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Nursery treatments with non-conventional products against crown and root rot, caused by *Phytophthora capsici*, on zucchini

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Abstract *Phytophthora capsici*, a pathogen causing crown and root rot of zucchini in southern Italy since the 1980s, has recently been observed in open field in northern Italy, causing severe losses. Since chemical control on zucchini is complicated by a limited availability of registered chemicals, as well as by the scalar harvest, a number of resistance inducers, organic amendments, biocontrol agents and fungicides were tested against *P. capsici*, under greenhouse conditions. Experiments were carried out at the nursery level, with different timing and number of applications. In the presence of a very high disease pressure, the best disease control was provided by mefenoxam, followed by the phosphite-based products, which acted as resistance inducers and also provided a positive effect on plant biomass. Acibenzolar-S-methyl, although effective, was sometimes phytotoxic. The biocontrol agents tested as well as the patented formulation of *Brassica carinata* defatted seed meals were not effective, providing results statistically similar to the untreated control. The efficacy of resistance inducers is interesting in view of their possible use in alternation with chemicals, or as stand-alone treatments in cultivation systems which do not adopt chemical control.

Keywords *Cucurbita pepo*; fungicides; resistance inducers; biocontrol agents; integrated control

Introduction

Zucchini (*Cucurbita pepo* L.) is an economically important crop in Italy, covering approximately 13,780 Ha: 10,000 ha in open field and 3,780 ha under protection (ISTAT 2011). In recent years,

symptoms of *Phytophthora capsici* on zucchini have been observed more frequently in northern Italy in open field (Garibaldi and Gullino, 2010). This pathogen, already reported on zucchini in southern Italy (Cristinzio and Novello, 1980), has also been recently reported in the intensively cropped area of Almerìa in southern Spain (Gómez *et al*., 2013), and causes crown, root and fruit rot. It is a very serious disease on bell pepper and cucurbits worldwide (Lamour *et al*., 2012), and it is able to attack other crops such as carnation, lima bean and weeds such as *Solanum nigrum* (Gubler and Davis, 1996; Hausbeck and Lamour, 2004).

The presence of this pathogen makes difficult the management of the crop, leading to increasing losses.

Chemical control on zucchini is complicated by a limited availability of registered chemicals, as well as by the scalar harvest, which makes the use of fungicides difficult. The adoption of genetic resistance is still at the early stage (Padley *et al*., 2008; Krasnow *et al*., 2014), while appropriate cultural practices need to be applied in integration with other control measures (Sanogo and Ji, 2012).

The induction of systemic acquired resistance is one of the approaches most intensively investigated for the management of a wide range of pathogens, particularly with the pathosystem *P. capsici*-squash (Koné *et al*., 2009; Ji., *et al*., 2011) and *P. capsici*–cucumber-pepper (Abbasi *et al*., 2011; Matheron and Porchas, 2002). Also, biological control methods, alternative to chemical fungicides, such as using microbial-based formulations, arbuscular mycorrhizal fungi (Ozgonen and Erkilic, 2007) are considered very interesting. Another approach used as an alternative to chemical soil disinfestation is the use of selected brassicas in biofumigation treatments (Ji *et al*., 2012).

This work was carried out in order to evaluate the efficacy of non-conventional products applied at nursery level as preventative treatments with different timing and number of applications, for the control of *P. capsici* of zucchini under greenhouse conditions.

Materials and methods

Experimental design and plant material in nursery trials A total of eight experimental trials were carried out in 2012 and 2013 under glasshouse conditions, at 23-27°C and 65-75% Relative Humidity (RH), as summarized in Table 1. Seeds of zucchini cv. Genovese (Furia sementi) were sown in 42-plug trays (5.5 cm Ø per pot, 4*l* soil capacity) filled with steamed (90°C for 30 minutes) peat mix substrate (blond peat: black peat 15:85, pH 5.5-6.0, 1,100 g m^{-3} of N:P:K and traces of molybdenum, Brill Type 5, Georgsdorf, Germany). The same substrate and fertilization were used

for the 20*l* plastic pots, used for transplanting zucchini seedlings 14-15 days after sowing (T14-T15). Five zucchini plants/pot were used with four replicates. Pots were filled before transplanting with the described substrate, and were artificially infested with the pathogen later. The experimental trials were arranged in a complete randomized block design.

Inoculum preparation and artificial inoculation The isolate of *Phytophthora capsici* PHC76 was obtained from infected zucchini (cv. Siltoza) plants in a field in northern-Italy and was maintained on a Phytophthora-semi-selective medium (Masago *et al*., 1977) at 12°C. The isolate was propagated by inoculation of a colonized agar–plug on to a sterile mixture of wheat-hemp kernels (2:1 v/v) in a 1*l* flask kept at room temperature in the dark. The 20-day-old culture of the pathogen was mixed with the previously described peat mix substrate Brill Type 5 at a rate of 1 g l^{-1} (Table 1). The 20*l* plastic pots containing the artificially infested substrate were maintained in the greenhouse under the same conditions as the 42-plug trays, and watered daily. The artificial inoculation of the substrate with the pathogen was carried out 7-8 days after the first treatment, as reported in tables 2-5.

Products tested Several fungicides, systemic inducers, organic amendments and biocontrol agents were compared with selected fungicides used as reference such as: azoxystrobin (Ortiva, 23.2% a. i., Syngenta Crop Protection), mefenoxam (Ridomil gold, 480 g l^{-1}, Syngenta Crop Protection) and propamocarb+fosethyl-Al (Previcur Energy, 47.2% + 27.6% a. i., Bayer Crop Science).

Phosphite-based products and resistance inducers The glucohumate complex (Glucoinductor + GlucoActivator, N 4%, P_2O_5 18%, International patent PCT, IB2004\001905, Fertirev) and a mineral fertilizer based on potassium phosphite (Alexin 95PS, P_2O_5 52%, K_2O 42%, Massò) were tested. Among products known for their capability to induce resistance mechanisms in plants, acibenzolar-S-methyl (Bion 50WG, 50% a.i., Syngenta Crop Protection) and phosethyl-Al (Alliette, 80% a.i, Bayer Crop Science) were used.

Organic amendments The patented formulation of *Brassica carinata* defatted seed meals (Biofence, N organic 3%, P 2.2%, K 2%, organic C 52%, Triumph) was used.

Biocontrol agents tested Bacillus subtilis QST 713 (Serenade Max, 15.6 % a.i., Bayer Crop Science), *Bacillus velezensis* (Cilus Plus IT45, 95%, Massò), *Trichoderma asperellum* ICC012+ *T. gamsii* ICC080 (Remedier WP, 2% a.i., Isagro Ricerca), a product based on arbuscular mycorrhizal

fungi combined with a microbial complex of *Trichoderma* and *Bacillus* (Rizocore, *Glomus* spp. 5%+*Bacillus megaterium* 10^4 $UFCg^{-1}$ + *Trichoderma* 10^{10} $UFCg^{-1}$, Biogard), and a microbial complex combined with arbuscular mycorrhizal fungi (Micosat, 14% a.i., CCS Aosta) were tested.

Products application and timing Most products tested were applied as a spray at high volume (1,500 *l* ha^{-1}), by using a hand sprayer. The first treatment was carried out on zucchini plants still in the plug tray, when they had reached the stage of second true leaves (7-10 days after sowing). The timing of application and the dosages are reported in tables 2-5. The zucchini seedlings grown in each tray were treated by spraying leaves at 5-7-day intervals. The number of spray treatments are reported in Tables 2-5. The two products based on arbuscular mycorrhizal fungi and microbial complex (Micosat and Rizocore) were mixed with 4*l* of the substrate used in the plug tray (Tables 2-5), while the organic amendment (Biofence) was mixed with the substrate used to fill the 20*l* plastic pots. These treatments were carried out one week before transplanting, at the same time of the artificial inoculation.

Disease and plant growth evaluation and analysis Assessments on the zucchini plants were carried out at 7 day intervals starting when the first symptoms, corresponding to yellow leaves caused by Phytophthora crown rot, were observed. The number of infected plants showing wilting and stem necrosis was counted to assess disease severity (DS). Disease severity ranging from 0 to 5 was evaluated at the end of each trial according to Padley *et al*., (2008). Disease severity was expressed by using the formula $[\sum(n° \text{ plants} \times x_{0\text{-}5}) / (\text{total } n^o \text{ of plants recorded})]$ with $x_{0\text{-}5}$ corresponding to the value reported: 0=no symptoms, healthy plants; 1=1 to 30% leaves slight wilted (midpoint 15%); 2=31 to 50% foliar wilting with crown lesion (midpoint 40%); 3=51 to 70% of plant is partially collapsed (midpoint 60%); 4=71 to 90% of plant is collapsed (midpoint 80%): 5=over 90% dead plant (midpoint 95%) (Tables 2-5).

At the end of the trials, the fresh weight of the zucchini plants was measured to evaluate the effect of different treatments carried out on plant growth.

The DS data were arcsine transformed to normalize their distribution, and then analysed by univariate ANOVA in SPSS 20.0. Means were separated by Tukey's test (P=0.05).

Results

Diseases symptoms started to be visible 5-7 days after transplanting into the artificially infested soil, and developed quickly under our experimental conditions (average air temperature ranging

from 23 to 28°C). In all trials, the artificial infestation of the soil led to a disease severity (DS) ranging from 53 to 93 in the untreated control plots.

In trials 1 and 2, the zucchini plants were treated at 0, 7 and 14 days (Table 2), and the untreated control showed a disease severity of 53 and 81, respectively. Acibenzolar-S-methyl provided the best efficacy, with a disease reduction from 87.7 to 100% at both dosages tested. These results were statistically similar to those provided by phosethyl-Al (97.6-93.8% disease reduction), *B. subtilis* (69% disease reduction), the phosphite-based products Alexine (100% disease reduction) and Glucohumate complex (97.5-78.5% disease reduction), and by the mixture of propamocarb + phosethyl-Al (98.9% disease reduction) in trial 1. In trial 2, in the presence of a very high disease incidence in the inoculated and untreated control, *B subtilis*, the phosphite-based Alexine, and phosethyl-Al + propamocarb only partially reduced the disease (44%, 40% and 58%, respectively). The other products tested (Rizocore and Micosat, *Trichoderma asperellum* ICC012+ *T. gamsii* ICC080, *Bacillus velenzensis* and *Brassica carinata* pellets) in trials 1 and 2 were not effective in disease control, providing results not statistically different from the untreated control (Table 2). In general, the fresh weight of zucchini plants at the end of trial 1 is related to disease control. Acibenzolar-S-methyl, applied three times at 0.0125 g l^{-1}, caused a phytotoxic effect, with a significant reduction in plant biomass compared with the non-inoculated and non-treated control, while the lowest fresh weight was observed in pots treated with arbuscular mycorrhizal fungi combined with a microbial complex of *Trichoderma* spp. and *Bacillus* spp. (Table 2).

In trials 3 and 4, four treatments were carried out at 0, 7 and 11 days in trays under nursery conditions, and at T26 under pot conditions (Tables 1 and 3). Phytophthora crown rot severely affected zucchini plants (DS of 70-76) in the inoculated and untreated control. In trials 3 and 4, acibenzolar-S-methyl at 0.0125 g l^{-1}, and the phosphite-based products (Alexine and Glucohumate complex) showed the best disease control (from 68 to 84% disease reduction), statistically similar to azoxystrobin and propamocarb + phosethyl-Al. The other products tested in trials 3 and 4 only partially controlled the pathogen (Table 3). The best disease reduction was provided by mefenoxam in trial 4. Both the phosphite-based products tested showed a high positive effect on plant biomass, whereas the four treatments with acibenzolar-S-methyl at 0.025 g l^{-1}, showed the most negative impact on the plant fresh weight (Table 3).

In trial 5 the products selected for their efficacy under different spray regimes were tested. In the presence of a very high disease severity (DS 90-93), 3 and 4 treatments with acibenzolar-S-methyl at 0.00625g l^{-1} provided results significantly similar to 2 and 3 treatments carried out with the same product at 0.0125 g l^{-1}, and to one treatment with mefenoxam (disease reduction ranging from 80 to 69%). Results statistically similar to those obtained with one treatment of mefenoxam were

provided by three treatments with phosethyl-Al, and with the phosphite-based product Alexin (Table 4). Two to four applications of acibenzolar-S-methyl gave statistically similar results in terms of fresh weight to phosethyl-Al and mefenoxam. The same trend was observed in trial 6. Three applications of the phosphite-based product Alexin significantly improved the plant biomass in trial 6 (Table 4).

In trials 7 and 8, where products selected from previous trials were applied in rotation in one to three treatments (Table 5), in the presence of a DS of 65 and 76 in the inoculated and non-treated control, all the combinations tested significantly reduced Phytophthora crown rot symptoms. A complete disease control, similar to that observed in the use of mefenoxam alone, was provided by three treatments with phosphite-K. Statistically similar results were also provided by two treatments of acibenzolar-S-methyl applied in the pre-inoculation stage and at the transplanting of zucchini into the pot (Table 5). The most effective treatments did not negatively affect plant biomass, whereas three treatments with acibenzolar-S-methyl at 0.025 g l^{-1} did significantly reduce plant biomass (Table 5). The fresh weight of zucchini plants was severely reduced in trial 8 compared with trial 7, probably due to less favourable environmental conditions, due for instance to light reduction (Table 5).

Discussion

The very limited availability of registered fumigants, coupled with the increasing restrictions in the availability of chemicals in general, due to the new European regulation, stimulates the search for different options, based on different types of control measures. Many studies have investigated management strategies using non-conventional means to control Phytophthora crown and root rot on several vegetables (Hausbeck and Lamour, 2004; Sanogo and Ji, 2012). However, the effect of preventative applications under nursery conditions of resistance inducers, biocontrol agents, *Glomus*-based products and *Brassica carinata* pellet in the pathosystem *P. capsici*-zucchini have not been described before. Their effectiveness depends on many factors, including the type of inoculum. For instance natural infestation of soil with oospores needs further investigations (Larkin *et al.*, 1995; Termorshuizen and Jeger, 2014). Since it is difficult to obtain consistent results under field conditions, it is useful to first develop their application under controlled conditions in the presence of artificial inoculation. Several studies have been carried out under this purpose with controlled conditions before field test (Kim *et al*., 2008; Sang *et al*., 2008; Gilardi *et al*., 2014). This study was carried out to obtain preliminary data by using artificial inoculation of *P. capsici* and will be followed by field trials.

The method of soil infestation used in this study, led to high disease severity in the untreated control plots, ranging from 45 to 93, thus permitting to test the efficacy of different products under severe conditions. Mefenoxam remains the best solution, providing complete control of the pathogen, even when applied only once, as in trials 5 and 6, while azoxystrobin, provided generally a lower effect in disease reduction. Several products, acting as resistance inducers, showed a very interesting activity. The good fungicidal activity of the phosphite-based product, coupled with the positive effect on plant biomass, is of special interest. Similar results against *P. capsici* were observed with the application of a phosphonates formulation as treatment of cucumber seeds (Abbasi *et al*., 2011). Among the resistant inducers tested, under our experimental conditions acibenzolar-S-methyl significantly controlled *P. capsici* of zucchini, as reported also against other Phytophthora crown and root rot agents (Matheron and Porchas, 2002; Koné *et al*., 2009; Ji *et al*., 2012; Gilardi *et al*., 2014). The rate and timing of application of resistance inducers are considered critical factors able to affect both the level of disease control as well as the yield (Walter *et al*., 2013). Unfortunately, acibenzolar-S-methyl, particularly when applied more than once, or under unfavourable environmental conditions, showed a phytotoxic effect that makes its application difficult under practical conditions. Also Romero *et al*. (2001) reported a similar negative effect of acibenzolar-S-methyl on plant growth on pepper.

The phosphite-based compound looks interesting in view of an integrated disease management approach. Because phosphite moves in the plant in the xylem and phloem, it can be applied as foliar spray or as soil treatment (Erwin and Ribeiro, 1996). Due to its complex mode of action (McDonald *et al*., 2001), can be applied closer to harvest, which on zucchini is scalar, and it has possible effects also against other diseases. It has been shown to be effective against downy mildew of basil, incited by *Peronospora belbahrii* (Gilardi *et al*., 2013; Mersha *et al*., 2012), *Phytophthora cinnamomi* on macadamia tree (Akinsanmi and Drenth, 2013), *Phytophthora nicotianae* on tomato (Gilardi *et al*., 2014), among others.

As reported for several pathogens, repeated applications of chemicals with a specific mode of action can easily induce the appearance of resistant populations of some pathogens including *P. capsici* (Jackson *et al*., 2012; Tamietti and Valentino, 2001). Azoxystrobin is widely used under field conditions on zucchini, while dimethomorph and mandipropamid are less commonly applied on this crop. The sensitivity of several strains of *P. capsici* against azoxystrobin requires further evaluation. The efficacy of resistance inducers is interesting in view of their possible use in alternation with chemicals, or as stand-alone treatments in cultivation systems which do not adopt chemical control. The availability of products acting by inducing resistance in the host plant represent a very valid option for growers. The results obtained under our experimental conditions, in the presence of a

high disease pressure, indicates that different compounds applied starting from the nursery conditions can satisfactorily control *P. capsici* on zucchini, providing growers a good range of treatment options.

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Table 1 General information of the operations carried out during the trials

Operation carried out	Protocol 1		Protocol 2		Protocol 3		Protocol 4	
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
Tray sowing	8/05/2012	18/06/2012	13/08/2012	27/09/2012	21/02/2013	10/05/2013	12/09/2013	15/10/2013
Tray treatments at the nursery level	T0: 14/05/2012 T7: 21/05/2012 T14: 28/05/2012	T0: 25/06/2012 T7: 2/07/2012 T11: 6/07/2012	T0: 20/08/2012 T7: 27/08/2012 T11: 31/08/2012	T0: 8/10/2012 T7: 15/10/2012 T11: 19/10/2012	T0: 28/02/2013 T5: 5/03/2013 T14: 13/03/2013	T0: 17/05/2013 T5: 22/05/2013 T11: 28/052013	T0: 19/09/2013 T6: 25/09/2013 T14: 2/10/2013	T0: 23/10/2013 T5: 28/10/2013 T12: 4/11/2013
Treatments at the 20*l* pot level	-	-	T26: 17/09/2012	T26: 2/11/2012	T21: 20/03/2013	T21: 7/06/2013	T21: 9/10/2013	T19: 11/11/2013
Artificial inoculation in the 20*l* pots	T8: 21/05/2012	T7: 2/07/2012	T7: 27/08/2012	T7: 15/10/2012	T7: 5/03/2013	T5: 2/05/2013	T7: 26/09/2013	T6 : 29/10/2013
Transplanting in 20*l*	T14: 28/07/2012	T11: 6/07/2012	T11: 31/08/2012	T11: 19/10/2012	T14: 13/03/2013	T14: 31/05/2013	T14: 2/10/2013	T12: 4/11/2013
Plants fresh weight, end of the trial	19/06/2012	7/08/2012	27/09/2012	15/11/2012	8/04/2013	20/06/2013	14/10/2013	15/11/2013

Table 2 Efficacy of different treatments against *Phytophthora capsici* on zucchini (cv. Genovese) in artificially infested soil, and plant biomass at the end of the trials (Protocol 1)

Treatment	Dosage a.i. g l^{-1}	Number and time of applications	Disease severity 0-100				Fresh weight g			
			Trial 1		Trial 2		Trial 1		Trial 2	
Inoculated non treated control	-	-	52.5	c[a]	81.3	f	184.3	c-e	199.1	a
Bacillus subtilis	0.58	3 (T0[c]; T7;T14)	16.3	ab	45.0	a-f	366.5	a-e	316.8	a
Bacillus velezensis	0.4[b]	3 (T0; T7;T14)	29.4	a-c	68.8	ef	270.9	a-e	248.9	a
T. asperellum + T. gamsii	0.02	3 (T0; T7;T14)	33.1	bc	51.3	c-f	243.8	a-e	365.5	a
Acibenzolar- S-methyl	0.025	3 (T0; T7;T14)	1.3	a	0.0	a	295.8	a-e	386.0	a
Acibenzolar- S- methyl	0.0125	3 (T0; T7;T14)	1.3	a	10.0	a-c	226.3	b-e	360.4	a
Phosethyl-Al	1.6	3 (T0; T7;T14)	1.3	a	5.0	ab	403.8	a-d	409.3	a
Arbuscular mycorrhizal + *Bacillus* +*Trichoderma*	0.08[b]	3 (T0; T7;T14)	31.9	bc	51.3	c-f	174.9	de	339.2	a
Glomus spp.+ microbial complex	1.5[b]	3 (T0; T7;T14)	26.3	a-c	58.8	d-f	343.7	a-e	336.3	a
Phosphite K	1.3+1.06	3 (T0; T7;T14)	0.0	a	48.8	b-f	316.8	a-e	303.2	a
Glucohumate complex fertilizer	1.6+0.72	3 (T0; T7;T14)	1.3	a	17.5	a-d	418.1	a-c	344.6	a
Propamocarb + phosethyl-Al	1.4+0.8	1 (T14)	0.6	a	33.8	a-e	433.2	ab	253.7	a
Brassica carinata pellet	0.15+0.055+0.05+1.13	1(T14)	41.3	bc	36.3	a-f	249.1	a-e	332.3	a
Non inoculated and non-treated control	-	-	0.0	a	0.0	a	481.6	a	326.7	a

[a] The mean values of the same column followed by the same letter, do not differ significantly according to Tukey's test (P=0.05)

[b] Corresponding to the dosage (g l^{-1}) reported on the commercial formulation

[c] Corresponding to the first treatment carried out on zucchini plants at the stage of second true leaves

Table 3 Efficacy of different treatments against *Phytophthora capsici* on zucchini (cv. Genovese) in artificially infested soil, and plant biomass at the end of the trials (Protocol 2)

Treatment	Dosage a.i. g l^{-1}	Number and time of applications	Disease severity 0-100				Fresh weight g			
			Trial 3		Trial 4		Trial 3		Trial 4	
Inoculated non treated control	-	-	76.3	d[a]	70.0	e	156.9	b-e	44.4	c
Bacillus subtilis	0.58	4 (T0[c];T7;T11;T26)	71.7	cd	73.3	e	106.0	de	101.7	bc
Bacillus velezensis	0.4[b]	4 (T0;T7;T11;T26)	63.3	b-d	66.7	de	149.5	c-e	142.5	a-c
T. asperellum + *T. gamsii*	0.02	4 (T0;T7;T11;T26)	41.3	a-d	74.0	e	181.9	b-e	89.5	c
Acibenzolar -S-methyl	0.025	4 (T0;T7;T11;T26)	46.3	a-d	45.3	a-e	81.3	e	29.8	c
Acibenzolar -S- methyl	0.0125	4 (T0;T7;T11;T26)	15.0	ab	22.0	a-c	159.9	b-e	59.3	c
Phosethyl-Al	1.6	4 (T0;T7;T11;T26)	55.0	b-d	37.0	a-e	225.3	b-d	260.7	ab
Arbuscular mycorrhizal + *Bacillus* +*Trichoderma*	0.08[b]	4 (T0;T7;T11;T26)	51.7	b-d	54.7	b-e	142.1	c-e	100.0	bc
Glomus spp.+ microbial complex	1.5[b]	4 (T0;T7;T11;T26)	60.0	b-d	64.0	c-e	255.7	bc	108.8	bc
Phosphite K	1.3+1.06	4 (T0;T7;T11;T26)	23.3	a-c	10.7	a-c	287.8	b	308.0	a
Glucohumate complex fertilizer	1.6+0.72	4 (T0;T7;T11;T26)	18.8	ab	33.3	a-c	277.3	bc	284.3	a
Propamocarb + phosethyl-Al	1.4+0.8	2 (T14; T26)	23.3	a-c	7.0	ab	212.8	b-e	178.3	a-c
Azoxystrobin	0.86	2 (T14; T26)	25.0	a-c	20.0	a-c	428.2	a	139.8	a-c
Brassica carinata pellet	0.15+ 0.055+ 0.05+1.13	1(T7)	63.3	b-d	90.7	e	159.5	b-e	31.4	c
Mefenoxam	0.48	2 (T14;T26)	-[d]		0.0	a	-		262.5	ab
Non inoculated and non-treated control	-	-	0.0	a	2.0	ab	431.8	a	152.3	a-c

[a] The mean values of the same column followed by the same letter, do not differ significantly according to Tukey's test (P=0.05)

[b] Corresponding to the dosage (g l^{-1}) reported on the commercial formulation

[c] Corresponding to the first treatment carried out on zucchini plants at the stage of second true leaves

[d] Not tested

Table 4 Efficacy of different treatments against *Phytophthora capsici* on zucchini (cv. Genovese) in artificially infested soil and plant biomass at the end of the trials (Protocol 3)

Active ingredient	Dosage a.i. g l^{-1}	Number and timings of spray applications (Total number)	Disease severity 0-100				Fresh weight g			
			Trial 5		Trial 6		Trial 5		Trial 6	
Inoculated non-treated control	-	-	93.0	c[a]	90.0	e	4.0	c	4.6	e
Acibenzolar-S-methyl	0.00625	2 pre-inoculation + 2 post (4)	29.0	ab	40.0	cd	57.3	bc	35.2	de
Acibenzolar-S-methyl	0.00625	2 pre-inoculazion + 1 post (3)	26.0	ab	33.0	c	32.1	bc	38.6	de
Acibenzolar-S-methyl	0.0125	2 pre-inoculazion + 1 post (3)	18.0	ab	16.0	b	60.5	bc	46.6	cd
Acibenzolar-S-methyl	0.0125	2 pre-inoculazion (2)	33.0	b	36.0	c	40.0	bc	49.1	cd
Acibenzolar-S-methyl	0.025	2 pre-inoculazion (2)	34.0	b	36.0	c	33.5	bc	38.3	de
Phosethyl-Al	1.6	2 pre-inoculazion + 1 post (3)	27.0	ab	50.0	d	58.0	bc	51.0	cd
Phosphite K	1.3+1.06	2 pre-inoculazion + 1 post (3)	26.0	ab	16.0	b	58.8	bc	213.9	a
Mefenoxam	0.48	1 pre-inoculazion (1)	0.0	a	0.0	a	78.9	ab	81.5	c
Non inoculated and non -treated control	-	-	0.0	a	0.0	a	123.9	a	135.3	b

[a] The mean values of the same column followed by the same letter, do not differ significantly according to Tukey's test (P=0.05)

Table 5 Efficacy of different spray programs against *Phytophthora capsici* on zucchini (cv. Genovese) in artificially infested soil and plant biomass at the end of the trials (Protocol 4)

Treatment	Dosage a. i. g l^{-1}	Time of applications			Disease severity 0-100				Fresh weight g			
		T0[b]	T5/6	T14	Trial 7		Trial 8		Trial 7		Trial 8	
Inoculated non-treated control	-		-		65.0	b[a]	76.0	b	136.6	de	7.3	d
Phosphite K+ acibenzolar-S-methyl	(1.3+1.6)+0.00625		X		6.0	a	0.0	a	251.5	a-e	29.0	ab
Phosphite K+ azoxystrobin	(1.3+1.6)+0.19		X		29.0	a	19.0	a	329.5	a-c	30.8	ab
Phosphite K+ mefenoxam	1.3+1.6		X		0.0	a	0.0	a	398.8	a	24.0	a-c
Azoxystrobin	0.19		X		30.0	ab	13.0	a	278.5	a-e	29.0	ab
Mefenoxam	0.48		X		0.0	a	4.0	a	394.2	a	18.8	b-d
Acibenzolar-S-methyl	0.0125		X	X	35.0	ab	2.0	a	181.4	b-e	24.3	a-c
Acibenzolar-S-methyl	0.00625		X	X	35.0	ab	7.0	a	285.1	a-e	26.8	ab
Phosphite di K	1.3+1.6		X	X	26.0	a	17.0	a	350.2	a-c	23.8	a-c
Phosphite di K	2.6+3.2		X	X	23.0	a	8.0	a	366.3	ab	25.0	a-c
Azoxystrobin	0.19		X	X	31.0	ab	63.0	b	253.7	a-e	19.8	b-d
Mefenoxam	0.48		X	X	0.0	a	1.0	a	349.4	a-c	23.8	a-c
Phosphite K+ acibenzolar-S-methyl	(1.3+1.6)+0.00625		X	X	17.0	a	9.0	a	241.1	a-e	24.5	a-c
Phosphite K+ azoxystrobin	(1.3+1.6)+0.19		X	X	15.0	a	10.0	a	337.6	a-c	19.8	b-d
Phosphite K+ mefenoxam	1.3+1.6		X	X	0.0	a	2.0	a	311.3	a-d	18.5	b-d
Acibenzolar-S-methyl	0.0125	X	X	X	31.0	ab	25.0	a	106.7	e	13.0	cd
Acibenzolar-S-methyl	0.00625	X	X	X	31.0	ab	19.0	a	167.5	c-e	19.0	b-d
Phosphite K	1.3+1.6	X	X	X	9.0	a	8.0	a	398.0	a	27.3	ab
Non inoculated non-treated control	-		-		0.0	a	0.0	a	428.5	a	34.5	a

[a] The mean values of the same column followed by the same letter, do not differ significantly according to Tukey's test (P=0.05)

[b] Corresponding to the first treatment carried out on zucchini plants at the stage of second true leaves