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Effect of biocontrol agents and potassium phosphite against Phytophthora crown rot, caused by *Phytophthora capsici,* on zucchini in a closed soilless system

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Abstract

Five biocontrol agents and potassium phosphite, used at various concentrations and at a different number of applications, have been tested to establish their ability to control *Phytophthora capsici* on hydroponically grown zucchini plants. In a first set of trials, various experimental biocontrol agents (*Trichoderma* sp. TW2, a mixture of *Pseudomonas* FC7B, FC8B, FC9B, *Fusarium solani* FUS25 and *Pseudomonas* sp. PB26) and a commercial formulation of *Trichoderma gamsii +T. asperellum* (Remedier) were applied at the artificial infestation with the pathogen of a peat substrate, 5-7 days before planting the zucchini seedlings, and later at 5-day-intervals. BCAs were compared with a potassium phosphite-based fertiliser. In a second set of trials, the potassium phosphite fertiliser was applied directly to the growing media or via a nutrient solution every 6 days, starting at the infestation with the pathogen and 5-7 days before planting, in order to select the optimal rate, type and number

of applications. Potassium phosphite reduced by 62 to 94% *Phytophthora* crown rot of zucchini, providing more consistant disease severity reduction than those achieved using the experimental BCAs, alone or in mixture, and the *Trichoderma gamsii* + *T. apserellum* formulated mixture (29 to 47% reduction in disease severity). One application of potassium phosphite, at the highest tested concentration, was less effective than three applications. Potassium phosphite consistently reduced the severity of Phytophthora crown rot under different disease pressure (by 48 to 79%) when applied via a treated peat growing media or via a nutrient solution with 3 to 6 applications, thereby offering growers an important opportunity to control *P. capsici* on soilless grown zucchini.

Keywords: Hydroponic; Phytophthora control, *Cucurbita pep*o, nutrient solution, microorganisms, phosphites.

1. Introduction

Zucchini (*Cucurbita pepo* L.) is an important crop throughout the world that is affected by several air- and soil-borne pathogens which cause severe losses (Gubler and Davis, 1996). Currently, soil-borne pathogens are a cause of particular concern in many geographical areas, including the Mediterranean, because of the difficulties encountered in their management, due to the increasing lack of effective, available control measures (Colla et al., 2014; Garibaldi et al., 2014; Katan, 2017). *Phytophthora capsici,* which causes the root and crown rot of zucchini (Lamour et al., 2012), has long been known in Italy (Cristinzio and Noviello, 1980) and remains one of the most critical pathogens of this crop (Gullino et al., 2018). This pathogen can also be spread through infected transplants, seeds and water resources (Granke et al., 2012; Lamour et al., 2012; Reistano and Stephens, 1999), and is thus also of concern for soilless systems, where oomycetes find an environment that is favourable for their survival and spread (Jenkins and Averre, 1983). In fact, despite having been developed and promoted to reduce the problems caused by soil-borne pathogens,

53 to reduce the release of nutrients into the environment and to improve water efficiency (Van Os,
54 1999). However, closed soilless systems, which are increasingly adopted in southern countries, are
55 often characterised by the presence of root diseases (Postma et al., 2008; Stanghellini and Rasmussen,
56 1994).
57 Owing to the limited availability of synthetic fungicides registered for soilless systems, it is necessary
58 to evaluate the efficacy of alternative disease control measures. Thus, disease management, based on
59 biocontrol agents, suppressive soils and inorganic salts, is increasingly being exploited in such
60 growing systems (Gullino et al., 2015; Paulitz, 1997; Van Os, 1999; Postma, 2004; 2010; Vallance et
61 al., 2001). Phosphite has been shown to be effective in the control of oomycete related diseases in
62 horticulture. Deliopoulos et al., (2010), for instance, showed that phosphite salts are effective against
63 several soil-borne pathogens in different pathosystems, such as *Pythium ultimum*-cucumber,
64 *Phytophthora cinnamomi*-lupin and *Phytophthora nicotianae*-tobacco. The protective effect induced
65 by phosphite, and its persistence *in planta*, may vary among species (Barrett et al. 2003; Shearer and
66 Crane, 2012), type of application (Guest and Grant 1991; Smillie et al., 1989), Phytophthora species
67 and strains (Coffey and Bower, 1984), and could be affected by concentration (Jackson et al., 2000;
68 Daniel and Guest, 2006). Although the extensive research carried out to better understand the mode
69 of action of phosphite in plant protection (Hardy et al., 2001; Thao et al., 2009; Alexanderson et al.,
70 2016), there is still a need to better understand their potential when applied in hydroponics. In the
71 case of biocontrol agents, different microorganisms have been tested in the past in soilless systems,
72 such as *Muscodor albus* against Rhizoctonia damping-off of broccoli, *Gliocladium virens* against
73 *Rhizoctonia solani* and *Pythium ultimum* of zinnia, cotton and cabbage (Lumsdenand Locke, 1989),
74 and non-pathogenic *Fusarium oxysporum* against *Fusarium oxysporum* f.sp. *basilici* on basil (Fravel
75 and Larkin 1999). Other studies have shown a positive effect of applying biocontrol agents to
76 hydroponic systems via recirculating nutrient solutions or in the growing-medium on different hosts
77 affected by oomycete pathogens; this is the case of bacterial isolates of fluorescent *Pseudomonades*
78 in the *Pseudomonas fluorescens, P. putida* and *P. aeruginosa* group against *Pythium ultimum* on

tomato (Alsanius et al., 1999), of a mixture of *Fusarium* spp. and *Trichoderma* spp. against *Phytophthora cryptogea* on gerbera (Garibaldi et al., 2003), of indigenous *Pseudomonas* spp. and *Trichoderma* sp. against *Pythium aphanidermatum* on cucumber (Postma et al., 2000, 2005), of *Muscodor albus* against P*hytophthora capsici* on bell pepper (Mercier and Manker, 2005) and of *Bacillus subtilis* against *Pythium aphanidermatum* on lettuce (Utkhede et al., 2000). However, despite many studies, their pratical application is still limited.

The type, rate and timing of the application of biocontrol agents and salts, which often act as resistance inducers, affect both the level of disease control and the yield (Paulitz, 1997; Walter et al., 2013; Bonanomi et al., 2018). Thus, finding the right application method for biocontrol agents and salts in soilless systems against zoospore producing pathogens merits further attention.

Although other studies have been carried out on the effect of biocontrol agents and salts against pathogens that are well adapted to soilless systems, such as *Pythium* spp., and *Phytophthora* sp. (Armitage, 1993; Förster et al., 1998; Garibaldi et al., 2003; Garibaldi and Gullino, 2010; Gullino et al., 2015; Stanghellini et al., 1994), there is still a lack of knowledge on their efficacy against *Phytophthora capsici* on zucchini grown in soilless systems, and the possible effect of combined BCAs on disease severity.

This work has been carried out in a closed soilless system, under controlled conditions, in order to evaluate the efficacy of experimental biocontrol agents used alone or in mixture, compared with a commercial formulation of *Trichoderma gamsii +T. asperellum*, and potassium phosphite salts, considering the long-term efficacy of different types and different numbers of applications to control *P. capsici* on zucchini, with the aim of developing practical solutions to manage the disease.

2. Material and methods

2.1. Experimental layout, soilless system and plant material

Thirteen trials were carried out in a glasshouse at the Agroinnova Centre of Competence of the University of Torino, in Grugliasco (Torino, Italy), at temperatures ranging from 20 to 28°C, in a fully automated closed soilless system. A small-scale hydroponic experimental system, with a recirculating nutrient solution, was used throughout the trials. Each hydroponic unit consisted of one channel (6 m long and 25 cm wide) connected to a storage tank (300 L) filled with a nutrient solution, which was automatically delivered to the plants, thanks to the use of an electronic control unit (Idromat2, Calpeda S.p.a., Vicenza, Italy). The nutrient solution was pumped at 1.5-1.6 mS cm^{-1} from the water storage tank, fed to the plants through drip emitters and left to drain back into the storage tank by means of gravity Nutrient solutions with the following compositions were used: 11.24 mM NO_3^-, 4.8 mM NH_4, 0.75 mM KH_2PO_4, 0.75 mM K_2SO_4, 0.012 mM Iron chelate EDTA, 2 mM MgO, 2 mM SO_3, 0.2 mM B, 0.001 mM Mo, 0.15 mM Zn, 3.1 mM CaO, 0.05 mM Cu^{++}, 0.25 mM Mn, 12.2 mM K. The pH and E.C. values were checked regularly by means of portable instruments, that is, a pH meter and a SevenGo DUO TM SG23 conductivity meter *(*Tettler, Toledo, Spain). The plants were irrigated with the solution as described above and treated.

The experimental unit consisted of six pots replicated five times (n= 30 pots each channel). Two plants were planted in each pot, and six pots corresponded to one sub-replicate of 12 plants each. Five replicates were used per treatment (60 plants/treatment).

Each trial included one untreated and inoculated control and different treatments with products tested alone or in mixture, according to the protocol tested in the first and second set of trials (Tables 1 and 2).

The susceptible Genovese zucchini cv. (Furia Sementi, Monticelli Terme (PR), Italy) was transplanted at 15 days of age into 3 L plastic pots filled with a growing medium based on blonde peat (Tecno 2, Turco S.r.l., Albenga, Italy) in all the trials.

2.2.Biological control agents (BCAs) and phosphite treatments

The following BCAs, isolated from suppressive composts and provided by AgriNewTech srl (Italy), were tested alone and in mixture (1:1:1 v/v) in the first set of trials (trials 1- 4): *Pseudomonas* sp. PB26 (Pugliese et al., 2008), *Fusarium solani* FUS25 (Gullino and Pugliese, 2011), *Trichoderma sp.* TW2 (Cucu et al., 2019).A mixture of three *Pseudomonas* spp. strains, *Pseudomonas* sp. FC7B (EU836174) - *Pseudomonas putida* FC 8B (EU836171) and *Pseudomonas* sp. FC 9B (EU836172), isolated from a suppressive rockwool substrate in a soilless system (Clematis et al., 2009; Srinivasan et al., 2009), was also used (Table 1).

The bacterial strains were maintained at 4°C in Luria Bertani (LB) slants throughout the study. The fresh bacterial suspensions were prepared by inoculating a loopful of bacterial cells into 30 ml of an LB medium in 100 ml Erlenmeyer flasks, and then incubating the suspension on a rotary shaker at 600 rpm for 48 h at 23°C. The cell suspension was centrifuged, and the pellets were re-suspended in sterile deionised water. The bacterial concentrations were checked by means of optical density (OD_{600}) before application. The density (OD600) was adjusted with sterile deionised water, by means of serial dilution, to $1x10^8$ cell ml^{-1} before application. *Trichoderma* sp. TW2 was grown in a 1000-ml-flask containing 250 ml of potato dextrose broth (SIGMA, Germany) and maintained under static conditions at 25°C. After 15 days, the produced mycelium was transferred to 200 ml sterile distilled water and homogenised using a hand-held rotary mixer. The conidia suspension obtained for the *Trichoderma* sp. TW2 isolate was standardised to 1×10^7CFU ml^{-1}.

The antagonistic *Fusarium solani* FUS25 was propagated into 1000-ml-flasks containing 250 ml of potato dextrose broth (Sigma, Germany) and maintained on a rotary shaker for ten days at 200 rpm. The cultures were centrifuged at 8,000 g for 20 min at 4°C. The conidia and mycelium pellet were transferred into 200 ml of sterile distilled water and homogenised using a rotary mixer. The conidial suspension was adjusted with sterile deionized water, by means of serial dilution, to $1x10^7$ conidia ml^{-1} before application.

In the first set of trials, each BCA suspension was applied to each pot and after planting around the base of 15 day-old seedlings at a final concentration of $1x10^7$CFUml^{-1}. The BCAs were applied six

155 times to the growing medium at 5 day-intervals using 100 ml/pot of the suspension, according to the
156 experimental protocol (Table 1).
157 The experimental BCAs were compared with a commercial formulation of *Trichoderma*
158 *asperellum* + *T. gamsii* (Remedier, Isagro, Milan, Italy), which had been applied at the label rate of
159 0.25 g l^{-1} of peat substrate and compared with the potassium phosphite fertilizer (Alexine 95PS P2O5
160 52% + K2O,42%, Alexine, Massò, Spain), which was labelled as a phosphorus supplement for
161 soilless application using 2.5 g/l (Table 1).
162 The second set of trials (trials 5 to 13) was conducted to select the optimal type, frequency and
163 number of potassium phosphite applications (Table 2). The fertilizer-based phosphite was added
164 directly to the nutrient solution (NS) or applied to each pot around the base of the seedlings using 100
165 ml/pot of the suspension prepared at 1.125 and 2.5 g/l, according to the experimental protocol. K-
166 phosphite was applied at 5-6 day intervals with 1, 3 and 6 applications (Table 2).
167 In both protocols, the first treatment,was carried out the same day of the artificial infestation of the
168 peat substrate.
169
170 *2.3. Artificial inoculation with the pathogen*
171 A highly virulent strain of *Phytophthora capsici* (coded PHC 1/16), isolated from zucchini and
172 taken from the AGROINNOVA collection, was cultured on a selective oomycete medium (Masago
173 et al., 1977) at 20°C for one week. One mycelium-agar plug (5 mm diameter), taken from an actively
174 growing colony, was transferred to a 1000-ml-flask containing the wheat-hempseed medium (200g
175 wheat kernels, 100g hempseeds and 320 ml water, sterilized at 121°C for 30 min) and incubated at
176 20°C in a growth chamber under a 12-h fluorescent photoperiod. The *P. capsici* produced after 10
177 days of incubation at 22°C was mixed into the soil substrate at a concentration of 1 g of fresh biomass
178 per litre of growing medium immediately before the first treatments was made.
179 Fifteen-day-old zucchini seedlings were transplanted into the treated and untreated pots 5-7 days after
180 the artificial infestation of the substrate with the pathogen.

181

182 *2.4.Disease assessment and statistical analysis*

183 The zucchini plants were assessed at 7-day-intervals, starting from when the first symptoms
184 caused by Phytophthora crown rot, corresponding to yellowing of the leaves, were observed. Disease
185 severity was evaluated at the end of the trials 7 to 14 days after the final treatment, according to
186 Padley et al., (2008). Disease severity was expressed using the [$\sum$(n° plants × x $_{0\text{-}5}$) / (total n^{o} of plants
187 recorded)] formula, with x $_{0\text{-}5}$ corresponding to the reported value: 0=no symptoms, healthy plants;
188 1=1 corresponding to 30% of the leaves being slightly wilted (midpoint 15%); 2=31 corresponding
189 to 50% of foliar wilting and crown lesions (midpoint 40%); 3=51 corresponding to 70% of the plants
190 being partially collapsed (midpoint 60%); 4=71 corresponding to 90% of the plants being collapsed
191 (midpoint 80%): 5=over 90% of dead plants (midpoint 95%).

192 The data obtained from the experiments were subjected to analysis of variance (ANOVA)
193 appropriate to the experimental design using SPSS, Version 25. The experimental unit consisted of a
194 3-L pot with two plants and sub-replicates with 12 plants each. Each set of treatments was repeated
195 at last three times in the first and second sets of trials according to protocols 1 and 2 (Tables 1 and 2).
196 The trials were combined when the 'trial' factor was not significant (P>0,05). The data were
197 compared using Tukey's test at a significance level of 5%. The considered factors were: five
198 experimental biocontrol agents and K-phosphite, and the type of application that is in pots to the peat
199 medium or via nutrient solution (NS), rate (1.125 and 2.5 g/l) and number of applications (1, 3 or 6).
200 The efficacy of the different treatments in controlling *P. capsici* was calculated as: % Disease
201 reduction (E%)= $\frac{LS_i - LS_t}{LS_i} \times 100$

202 where LS = percentage of plants affected by DS; i = inoculated and untreated control; t = treatments.

203

204 **3. Results**

205 *3.1. Effect of the biocontrol agents*

206 The data from the first set of trials (1- 4) were analysed separately for each experimental run
207 because there was evidence of heterogeneity (P <0.05) between the trial runs. *Phytophthora* crown
208 root severity ranged from 30 to 47.9% (Table 1); the experimental biocontrol agents applied to the
209 growing media every 4-5 days only partially reduced disease severity, with inconsistent results
210 throughout the trials. For instance, the disease reduction of *Pseudomonas* Ant P28, compared to the
211 untreated control, was from 17 to 47%, while it was from 8 to 54% for *Fusarium solani* FUS25, from
212 12 to 54% for *Trichoderma* sp. TW2 and from 4 to 46% for *Pseudomonas* (FC7, FC 8, FC 9). The
213 tested biocontrol generally provided results that were statistically comparable with the results for the
214 formulated mixture of *Trichoderma asperellum* + *T. gamsii* (29 to 43% efficacy) used as reference.
215 The co-application of the mixture of *Pseudomonas* PB26+ *F. solani* A25F+*Trichoderma* sp. TW2
216 tested in this study did not enhance the disease control efficacy, compared to the efficacy achieved
217 when BCAs were used on their own. The highest and most consistent *P. capsici* control was provided
218 by K-phosphite (62 to 94% efficacy).
219
220 *3.2. Effect of the dosage, type and number of applications of potassium phosphite*
221 The data from trials 5-13 were combined when no significant differences in disease severity were
222 found among the trials (Table 4). Phytophthora crown rot severity differed throughout the trials and
223 resulted in an average disease severity in the untreated control of 20.3, 40.1 and 59.1, respectively
224 (Table 5). The application of potassium phosphite significantly reduced disease severity in all the
225 experiments. One-way analysis of variance showed that the tested dosages and the type of application
226 (to the peat growing medium or to the nutrient solution) were not significant factors in the trials (Table
227 4), while the number of applications (1, 3 and 6) and the interaction of all the considered factors
228 significantly influenced disease severity under different disease pressures (P < 0.001). The efficacy
229 of potassium phosphite at the lowest tested rate increased by 30% and almost doubled when the
230 number of treatments was increased from one to six (Table 5). Moreover, one application of
231 potassium phosphite at the highest tested concentration resulted in a much lower effect than the three

applications in trials 5-7 and 8-10, while no significant effect (P = 0.259) was found in trials 11-13 (Tables 4 and 5). Three more applications (for a total of six) generally had little or no further effect on the efficacy of the treatment in two out of the three sets of trials that were carried out, and an efficacy of 43.2% to 78.8% was reached (Table 5). No effect of phosphite on plant growth was found at both tested rates and frequency of applications throughout the trials (data not shown).

The effect of the interaction of all the factors (concentration, number of application and application method) was significant in all the experiments.

4. Discussion

Hydroponic systems have become a standard cultivation method in Southern Europe for several intensively grown vegetables, in part because, despite the high initial investments, they can provide growers with higher incomes (Savvas et al., 2013; Sambo et al., 2019). In fact, soilless cultivation, apart from being a solution from a technical and agronomical point of view, in many cases represents the grower's choice when crop rotation is not feasible and resistant cultivars are not available, and when chemical control becomes complicated due to increasing limitations in the availability of fumigants and registered fungicides (Garibaldi et al., 2014; Vallance et al., 2011).

Unfortunately, soilless grown plants may be attacked by the same pests and diseases as plants cultivated traditionally in soil, even though the occurrence and degree of severity may be different (Schnitzler 2004), and one of the main concerns of closed systems is the potential spread of root pathogens with the recirculation of the nutrient solution (Postma et al., 2008). The very limited availability of traditional fungicides for soilless systems has stimulated the adoption of other options. For instance, some biocontrol agents have been labelled for applications in irrigation systems and phosphite fertilizers, when labelled as phosphorus supplements, are admitted for application in soilless systems (Gómez-Merino and Trejo-Télle, 2015). Hydroponic is a complex environment and

multiple chemical and biological equilibria must be taken into account for developing practical solutions to manage diseases of plants grown soilless.

In the present study, the experimental biocontrol agents obtained from suppressive compost (*Pseudomonas* sp. PB26, *F. solani* A25F and *Trichoderma* sp. TW2) and from a soilless rockwool medium (*Pseudomonas* FC7B, FC8B, FC9B mixture) have provided a certain degree of control and have led to results that are comparable with those obtained for the formulated mixture of *Trichoderma gamsii* + *T. apserellum*. Among the huge diversity of microorganisms that have shown to suppress the growth of *P. capsici*, the most explored belong to rizhobacteria (Thomashow et al., 1995; Sheoran et al., 2015; Agisha et al., 2019) and *Trichoderma* (Harman, 2006; Kaewchai et al., 2009; Segarra et al., 2016; Bae et al., 2011). *Trichoderma* spp. have shown high biocontrol potential through one or more mechanisms such as mycoparasitism, competition for key nutrients and colonization of sites, production of antibiotics, or by stimulating plant defense mechanisms (Benítez et al., 2004; Elad et al., …). The disease suppression and plant-growth promotion activities of various strains might be related to the production of different antibiotics, secondary metabolites, lytic enzymes, phytohormones, siderophores, and volatiles (Bae et al., 2011; Li et al., 2019). *Trichoderma* species are well known for their capacity to produce secondary metabolites, including peptaibols, terpenes, diketopiperazines, steroids, amides, lactones, polyketides, tetronic acid derivatives, peptides, pyranone derivatives, pyridines, and cyclopentenones, which may have numerous biological activities, including antifungal, antibacterial, plant-growth-enhancing/inhibitoring, bioinducer, antagonism and plant resistance effects (Li et al., 2019), suggesting a potential role also in the control of *P. capsici*, as demonstrated by Bae et al., 2011 *Pseudomonas* strains are known for producing metabolites active against *P. capsici,* like pyrazines, dimethyl trisulphide and dimethyl disulfide (Sheoran et al., 2015; Agisha et al., 2019). Among different mecchanisms, the ability in inducing a motility inhibitory effect of zoospore of *P. capsici* provided by *Pseudomonas* has been demostred (Zohara et al., 2016). Non-pathogenic *Fusarium oxysporum* and *F. solani* collected from wilt-

suppressive soil have been reported as biocontrol agents against Fusarium wilt diseases of tomato, watermelon and muskmelon (Larkin and Fravel 1998; Malandrakisa et al., 2018).

Most biological control studies in hydroponics deal with one antagonist, although attempts to apply more than one antagonist helped in disease control efficiency. Indeed, the efficiency of biological control agents in mixtures may be related to complementary modes of action of combined organisms (Xu et al., 2011). For instance, a mixture of fluorescent pseudomonads and nonpathogenic isolates of *F. oxysporum* were effective in reducing the density of pathogenic *F. oxysporum* f.sp. *gladioli* populations in soils (Lemanceau and Alabouvette, 1993). Other studies have demonstrated that the combination of fungi and bacterial species, respectively, *Trichoderma hamatum* and *Pseudomonas aeruginosa*, is able to significantly reduce the incidence of *P. capsici* disease in chili pepper (Chemeltorit et al. 2017). In the present study the co-application of the mixture of *Pseudomonas* sp. PB26 + *F. solani* A25F + *Trichoderma* sp. TW2 did not generally enhance the efficacy of the BCA used alone. However, in agreement with another study (Xu et al., 2011), combinations may be valuable for other reasons, including control of various pathogens, more consistent efficacy, or control over different environments and stress conditions, which were not evaluated in this study. Since inconsistent results were observed for the tested biocontrol agents, further investigations are needed under various environmental conditions. Indeed, introduction of single or mixtures of biocontrol agents that are not native to that microenvironment fail to sustain its population high enough for being effective. The presented results provided evidence of a new application potential of *Pseudomonas* sp. PB26, *F. solani* A25F and *Trichoderma* sp. TW2 for controlling *Phytophthora capsici* in soilless. Hence, future research on the dosage/frequency and on possible combinations with other control measures is suggested.

Among the options that were tested, the one with potassium phosphite provided a good control of the pathogen. Although studies have long been made on phosphite in order to understand its role in agriculture (McDonald et al., 2001; Ouimette and Coffey, 1989; 1990; Ristaino and Johnston, 1999; Thao and Yamakawa, 2009), its exploitation in soilless systems has been limited. Phosphite has, for

instance, been investigated under hydroponic conditions against the Phytophthora crown rot of tomato and pepper-*P. capsici* (Förster et al., 1998; Stanghellini et al., 1994) and lettuce- *Phytophthora drechsleri* (Jee et al., 2002). These compounds are systemic, can be transported upward in the xylem and downward in the phloem to the roots (Cohen and Coffey, 1986), and have both protective and curative properties (Barrett et al., 2003). Both direct and indirect modes of action may occur, depending on the time interval between the phosphite application and the inoculation, the applied phosphite concentration and the tolerance of the host and pathogen to phosphite (Jackson et al., 2000; Smillie et al., 1989). The high water solubility of phosphite allows different type of application, however, there is still a lack of information involving phosphite in soilless. Results from the present study help in elucidating the possible long-term effect of phosphite applied in soilless against *P.capsici*. Many phosphite application methods, including foliar, seed and root immersion, trunk injections and soil applications, have resulted to be efficient against several soilborne and foliar pathogens in horticultural crops (Alexandersson et al., 2016; Barrett et al., 2003; Carmona et al., 2018; Föster et al., 1998; Greenhalghet al. 1994; Yandoc-Ables et al. 2007; Liljeroth et al., 2016; Lobato et al., 2010; Smillie et al., 1989). Under field conditions, phosphite-based fertilizers are normally applied as drenches or through an irrigation system. However, the application of phosphites should be timed carefully on the basis of the plant genotype, phenological stage and environmental conditions (Alexandersson et al., 2016). In fact, excessive phosphite concentrations have resulted in phytotoxicity in some horticultural crops (Barrett et al., 2003; Pilbeam et al., 2000; Walker, 1991). In the present study, potassium phosphite has been applied to zucchini plants by adding soluble forms of the element to the nutrient solution of a closed hydroponics system and the results have been compared with the results of its application to the growing media. The obtained results have pointed out that the type of application of potassium phosphite and the concentration of application did not affect the Phytophthora crown rot control in the trials carried out under different disease pressures. The results obtained in this study are in agreement with those of Pilbeam et al. (2000), who showed a slight improvement in the control of *Phytophthora cinnamomi* on *Eucalyptus marginata* above a

certain application rate. However, the efficacy of potassium phosphite was improved when the number of applications was increased from 1 to 3. When the number of application was increased, the protection provided by potassium phosphite was generally doubled, without any negative effect on plant growth. Increasing the number of applications from 3 to 6 did not provide any significant additional advantage. Because potassium phosphite acts systemically and is known for its direct effect on the pathogen (Guest and Bompeix, 1990; Smillie et al., 1989), its application in a closed soilless system under controlled conditions should be a topic of continuous research on different hosts and pathogens. Indeed, potassium phosphite acts primarily on the pathogen, inducing the release of stress metabolites to elicit the defence response (Guest and Grant, 1990) and some host plants are more responsive to phosphonate than others. In the present study, we did not evaluate the mechanism of action of phosphite. The results obtained consistently show that potassium phosphite, applied to the nutrient solution, represents an important option for growers to control *P. capsici* on soilless gown zucchini. Moreover, it is possible that the level of control provided by the here tested biocontrol agents may be improved in IPM programmes. The impact of combined application of BCA with reduced dosage of phosphite merits further evaluations.

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Conflict of Interest

Massimo Pugliese declares he has a financial interest (shareholder) in the company AgriNewTech that provided three microorganisms (*Trichoderma* sp. TW2, *Fusarium solani* FUS25 and *Pseudomonas* sp. PB26) tested in this study.

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Tables

Table 1.

Main operations carried out during the first set of trials

Operation	Trial 1	Trial 2	Trial 3	Trial 4
Sowing in nursery	30.12.1016	30.01.2017	15.03.2017	27.09.2017
Artificial inoculation with *Phytophthora capsici*	12.01.2017	9.02.2017	28.03.2017	11.10.2017
Treatments with BCAs and K-phosphite	12.01.2017 16.01.2017 20.01.2017 25.01.2017 30.01.2017 6.02.2017	09.02.2017 15.02.2017 20.02.2017 24.02.2017 01.03.2017 6.03.2017	28.03.2017 03.04.2017 07.04.2017 12.04.2017 18.04.2017 21.04.2017	11.10.2017 17.10.2017 23.10.2017 27.10.2017 02.11.2017 07.11.2017
Transplanting	16.01.2017	15.02.2017	03.04.2017	17.10.2017
End of the trial	13.02.2017	13.03.2017	28.04.2017	14.11.2017

Table 2.

Main operations carried out during the second set of trials

Operation	Trials carried out under different disease pressures.								
	Low[a]			Average			High		
	5	6	7	8	9	10	11	12	13
Artificial inoculation with *Phytophthora capsici*	16.06.17	08.09.17	08.06.17	13.04.17	09.05.17	30.05.17	05.05.17	15.09.17	05.04.17
Treatments with K-phosphite	16.06	08.09	08.06	13.04	09.05	30.05	05.05	15.09	05.04
	22.06	15.09	13.06	19.04	15.05	05.06	11.05	22.09	10.04
	26.06	20.09	16.06	24.04	19.05	09.06	15.05	27.09	14.04
	30.06	25.09	21.06	28.04	24.05	14.06	19.05	02.10	19.04
	05.07	29.09	26.06	03.05	29.05	19.06	24.05	06.10	24.04
	10.07	04.10	30.06	08.05	02.06	23.06	29.05	11.10	28.04
Transplanting	22.06.17	15.09.17	13.06.17	19.04.17	15.05.17	05.06.17	11.05.17	22.09.17	6.04.17
End of the trial	24.07.17	18.10.17	14.07.17	22.04.17	16.06.17	7-07.17	12.06.17	25.10.17	12.05.17

[a] Disease severity in the untreated control as average of three trials: low, DS 20.3; average: DS 40.1 and high DS 59.1.

Table 3.

Effect of the experimental BCA treatments on Phytophthora crown rot caused by *P. capsici* on soilless grown zucchini (cv. Genovese) . The data shown are expressed as disease severity (DS0-100) at the end of trials 1-4. Standard errors and the efficacy, compared with the untreated control (E%), are reported.

Treatments	DS 0-100															
	Trial 1				Trial 2				Trial 3				Trial 4			
Untreated control	30.0	±5.6	b [a]	**E%[b]**	46.7	±5.0	b	**E%**	40.0	±7.2	c	**E%**	47.9	±2.2	c	**E%**
Pseudomonas sp. PB26	25.0	±3.7	b	**17**	25.0	±2.6	ab	**47**	25.5	±3.6	ab	**36**	30.2	±3.2	ab	**37**
Fusarium solani FUS25	23.3	±5.5	b	**22**	26.7	±3.1	ab	**43**	36.7	±4.3	bc	**8**	29.2	±3.9	ab	**39**
Trichoderma sp. TW2	20.0	±2.0	ab	**33**	21.7	±3.5	ab	**54**	35.0	±4.1	b	**12**	35.4	±4.4	bc	**26**
Pseudomonas sp. PB26+ *F. solani* FUS25+*Trichoderma* sp. TW2	30.0	±7.3	b	**0**	25.0	±3.7	ab	**47**	21.7	±5.7	ab	**46**	31.8	±4.4	a-c	**34**
Pseudomonas (FC7,8,9)	18.4	±1.7	ab	**39**	25.0	±2.5	ab	**46**	38.3	±7.3	c	**4**	30.4	±2.5	ab	**37**
Trichoderma asperellum + T,gamsii	18.3	±4.9	ab	**39**	26.7	±3.2	ab	**43**	28.3	±2.0	ab	**29**	25.5	±4.1	ab	**47**
K-phoshite (Alexine at 2.5g/l)	1.7	±1.7	a	**94**	8.3	±1.6	a	**82**	13.8	±1.8	a	**65**	18.3	±2.3	a	**62**

[a] Means in the same column, followed by the same letter, do not differ according to Tukey's Test ($P < 0.05$)

[b]E%: percentage of disease reduction, compared to the untreated control, at the end of the trial.

Table 4.

Effect of the K-phospite, dosage, type of application, number of treatments and their interaction on the disease severity average (DS) for trails under low (trials 5-7), average (trials 8-10) and high (trials 11-13) disease pressure according to the analysis of variance.

Considered factors and their interaction	at $P < 0.05$
Trials 5-7	0.456
Dosage (1.25 and 2.5 g/l)	<0.0001
Type of application (pot or NS)	0.353
Number of treatment (1, 3 and 6)	0.002
Dosage × Number × type of application	<0.0001
Trials 8-10	0.338
K-Phosphite dosage (1.25 and 2.5 g/l)	<0.0001
Type of application (pot or NS)	0.227
Number of treatment (1, 3 and 6)	<0.0001
Dosage × Number × type of application	<0.0001
Trials 11-13	0.574
K-Phosphite dosage (1,25 and 2,5 g/l)	<0.0001
Type of application (pot or NS)	0.181
Number of treatment (1, 3 and 6)	<0.0001
Dosage × Number × type of application	<0.0001

Table 5.

Effect of the dosage (1.125 and 2.5 g/l), type (pot and nutrient solution NS) and numbers of applications (1, 3 and 6) of K-phosphite against *Phytophthora capsici* on zucchini (cv. Genovese). Data shown are mean of three trials each carried out under different disease pressure.

Treatments and dosage (g/l)	Type of Application	Number of applications and intervals between (days)	DS 0-100											
			Trials 5-7				Trials 8-10				Trials 11-13			
Untreated	-	-	20.3	±2.4	b[a]	***E%****	40.1	±3.6	c	***E%***	59.1	±5.3	b	***E%***
K phosphite, 1.125	Pot	1	12.9	±1.6	ab	***37***	32.2	±3.7	bc	***20***	33.9	±5.2	ab	***43***
K phosphite, 1.125	Pot	6× 5 d.	8.1	±1.9	a	***60***	20.9	±3.2	ab	***48***	29.5	±4.7	a	***50***
K phosphite, 2.5	Nutrient solution	1	13.3	±2.9	ab	***35***	22.9	±5.0	ab	***43***	32.1	±5.3	ab	***46***
K phosphite, 2.5	Pot	1	12.2	±2.4	ab	***40***	20.3	±0.5	ab	***49***	35.8	±5.3	ab	***39***
K phosphite, 2.5	Nutrient solution	3 ×5 d.	10.0	±2.0	a	***51***	10.4	±3.1	a	***74***	24.0	±5.1	a	***59***
K phosphite, 2.5	Pot	3 ×5 d.	5.8	±1.5	a	***72***	8.2	±2.0	a	***80***	28.0	±6.1	a	***53***
K phosphite, 2.5	Nutrient solution	6 ×5 d.	6.0	±2.5	a	***71***	22.7	±4.7	ab	***43***	21.0	±6.3	a	***64***
K phosphite, 2.5	Pot	6 ×5 d.	5.0	±1.4	a	***75***	8.5	±1.6	a	***79***	28.1	±6.5	a	***53***

[a] Means in the same column, followed by the same letter, do not differ according to Tukey's Test (P <0.05)

[b]E%: percentage of disease reduction, compared to the untreated control, at the end of the trial.

Fig 1.

Effect of the numbers of applications (1, 3 or 6) of K-phosphite against *Phytophthora capsici* on zucchini (cv. Genovese) under different disease severity pressures in the three set of trials. The data are expressed as the mean values of disease severity in trials 5-7, 8-10 and 11-13.