5	61.36a	32.85b	4.11c	<0.0001
Chitosan	1.5	62.99a	24.68b	1.92c	<0.0001
H_2O (control)	-	60.40a	29.57b	0.00c	<0.0001

Means for each treatment followed by the different letters indicate differences ($P \leq 0.05$), according to Fisher's LSD tests. CMC = carboxymethylcellulose.

growth, or establishment in plants (Guijarro *et al*., 2008; Sabuquillo *et al*., 2009). The type of formulation will also depend on the disease and the phytopathogen to be controlled (Bernhard *et al*., 1998).

The different tested drying support or polymer compounds tested here did not modify the antagonistic activity of *T. harzianum* or *C. rosea* towards *D. seriata*. Thus, their use in formulations at the concentrations tested is likely to modify viability or growth of the BCAs. Nevertheless, final selection of specific adjuvants to be included in formulations should consider characteristics of each compound. For example, inulin which neither modified conidium viability of growth nor interfered with antagonistic activities of *T. harzianum* and *C. rosea* against *D. seriata*, could be used as a formulation adjuvant. Inulin is a suitable carbon source for microorganisms (Kelly, 2008), it stabilizes proteins, and protects conidia during freeze-drying (Mensink *et al*., 2015; Nunes *et al*., 2018). However, some *Trichoderma* isolates are not able to hydrolyze inulin (Cordeiro *et al*., 1997; Souza-Motta *et al*., 2003); so, this compound may not be useful unless adequately evaluated. The drying support maltodextrin, that has been widely used in formulations (Samborska *et al*., 2007; Du *et al*., 2014; Wenzel *et al*., 2017; Fernández and Sepúlveda, 2019) did not modify the parameters analyzed in the present study, except at greater than 4% w/v, an increase of *C. rosea* conidium germination was detected, indicating a protective effect. These results are like those previously described for *T. harzianum*, where maltodextrin protected from protein denaturation increased shelf-life by up to 6 months at temperatures between 15°C and 35°C (Rai and Tewari, 2016).

Lactose is a drying support used in production of bioformulations, for minimization microorganism viability losses from high temperatures during spray drying or low temperatures during freeze-drying (Tan *et al*., 2007; Higl *et al*., 2008; Cabrefiga *et al*., 2014). Results in the present study showed that addition of lactose decreased *T. harzianum* conidium germination at concentrations less than 2%, and mycelium growth at less than 8% w/v. However, results were different for *C. rosea*, where lactose favored mycelium growth and did not modify conidium viability. These results could be due to different use of lactose by the microorganisms, as has been reported for filamentous fungi which use lactose at low rates (Swartz, 1985), and in two possible ways: direct absorption of the disaccharide and subsequent intracellular hydrolysis, or extracellular hydrolysis and subsequent absorption of the resulting monosaccharides (Seiboth *et al*., 2007). The present study results could be explained by the biocontrol agents using different routes

of lactose utilization from the culture medium. It has also been reported that incorporation of lactose in bioformulations can favor stability and survival of *T. harzianum* over a wide range of storage temperatures (-20 to 30°C; Kumar *et al*., 2016), since lactose protects against desiccation, stabilizes proteins and lipids in cell membranes (Santos *et al*., 2011), or can be a prebiotic (Chávez and Ledeboer, 2007).

Talc, although showing no effects on mycelium growth of *C. rosea* or *T. harzianum*, decreased the conidium germination of *T. harzianum*, probably because this substance can form barriers surrounding conidia, reducing the water and nutrient, and germination of the conidia (IARC, 2010). However, the 80% conidium germination obtained in the presence of talc indicates that it could be used in formulations, since it favored survival and storage of CFUs for up to 150 d at temperatures 0 to 40°C (Bhat *et al*., 2009; Kumar *et al*., 2013). Similarly, Rai and Tewari (2016) used talc for moisturizing and liquid formulations containing *T. harzianum*. However, use of talc must be accompanied by other adjuvants such as CMC, since single component adjuvants cause short shelve lives (approx. 3 months) and dehydration (Jayaraj *et al*. 2006; Sallam *et al.* 2013).

The results obtained with up to 0.5% CMC, where conidium viability or mycelium growth of both BACs were not affected, are like those from other studies for bioformulations containing *T. harzianum* (Mukherjee *et al*., 2014) or *C. rosea* (Musiet, 2015; Wu *et al*., 2018), where CMC was used as a binder or adherent.

Chitosan did not adversely affect conidium viability or mycelium growth of both the biocontrol agents. However, chitosan has been reported to inhibit *T. harzianum* spp. conidium germination at 2.0% w/v (Palma-Guerrero *et al*. 2008). This could be related to chitosan prevention of *T. harzianum* conidium germination (Ruiz-de-la-Cruz *et al*. 2017) or to the presence of antibiotic activity (El-Mohamedy *et al*. 2014).

Concentrations of Aloe vera gel greater than 1% w/v decreased in conidium viability and mycelium growth of both BCAs, which agrees with the results of Sitara *et al.* (2011). This could be due to the diversity of bioactive molecules in *Aloe vera* gel that have antimicrobial and antioxidant properties (Davis 1997; Vega-Gálvez *et al*., 2011), that have been used for medicinal and therapeutic purposes (Ahlawat and Khatkar, 2011).

The greatest concentrations of drying supports or polymers that did not affect conidium germination or mycelium growth of both BCAs did not affect growth of *D. seriata* growth.

This study has shown that the maximum concentrations of the drying supports used in bioformulations containing *T. harzianum* and *C. rosea* were 10.0% w/v for inulin, 10.0% w/v for maltodextrin, 6.0% w/v for lactose, and 4% w/v for talc. Similarly, maximum concentrations for polymer additives were 0.5% w/v for carboxymethylcellulose, 0.5% w/v for Aloe vera gel, and 1.5% w/v for chitosan. These compounds did not affect *D. seriata* development in the absence of the two various BCAs assessed.

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