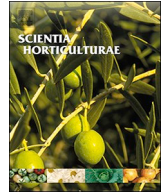




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Effect of biocontrol agents and potassium phosphite against *Phytophthora* crown rot, caused by *Phytophthora capsici*, on zucchini in a closed soilless system

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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 *Phytophthora* crown rot of zucchini by 62–94%, providing more consistent disease severity reduction than those achieved using the experimental BCAs, alone or in mixture, and the *Trichoderma gamsii* + *T. asperellum* formulated mixture (29–47% 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 48–79%) when applied via a treated peat growing media or via a nutrient solution with 3–6 applications, thereby offering growers an important opportunity to control *P. capsici* on soilless grown zucchini.

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 (Gómez et al., 2013; 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 (Garibaldi et al., 2014). This pathogen can also be spread through infected transplants, seeds and water resources (Granke et al., 2012; Hausbeck and Lamour, 2004; 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, to reduce the release of nutrients into the environment and to improve water efficiency (Van Os, 1999), closed soilless systems, which are increasingly adopted in southern countries, are often characterised by the presence of root diseases (Jarvis, 1991; Postma et al., 2008; Stanghellini and Rasmussen, 1994).

Owing to the limited availability of synthetic fungicides registered for soilless systems, it is necessary to evaluate the efficacy of alternative disease control measures. Thus, disease management, based on biocontrol agents, suppressive soils and inorganic salts, is increasingly being exploited in such growing systems (Gullino et al., 2015; Paulitz, 1997; Postma et al., 1999; Van Os, 1999; Postma, 2010; Vallance et al., 2011). Phosphite has been shown to be effective in the control of oomycete related diseases in horticulture. Deliopoulos et al. (2010), for

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instance, showed that phosphite salts are effective against several soil-borne pathogens in different pathosystems, such as *Pythium ultimum*-cucumber, *Phytophthora cinnamomi*-lupin and *Phytophthora nicotianae*-tobacco. The protective effect induced by phosphite, and its persistence in planta, may vary among species (Barrett et al., 2003; Shearer and Crane, 2012), type of application (Guest and Grant, 1991; Smillie et al., 1989), *Phytophthora* species and strains (Coffey and Bower, 1984), and could be affected by concentration (Jackson et al., 2000; Daniel and Guest, 2006). Although the extensive research carried out to better understand the mode of action of phosphite in plant protection (Hardy et al., 2001; Thao and Yamakawa, 2009; Alexandersson et al., 2016), there is still a need to better understand their potential when applied in hydroponics. In the case of biocontrol agents, different microorganisms have been tested in the past in soilless systems, such as *Muscodor albus* against *Rhizoctonia damping-off* of broccoli, *Gliocladium virens* against *Rhizoctonia solani* and *Pythium ultimum* of zinnia, cotton and cabbage (Lumsden and Locke, 1989), and non-pathogenic *Fusarium oxysporum* against *Fusarium oxysporum* f.sp. *basilici* on basil (Fravel and Larkin, 1999). Other studies have shown a positive effect of applying biocontrol agents to hydroponic systems via recirculating nutrient solutions or in the growing-medium on different hosts affected by oomycete pathogens; this is the case of bacterial isolates of fluorescent *Pseudomonades* 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 *Phytophthora capsici* on bell pepper (Mercier and Manker, 2005) and of *Bacillus subtilis* against *Pythium aphanidermatum* on lettuce (Utthede et al., 2000). However, despite many studies, their practical 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; Walters 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; Gullino et al., 2015; Stanghellini et al., 1996), 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 for the control of *P. capsici* on zucchini, compared with a commercial formulation of *Trichoderma gamsii* + *T. asperellum*, and potassium phosphite salts. Moreover, different types and different numbers of applications of potassium phosphite have been considered, with the aim of developing practical solutions to manage the disease.

Table 1

Main operations carried out during the first set of trials.

Operation	Trial 1	Trial 2	Trial 3	Trial 4
Sowing in nursery	30.12.2016	30.01.2017	15.03.2017	27.09.2017
Artificial inoculation with <i>Phytophthora capsici</i>	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

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 NO₃⁻, 4.8 mM NH₄, 0.75 mM KH₂PO₄, 0.75 mM K₂SO₄, 0.012 mM Iron chelate EDTA, 2 mM MgO, 2 mM SO₃, 0.2 mM B, 0.001 mM Mo, 0.15 mM Zn, 3.1 mM CaO, 0.05 mM Cu⁺⁺, 0.25 mM Mn and 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 and treated as described above.

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 mixtures, according to the protocol tested in the first and second set of trials (Tables 1 and 2).

A 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 blond peat-based growing medium (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 mixtures (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), and *Trichoderma* sp. TW2 (Cucu et al., 2019). A mixture of three *Pseudomonas* spp. strains, *Pseudomonas* sp. FC7B (EU836174) - *Pseudomonas putida* FC8B (EU836171) and *Pseudomonas* sp. FC9B (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

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 <i>Phytophthora capsici</i>	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; 22.06	08.09; 15.09	08.06; 13.06	13.04; 19.04	09.05; 15.05	30.05; 05.06	05.05; 11.05	15.09; 22.09	05.04; 10.04
	26.06; 30.06	20.09; 25.09	16.06; 21.06	24.04; 28.04	19.05; 24.05	09.06; 14.06	15.05; 19.05	27.09; 02.10	14.04; 19.04
	05.07; 10.07	29.09; 04.10	26.06; 30.06	03.05; 08.05	29.05; 02.06	19.06; 23.06	24.05; 29.05	06.10; 11.10	24.04; 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.

medium in 100 ml Erlenmeyer flasks, and then incubating the suspension in a rotary shaker at 600 rpm for 48 h at 23 °C. The cells suspension was centrifuged, and the pellets were re-suspended in sterile deionised water. The bacterial concentrations were checked by means of optical density (OD₆₀₀) before application. The density (OD₆₀₀) was adjusted with sterile deionised water, by means of serial dilution, to 1×10^8 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 of sterile distilled water and homogenised using a hand-held rotary mixer. The conidia suspension obtained for the *Trichoderma* sp. TW2 isolate was adjusted by means of serial dilution to a final concentration of 1×10^7 CFU ml⁻¹.

The antagonistic *Fusarium solani* FUS25 strain was propagated into 1000-ml-flasks containing 250 ml of potato dextrose broth Sigma, Germany and maintained in a rotary shaker for ten days at 200 rpm. The cultures were centrifuged at 8000 g for 20 min at 4 °C. The conidia and mycelium pellets 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 1×10^7 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 1×10^7 CFU ml⁻¹. The BCAs were applied six times to the growing medium at 5 day-intervals using 100 ml/pot of the suspension, according to the experimental protocol (Table 1).

The experimental BCAs were compared with a commercial formulation of *Trichoderma asperellum* + *T. gamsii* (Remedier, Isagro, Milan, Italy), which had been applied at the label rate of 0.25 g l⁻¹ of peat substrate. They were also compared with 2.5 g l⁻¹ of potassium phosphite fertilizer (Alexine 95PS P₂O₅ 5₂ % + K₂O, 42 %, Alexine, Massò, Spain), which was labelled as a phosphorus supplement for soilless applications (Table 1).

The second set of trials (trials 5–13) was conducted to select the optimal type, frequency and number of potassium phosphite applications (Table 2). The fertilizer-based phosphite was added directly to the nutrient solution (NS) or applied to each pot around the base of the seedlings using 100 ml/pot of the suspension prepared at 1.25 and 2.5 g l⁻¹, according to the experimental protocol. K-phosphite was applied at 5–6 day intervals with 1, 3 and 6 applications (Table 2).

The first treatment in both protocols was carried out the same day as the artificial infestation of the peat substrate.

2.3. Artificial inoculation with the pathogen

A highly virulent strain of *Phytophthora capsici* (coded PHC 1/16), isolated from zucchini and taken from the AGROINNOVA collection, was cultured on a selective oomycete medium (Masago et al., 1977) at 20 °C for one week. One mycelium-agar plug (5 mm diameter), taken from an actively growing colony, was transferred to a 1000-ml-flask containing a

wheat-hempseed medium (200 g of wheat kernels, 100 g of hempseeds and 320 ml of water, sterilized at 121 °C for 30 min) and incubated at 20 °C in a growth chamber under a 12-h fluorescent photoperiod. The *P. capsici* produced after 10 days of incubation was mixed into the soil substrate at a concentration of 1 g of fresh biomass per litre of growing medium immediately before the first treatment was made.

Fifteen-day-old zucchini seedlings were transplanted into the treated and untreated pots 5–7 days after the artificial infestation of the substrate with the pathogen.

2.4. Disease assessment and statistical analysis

The zucchini plants were assessed at 7-day-intervals, starting from when the first symptoms of *Phytophthora* crown rot, that is yellowing of the leaves, were observed. Disease severity was evaluated 7–14 days after the final treatment, according to Padley et al. (2008). Disease severity (DS) was expressed using the $[\sum(n^{\circ} \text{ plants} \times x_{0-5}) / (\text{total } n^{\circ} \text{ of plants recorded})]$ formula, with x_{0-5} corresponding to the reported value: 0 = no symptoms, healthy plants; 1 = 1 corresponding to 30 % of the leaves being slightly wilted (midpoint 15 %); 2 = 31 corresponding to 50 % of foliar wilting and crown lesions (midpoint 40 %); 3 = 51 corresponding to 70 % of the plants being partially collapsed (midpoint 60 %); 4 = 71 corresponding to 90 % of the plants being collapsed (midpoint 80 %); 5 = over 90 % of dead plants (midpoint 95 %).

The data obtained from the experiments were subjected to an analysis of variance (ANOVA) using SPSS, Version 25. The experimental unit consisted of a 3-L pot with two plants and sub-replicates with 12 plants each. Each set of treatments was repeated at last three times in the first and second sets of trials according to protocols 1 and 2 (Tables 1 and 2). The trials were combined when the 'trial' factor was not significant ($P > 0.05$). The data were compared using Tukey's Test at a significance level of 5 %. The considered factors were: five experimental biocontrol agents and K-phosphite, and the type of application, that is, directly to the peat medium in the pots or via nutrient solution (NS), the rate (1.25 and 2.5 g l⁻¹) and the number of applications (1, 3 or 6). The efficacy of the different treatments in controlling *P. capsici* was calculated as:

$$\% \text{ Disease reduction (E)} = \frac{LS_i - LS_t}{LS_i} \times 100$$

where LS = DS of plants affected by *P. capsici*; i = inoculated and untreated control; t = treatments.

3. Results

3.1. Effect of the biocontrol agents

The data from the first set of trials (1–4) were analysed separately for each experimental run because of the evidence of heterogeneity ($P < 0.05$) between the trial runs (Table 1). *Phytophthora* crown root

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 (DS 0-100) at the end of trials 1-4. Standard errors and the efficacy (E%), compared with the untreated control, are reported.

Treatments	DS 0-100															
	Trial 1		Trial 2		Trial 3		Trial 4		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%
<i>Pseudomonas</i> 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
<i>Fusarium solani</i> 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
<i>Trichoderma</i> 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
<i>Pseudomonas</i> sp. PB26 + <i>F. solani</i> FUS25 + <i>Trichoderma</i> 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
<i>Pseudomonas</i> (FC7B,FC8B,FC9B)	18.4	± 1.7	ab	39	25.0	± 2.5	ab	46	38.3	± 7.3	c	4	30.4	± 2.5	ab	37
<i>Trichoderma asperellum</i> + <i>T. gamsii</i>	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-phosphite (Alexine at 2.5 g l ⁻¹)	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.

severity ranged from 30 to 47.9 % (Table 3); the experimental biocontrol agents applied to the growing media every 4–5 days only partially reduced the disease severity, and inconsistent results were observed throughout the trials. For instance, the disease reduction of *Pseudomonas* PB26, compared to the untreated control, was from 17 to 47 %, while it was from 8 to 43 % for *Fusarium solani* FUS25, from 12 to 54 % for *Trichoderma* sp. TW2 and from 4 to 46 % for the mixture of *Pseudomonas* (FC7B, FC8B, FC9B). The tested biocontrol generally provided results that were statistically comparable with the results of the formulated mixture of *Trichoderma asperellum* + *T. gamsii* (29–43% efficacy) used as a reference. The co-application of *Pseudomonas* PB26 + *F. solani* FUS25 + *Trichoderma* sp. TW2 mixture tested in this study did not enhance the disease control efficacy, compared to the efficacy achieved when the BCAs were used on their own. The highest and most consistent *P. capsici* control was provided by K-phosphite (62–94% efficacy) (Table 3).

3.2. Effect of the dosage, type and number of potassium phosphite applications

The data from trials 5–13 were combined when no significant differences in disease severity were found among the trials (Table 4). Phytophthora crown rot severity differed throughout the trials, and resulted in average disease severity values of 20.3, 40.1 and 59.1, respectively, in the untreated control (Table 5). The application of potassium phosphite significantly reduced disease severity in all the experiments. One-way analysis of variance showed that the tested dosages and the type of application (to the peat growing medium or to the

nutrient solution) were not significant factors in the trials (Table 4), while the number of applications (1, 3 and 6) and the interaction of all the considered factors significantly influenced disease severity under different disease pressures ($P < 0.001$). The efficacy of potassium phosphite at the lowest tested rate increased by 30 % and almost doubled when the number of treatments was increased from one to six (Table 5). Moreover, one application of potassium phosphite at the highest tested concentration resulted in much less 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, and an efficacy of 43.2%–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 applications and application method) was significant in all the experiments (Fig. 1).

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 option 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 through 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* FUS25 and *Trichoderma* sp. TW2) and from a soilless rockwool medium (*Pseudomonas* FC7B, FC8B, FC9B mixture) have provided a certain

Table 4

Effect of the K-phosphite, 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 ⁻¹)	< 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 ⁻¹)	< 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 ⁻¹)	< 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 (1.25 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 ⁻¹)	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	<i>E</i> %*	40.1 ± 3.6	c	<i>E</i> %	59.1 ± 5.3	b	<i>E</i> %
K-phosphite, 1.25	Pot	1	12.9 ± 1.6	ab	37	32.2 ± 3.7	bc	20	33.9 ± 5.2	ab	43
K-phosphite, 1.25	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

^aMeans 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.

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 and Weller, 1995; Sheoran et al., 2015; Agisha et al., 2019) and *Trichoderma* (Harman, 2006; Kaewchai et al., 2009; Segarra et al., 2013; 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; Berg, 2009; Elad, 2000). 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; Berg, 2009; Li et al., 2019; Sid Ahmed et al., 2003). *Trichoderma* species are well known for their capacity to produce secondary metabolites, including peptaibols, terpenes, diketopiperazines, steroids, amides, lactones, polyketides, tetrone acid derivatives, peptides, pyranone derivatives, pyridines, and cyclopentenones, which may have numerous biological activities, including antifungal, antibacterial, plant-growth-enhancing/inhibiting, bioinducer, antagonism and plant resistance effects (Berg, 2009; Li et al., 2019), suggesting a potential role also in the control of *P. capsici*, as demonstrated by Bae et al. (2011). Moreover, *Pseudomonas* strains are known for producing

metabolites active against *P. capsici*, like pyrazines, dimethyl trisulphide and dimethyl disulfide (Lemanceau and Alabouvette, 1993; Sheoran et al., 2015; Agisha et al., 2019). Among different mechanisms, the ability in inducing a motility inhibitory effect of zoospore of *P. capsici* provided by *Pseudomonas* has been demonstrated (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; Kavroulakis et al., 2007; Malandrakisa et al., 2018). Only a few papers have reported efficacy against *Pythium ultimum* (Benhamou et al., 2002), *Phytophthora capsici* (Silvar et al., 2009). In the present study the impact of antagonistic *F. solani* was demonstrated for the first time in soilless and against *P. capsici*.

Most biological control studies in hydroponics deal with one antagonist, although efforts to study the impact of more than one antagonist on disease control efficiency received an increased attention. Indeed, the efficiency of biological control agents in mixtures may be related to complementary modes of action of combined organisms (Manasfi et al., 2018; Xu et al., 2011). For instance, a mixture of fluorescent *Pseudomonas* and nonpathogenic isolates of *F. oxysporum* were effective in reducing fusarium crown and root rot of tomato (Lemanceau and Alabouvette, 1991). Similar results were observed using the combination of *Trichoderma hamatum* and *Pseudomonas aeruginosa* against *P. capsici* disease in chili pepper (Chemeltorit et al.,

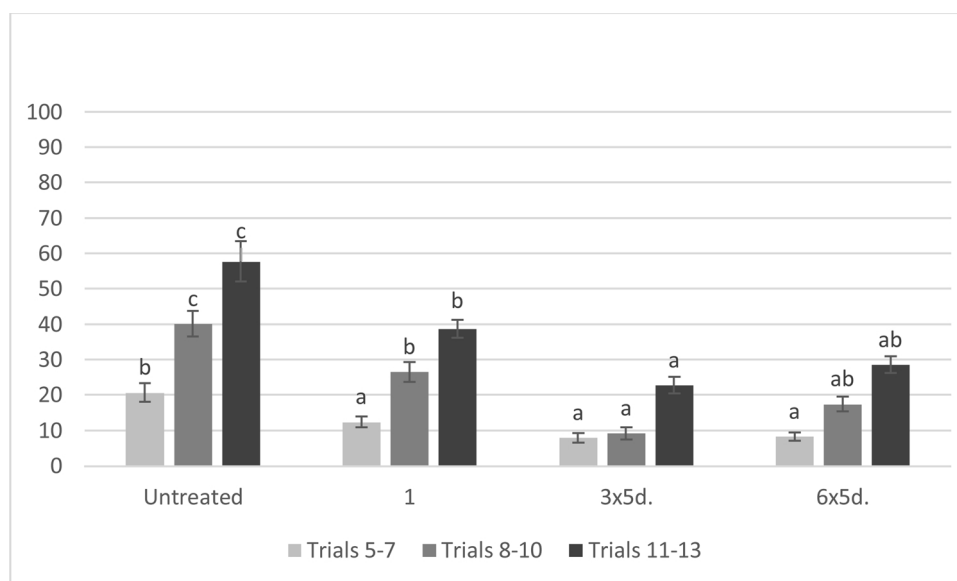


Fig. 1. Effect of the numbers of applications (1, 3 or 6) of K-phosphite against *Phytophthora capsici* on zucchini (cv. Genovese) applied every 5 days (5d.) under different disease severity pressures in the three set of trials. The data are expressed as the mean values of disease severity in trials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

2017). However, other studies have demonstrated that combinations of antagonists did not provide significantly better control than BCA antagonists alone in different pathosystems (Larkin and Fravel, 1998; Xu et al., 2011). In the present study the co-application of the *Pseudomonas* sp. PB26 + *F. solani* FUS25 + *Trichoderma* sp. TW2 mixture 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* FUS25 and *Trichoderma* sp. TW2 for controlling *P. 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 potassium phosphite one 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 been investigated under hydroponic conditions against the Phytophthora crown rot of tomato and pepper-*P. capsici* (Föster et al., 1998; Stanghellini and Rasmussen, 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; Greenhalgh et 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., 2002, 2003; Pilbeam et al., 2000; Walker, 1991). In the present study, potassium phosphite has been applied to zucchini plants by adding soluble forms 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 control of *Phytophthora* crown rot 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* for a certain application rate. However, the efficacy of potassium phosphite improved when the number of applications was increased from 1 to 3. When the number of applications was increased, the protection provided by potassium phosphite generally doubled, without any negative effect on plant growth. However, 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 Bompeix, 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 show that potassium phosphite, applied via the nutrient solution, represents an important option for growers to control *P. capsici* on soilless grown 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.

CRedit authorship contribution statement

G. Gilardi: Conceptualization, Data curation, Investigation, Methodology, Software, Writing - original draft, Writing - review & editing. **M. Pugliese:** Conceptualization, Writing - review & editing. **M.L. Gullino:** Conceptualization, Funding acquisition, Project administration, Writing - original draft, Writing - review & editing. **A. Garibaldi:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

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