**Evaluation of the short term effect of nursery treatments with phosphite-based products, acibenzolar-S-methyl, pelleted Brassica carinata and biocontrol agents, against lettuce and cultivated rocket fusarium wilt under artificial inoculation and greenhouse conditions**

# Evaluation of the short term effect of nursery treatments with phosphite-based products, acibenzolar-S-methyl, pelleted *Brassica carinata* and biocontrol agents, against lettuce and cultivated rocket Fusarium wilt under artificial inoculation and greenhouse conditions

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## ABSTRACT

Experimental trials have been carried out in order to evaluate the efficacy of preventative treatments based on plant defense activator products, biocontrol agents, a microbial complex with arbuscular mycorrhizal fungi, and *Brassica carinata* pellets against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce and *Fusarium oxysporum* f. sp. *raphani* on cultivated rocket under greenhouse conditions. These products were compared with fungicides known for their ability to induce host resistance (phosethyl-Al and acibenzolar-S-methyl), and with azoxystrobin. Three and four applications of the tested products were carried out on lettuce and rocket seedlings grown in nursery conditions. Treated and untreated plants were transplanted into soil infested with Fusarium wilt agents to obtain an average disease severity (DS) of 65.6-69.2 and of 56.9-62.1 on the untreated lettuce and rocket plants, respectively. The best Fusarium wilt biocontrol was obtained after four

applications of *Bacillus subtilis* Qst713 and with the Glomas microbial complex (42 and 46.7%, efficacy, respectively). *Brassica carinata* pellets provided a consistent control when applied 14 days before the rocket and lettuce were transplanted into the infested soil. Acibenzolar-S-methyl, applied at 0.025 g/liter, showed a DS reduction in *F. oxysporum* f. sp. *lactucae* from 36 to 61% and of *F. oxysporum* f. sp. *raphani* from 54 to 73%, thus showing statistically similar results to those of azoxystrobin, which was used as a reference (DS reduction from 59 to 65%). Although the Fusarium wilt control provided by such products was not complete in the present experimental conditions, these products can be considered interesting components for an integrated pest management of the Fusarium wilt of leafy vegetables, starting from nursey applications. Moreover, the tested BCAs could become potentially useful, especially for plant monocultures. This study has been produced new information on the effects of potassium phosphite, applied at the nursery level, on reducing lettuce and rocket fusarium wilt. An average efficacy of 69.5% was observed for lettuce, while an average efficacy of 65.2% was observed for cultivated rocket. The good fungicidal activity of the phosphite-based product, coupled with the positive effect on plant biomass, is of special interest.

**Keywords:** *Lactuca sativa*, *Eruca vesicaria*, *Fusarium oxysporum* f. p. *lactucae; Fusarium oxysporum* f. sp. *raphani*, integrated control

## 1. Introduction

In recent years, the economic relevance of lettuce (*Lactuca sativa* L.) and cultivated rocket [*Eruca vesicaria* (L.) Cav.] has increased, since many farms produce fresh cut and ready-to-eat salads. In these intensive systems, where leafy vegetables are continuously grown in the same soil, the phytopathological situation is constantly in evolution, as a consequence of the dynamism and specialization of such crops (Garibaldi and Gullino, 2010; Garibaldi et al., 2014). Fusarium wilt

50 incited by *Fusarium oxysporum* f. sp. *lactucae* on lettuce and by *Fusarium oxysporum:* ff. spp.
51 *raphani* and *conglutinans* on cultivated rocket, can lead to serious losses (Matheron and Gullino,
52 2012). The Fusarium wilt of lettuce was detected for the first time in Europe, in northern Italy, in
53 2002 (Garibaldi and Gullino, 2010). The two *formae speciales* that affect cultivated rocket, that is,
54 *Fusarium oxysporum* ff.spp. *raphani* and *conglutinans,* the first of which is more frequently
55 detected (Garibaldi et al., 2006; Srinivasan et al., 2012), also affect other genera belonging to
56 Brassicaceae, such as cabbage, brussel sprouts, broccoli, turnip, radish and stock (Garibaldi et al.,
57 2006). The Fusarium wilt of lettuce and rocket are easily and frequently seed-transmitted (Gullino
58 et al., 2014), thus suggesting the importance of preventative disease management strategies.
59 Lettuce varieties that are resistant or at least tolerant to Fusarium wilt are available (Scott et al.,
60 2010; Matheron and Gullino, 2012; Gilardi et al., 2014b), but their effective use is complicated by
61 the presence of three races of the pathogen (Fujinaga, 2005). In the case of rocket, the use of
62 resistant varieties is still very limited (Gilardi et al., 2007).
63 In general, the management of soil-borne pathogens is complicated by the limited number of
64 registered chemicals and by the restrictions in the use of pre-plant fumigants, including metam
65 sodium and dazomet (Colla et al., 2012). Several approaches to soil-borne disease management
66 have been investigated intensively in an attempt to find an answer to the many practical problems
67 associated with the loss or limitation of use of effective fumigants encountered by growers.
68 Moreover, more emphasis is now given to crop and soil health instead of disease control (Barrière
69 et al., 2014). Among the exploited strategies for disease management, systemic acquired resistance
70 (SAR) and induced systemic resistance (ISR), which are mainly triggered by microorganisms, such
71 as plant growth-promoting rhizobacteria, by the metabolic products of affected plants, or by
72 chemicals (Sticher et al., 1997; Oostendorp et al., 2001; Vallad and Goodman, 2004; Shoresh et al.,
73 2005) are at present attracting a great deal of interest. Induced systemic and localized resistance to
74 soil-borne pathogens, by means of *Trichoderma* treatments, has been well documented (Shoresh et

al., 2005; Vinale et al., 2008). Kloepper et al., (2004) proved the ability of *Bacillus* spp. to induce systemic resistance, and in most cases, to also elicit plant growth promotion. Moreover, arbuscular mycorrhizal fungi have been reported to be implicated in protecting plants against soil-borne pathogens, through different mechanisms, including an improvement in plant nutrition, damage compensation, competition, changes in the root system and activation of plant defense signaling (Whipps, 2004; Pozo and Azcón-Aguliar, 2007). Moreover, among the chemical resistance inducers, the phosphite-based fertilizers and acibenzolar-S-methyl have been shown to lead to a reduction in disease against soil-borne pathogens, on vegetable and ornamental crops (Eikemo et al., 2003; Elmer 2004; 2006; Hyeon et al., 2009; Bubici et al., 2006; Walters, 2012; 2013; Gilardi et al., 2014a), but no data are available concerning their efficacy against the Fusarium wilts of lettuce and rocket.

This study has been carried out in order to evaluate the efficacy of preventative treatments, including SAR and IRS activator products, biocontrol agents, a microbial complex based on arbuscular mycorrhizal fungi, and *Brassica carinata* pellets against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce and *Fusarium oxysporum* f. sp. *raphani* on cultivated rocket under simulated nursery conditions, in a greenhouse.

## 2. Material and methods

### *2.1. Plant material and experimental layout*

Experimental trials (Table 1) were carried out in 2012 and 2013 under greenhouse conditions in order to test the efficacy of different products against the Fusarium wilts of lettuce and cultivated rocket. Lettuce (cv. Crispilla) and cultivated rocket seeds (cv. Coltivata), which are very susceptible to Fusarium wilt (Garibaldi et al., 2004; Gilardi et al., 2014,), were sown in 100-plug trays (2.5 cm Ø per pot, 4-L of soil capacity) filled with a steamed (90°C for 30 minutes) peat mix substrate

99 (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
100 Type 5, Georgsdorf, Germany).
101 Fifteen-day-old lettuce and cultivated rocket seedlings were transplanted into the same substrate in
102 12-L plastic pots. Ten plants/pot of each tested crop were kept in a greenhouse on benches with air
103 temperatures ranging from 26 to 34°C during the day and from 20 to 25°C during the night (Table
104 1). The substrate was artificially infested with the Fusarium wilt agents, as described hereafter.
105 Forty plants per treatment were arranged in a complete randomized block design in each trial, which
106 represented the experimental unit, with three (Protocol 1) and four replicated trials (Protocol 2) as
107 reported in Table 1.
108
109 *2.2. Fungal strains and artificial inoculation*

110 A highly virulent strain of *F. oxysporum* f. sp. *lactucae*, isolated in 2002 from infected lettuce plants
111 in north-western Italy, ATCCMYA3040, belonging to Race 1 of the pathogen (Garibaldi et al.,
112 2002), and the FusRuc 13/03 strain, isolated from rocket grown in a commercial plastic greenhouse
113 in northern-Italy in 2003 and identified as *F. oxysporum* f. sp. *raphani* (Garibaldi et al., 2006), were
114 used throughout the experiments. The single-spore culture of each isolate was stored in glycerol at -
115 80°C.
116 These strains were grown in potato dextrose broth (Sigma-Aldrich, St. Luis, USA) and kept in a
117 rotatory shaker working at 90-100 rpm for 10 days at 25°C. The biomass produced after
118 centrifugation (9,600 g at 4°C) was prepared as a dry talc-based powder (biomass: talc 2:1 w/w), as
119 described by Locke and Colhoun (1974). After 20 days at 22-25°C, the number of chlamydospores
120 per gram of talc was assessed by serial plating on potato dextrose agar, PDA (Merck, Darmstadt,
121 Germany), which contained 25 mg $L^{-1}$ streptomycin sulphate. The talc formulations of *F.*
122 *oxysporum* f.sp. *lactucae* (strain MYA3040) and of *F. oxysporum* f.sp. *raphani,* (strain

123 FusRuc13/03) at 2 and $5x10^7$ chlamidospores/g, respectively, were used. These formulated
124 pathogens (2 and 1 g liter/respectively) were mixed into the steamed substrate as chlamydospores
125 dispersed in talc to achieve a final concentration of $5x10^4$ chlamydospores $ml^{-1}$ of substrate. A
126 non-infested substrate was used as a control (Table 1).
127

128 *2.3. Products used in the test*

129 Different compounds known for their capability to induce resistance in the host, that is, phosphite-
130 based fertilizers, organic amendments, biocontrol agents (BCAs) and fungicides were tested (Table
131 2).
132 Among the considered phosphite-based products and organic amendments, a phosphite-based
133 glucohumate complex (Glucoinductor + GlucoActivator, N 4%, $P_2O_5$ 18%, International patent
134 PCT, IB2004\001905, Fertirev, Torino, Italy), a mineral fertilizer based on potassium phosphite
135 (Alexin 95PS, $P_2O_5$ 52%, $K_2O$ 42%, Massò, Spain), and a patented formulation of *Brassica*
136 *carinata* defatted seed meal (Biofence, N organic 3%, P 2.2%, K 2%, organic C 52%, Triumph,
137 Spain) were tested.
138 Among the BCAs, *Bacillus subtilis* QST 713 (Serenade, 14.6 % a.i., BayerCropScience, Italy),
139 *Bacillus velezensis* (Cilus Plus IT45, 95%, Massò, Spain), *Trichoderma asperellum* +*T. gamsii*
140 (Remedier WP, Isagro Ricerca, Milano, Italy), a product based on arbuscular mycorrhizal fungi
141 combined with a microbial complex of *Trichoderma* and *Bacillus* (Rizocore, *Glomus* spp.
142 5%+*Bacillus megaterium* $10^4$ $UFCg^{-1}$ +*Trichoderma* $10^{10}$ $UFCg^{-1}$, Biogard, division of CBCEurope,
143 Italy) were tested.
144 The chemicals known for their ability to induce resistance, that is, acibenzolar-S-methyl (Bion
145 50WG, 50% a.i., Syngenta Crop Protection, Italy) and phosethyl-Al (Alliette, 80% a.i, Bayer Crop

146 Science, Italy) were tested, while azoxystrobin (Ortiva, 23.2% a. i., Syngenta Crop Protection, Italy)
147 was used as traditional chemical control (Gullino et al., 2002).
148
149 *2.4. Type and timing of the treatments*
150 The biocontrol agents, acibenzolar-S-methyl, the phosphite based products, as well as the tested
151 fungicides phosethyl-Al and azoxystrobin were applied as a leaf spray with a high volume of water
152 (1,500 L $ha^{-1}$), using a 1 L capacity hand sprayer.
153 The temporal organization of the experimental trials, as well as, the timing of the artificial
154 infestation of the soil and treatments are summarized in Table 1. The timing and the application
155 dosages of the tested products, which were based on the manufacturer's suggestions, are given in
156 table 2. The lettuce and cultivated rocket seedlings grown in each tray were treated by leaf spraying
157 with three applications at 6-7 day intervals according to protocol 1 (Trial block 1 to 3), or with one
158 more spray applications in the 12 L plastic pots, as in protocol 2 (Trial block 4 to 7), seven days
159 after transplanting the lettuce and cultivated rocket seedlings. Azoxysrobin and *B. carinata* pellets
160 were only applied once (Tables 1 and 2).
161 The first treatment (T0) was carried out in a greenhouse, at a temperature of 22-24°C, on plants still
162 in the plug tray, at the second true leaf stage, 7-10 days after sowing.
163 The product based on arbuscular mycorrhizal fungi and the microbial complex (Rizocore) was
164 mixed with 4 L of the substrate that was used in the plug tray at T0, than leaf sprayed at 6-7 day
165 intervals (Table1). The patented formulation of *B. carinata* defatted seed meals was mixed with the
166 substrate used to fill the 12 L plastic pots at T0 (14 days before the crops transplant) and at the same
167 time as the artificial infestation of the soil at T7.
168
169

170 *2.5. Data collection and analysis*

171

172 The plants were monitored weekly and the data were recorded, starting 10-14 days after the lettuce
173 and rocket had been transplanted into the infested substrate, at the appearance of the first yellow
174 leaf symptoms and reduced growth. The number of infected plants showing wilting and stem
175 necrosis was counted to assess disease incidence. The totally wilted (dead) plants were removed.
176 The final disease rating was made four weeks after the soil infestation by dissecting each plant. The
177 used disease severity (DS) index was: 0 = healthy plant, 25 = initial leaf chlorosis, 50 = severe leaf
178 chlorosis and initial symptoms of wilting during the hottest hours of the day, 75 = severe wilting
179 and severe symptoms of leaf chlorosis; 100 = plant totally wilted, leaves completely necrotic. At the
180 end of the trials, the total biomass was weighed in order to evaluate the effect of the tested
181 treatments on the yield. DS data were arcsine transformed in order to normalize the distribution of
182 variance. The data from the non-inoculated and untreated controls were not included in the
183 statistical evaluation of the DS data (Figure 1).

184 The efficacy of the different treatments in controlling the Fusarium wilt of lettuce and rocket,
185 corresponding to the percentage of DS reduction, was calculated as:

186

187 % efficacy =100 - (DS $_{t}$ x 100/DS $i_{control}$)

188

189 where,
190 $i$ = inoculated and untreated control

191 $t$ = tested treatments.

192

193 All the data were analysed by univariate ANOVA in SPSS 22.0, and the means were separated by
194 means of Tukey's multiple comparison test (p=0.05). The standard errors are marked with error
195 bars in all the figures.

196 **3. Results**

197

198 **Effect of pre-planting treatment on lettuce fusarium wilt**

199 The average disease severity (DS) at the end of the lettuce trials in the inoculated non-treated
200 control plots was 62.1 and 56.9, respectively (Figures 1a and 2a). When at least three treatments
201 were carried out, the best control of Fusarium wilt on lettuce was provided by the phosphite-based
202 product Alexin (69.2% efficacy), and this was followed by acibezolar-S-methyl at 0.0125 (59.9%
203 efficacy), by phosethyl-Al (57.8% efficacy) and the phosphite-based glucohumate complex (52.4%
204 efficacy).These treatments were effective as one application of azoxystrobin, which was found to
205 protect the lettuce from Fusarium wilt with an efficacy 55.7%. The biocontrol agents *Trichoderma*
206 *asperellum* + *T. gamsii* and *B. subtilis* provided a partial disease reduction, with statistically
207 different results compared to the inoculated and untreated control (32.8 and 30.7% efficacy,
208 respectively), while *B. velenzensis* and the microbial *Glomus* spp. + *B. megaterium* + *Trichoderma*
209 complex (Rizocore) were similar to the inoculated and untreated control. The *Brassica carinata*
210 pellets, applied once seven days before transplanting, were not effective (Figure 1a). The higher
211 fresh weight of lettuce plants obtained with the first protocol reflected the greatest efficacy in
212 disease reduction, which was provided by phosphite-based products, by acibenzolar-S-methyl at
213 the lower tested dosages, and by phosethyl-Al (Figure 1b).
214 In general, when an extra treatment was applied to the potted plants after transplanting, according
215 to protocol 2 (Figure 2a), all the tested biocontrol products differed significantly from the untreated
216 control, and an improved efficacy for *Bacillus subtilis* (42.7% efficacy), *T. asperellum* + *T. gamsii*
217 (38.3%, efficacy), and the microbial complex based *Glomus* spp complex (38.3%, efficacy) was
218 observed. On the other hand, the extra treatment with acibenzolar-S-methyl and the phosphite-
219 based products did not improve their efficacy. The application of *B. carinata* pellets led to a better
220 control of Fusarium wilt when they were applied 14 days before transplanting at T0, with an

221 average efficacy of 56% (Figure 2a). As far as the fresh weight of the lettuce plants at the end of
222 the trials is concerned, the best results, in terms of production, were observed in the plots in which
223 the best disease control was achieved (Figure 2b).
224
225 **Effect of the pre-planting treatment on rocket fusarium wilt**
226 Similar results, in terms of disease control, to those observed on lettuce, were observed in the trials
227 performed to evaluate the effects of different pre-planting treatments against *F. oxysporum* f. sp.
228 *raphani* on cultivated rocket, with an average disease severity at the end of the trials in the infested
229 and untreated control of 62.1 and 56.9, respectively (Figures 3a and 4a). When at least three
230 treatments were carried out, according to protocol 1, that is, acibenzolar-S-methyl at 0.0125 g $L^{-1}$,
231 the phosphite based products (Alexin and the Glucohumate complex), provided similar results to
232 one application of azoxystrobin, which reduced Fusarium wilt by 65%. All the tested biocontrol
233 agents were ineffective, and showed similar results to the inoculated and untreated control (Figure
234 3a). In general, there were no significant differences in plant fresh weight between treatments and
235 the inoculated and non-treated control plants, with the exception of the results shown for *Bacillus*
236 *subtilis*, *T. asperellum* + *T. gamsii* and the *Glomus* spp. +*Bacillus megaterium* +*Trichoderma*
237 product, in one out of four trials (Figure 3b).
238 The tested products, which were applied in four treatments (protocol 2), as previously described,
239 significantly reduced the Fusarium wilt of rocket compared to the infested and untreated control
240 (Figure 4a). The best rocket fusarium wilt control result was provided by potassium phosphite
241 (65%, efficacy), and this was followed by acibenzolar-S-methyl (57.5%, efficacy) and by the
242 Glucohumate complex (56.8%, efficacy), which were statistically similar to azoxystrobin, applied at
243 transplanting as a chemical reference, which showed 56.3% efficacy.
244 The *B. carinata* pellets also significantly reduced disease severity when they were applied 14 days
245 before transplanting (60.3%, efficacy). In general, the four treatments carried out with the

246 biocontrol agents *B. subtilis, B. velenzensis*, *T. asperellum* +*T. gamsii,* and by the microbial
247 *Glomus* spp. + *B. megaterium* + *Trichoderma* complex, significantly reduced the disease, compared
248 to the untreated control (42.7%, 42.3%, 34.5%, 46.7% of efficacy, respectively).
249 The *Brassica carinata* pellets led to a positive effect on rocket fresh weigh, compared to the non-
250 inoculated and non-treated control, when applied 7-14 days before transplanting. The phosphite-
251 based products led to an improvement in fresh weight after four treatments were applied (Figures
252 3b and 4b).

253 None of the products tested under the present experimental conditions affected the fresh weight of
254 the lettuce or rocket plants, thus confirming the absence of phytotoxicity.

255

256 **4. Discussion**

257

258 According to several authors, Fusarium wilt is a devasting disease that affects many economically
259 important vegetables and it is responsible for severe losses to growers worldwide (Elmer, 2006;
260 Katan et al., 2012; Matheron and Gullino, 2012; Walters, 2012; Garibaldi et al., 2014).
261 The tactics adopted to control this plant disease mainly involve the use of preventative measures,
262 such as the minimization of dissemination in the field, the use of plant resistant cultivars whenever
263 possible, the adoption of a proper crop rotation, and the removal and destruction of infested plant
264 material. Like other soil-borne pathogens, Fusarium wilt is difficult to manage with a single
265 approach and/or with a single product (Katan et al., 2012). Moreover, different measures are needed
266 for different cropping systems. Gordon and Koike (2015) have reviewed different ways of
267 managing lettuce fusarium wilt and they have pointed out the importance of the adoption of proper
268 practices in order to reduce the inoculum level. Among these measures, it has been found that 1-
269 month of solarization, with an average temperature of 47 to 49°C, at a depth of 5 cm, provides a

reduction of 41 to 92% of Fusarium wilt on lettuce in naturally infested fields (Matheron and Porchas, 2012). Even in sub-optimal temperature regimes (50 to 48°C for 6 h, 45 to 43°C for 8 h and 40 to 38°C for 10 h/day), 14 days of thermal treatment under controlled conditions has provided very valuable results, in terms of disease control on rocket (Gilardi et al., 2014). Moreover, recent investigations on the antagonistic microorganisms *Trichoderma asperellum* and *T. gamsii*, with a *Glomus* spp. +*Bacillus megaterium* +*Trichoderma* product used as a dressing on lettuce seeds, have shown promising results in the control of fusarium wilt on lettuce (Lopez et al., 2014). On the contrary, crop rotation is not an effective measure, because *F. oxysporum* f.sp. *lactucae* can colonise the roots of other crops and, there is evidence of an expanded host range of *Fusarium oxysporum* f.sp. *raphani* (Garibaldi et al., 2006; Scott et al., 2014).

Among the various alternatives to chemicals that have been tested so far, it has here been shown that any solution on its own is able to provide a viable level of disease control of the Fusarium wilt of lettuce and rocket, under short-cycles and for high levels of infestation. The success of the biological control strategies used against Fusarium wilt agents, through the adoption of various antagonist, organic amendments and biofumigation, in fact depends on many different factors, including the pathogen infestation level in the soil and the type of inoculum (Termorshuizen and Jeger, 2014). Soil temperature also has an important effect on the disease expression of both of these Fusarium wilt agents, and it has been shown to be a key factor that can influence the success of the control measures (Bosland et al. 1988; Scott et al., 2010a).

The present results provide evidence of the capability of commercial BCAs to reduce lettuce and rocket Fusarium wilt by 42 to 47%, respectively, under high disease pressure, when applied four times, starting from the nursery. The limited efficacy of lettuce and rocket Fusarium wilt control, after three applications of *Glomus* spp. +*Bacillus megaterium* +*Trichoderma,* could be due to an unsuccessful establishment of the mycorrhizal symbiosis. However, the mechanism involved in the control of Fusarium wilt by *Glomus* is complex and seems to be host specific. In the present study,

four applications of the *Glomus* spp. +*Bacillus megaterium* +*Trichoderma* product provide a better Fusarium wilt reduction on rocket with 47% of efficacy. Martinez-Medina et al., (2010) have suggested that the mechanism involved in *Fusarium oxysporum* f.sp. *melonis* control by *Glomus intraradices* is independent of the SA and JA pathways, while the Fusarium wilt control of tomato by means of root colonization with mycorrhizae has been attributed to a possible plant-mediated phenomenon (Kapoor, 2008).

The present study provides new information on the effect of resistance inducers, based on either phosphites or acibenzolar-S-methyl, applied as a pre-plant treatment in the nursery, against *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *raphani* on lettuce and cultivated rocket, respectively. Among the tested chemical resistance inducers, acibenzolar-S-methyl has been found to be effective in controlling the Fusarium wilt of both lettuce and rocket at the lowest tested dosage, with a positive effect on the yield. Encouraging results pertaining to the control of Verticillium wilt on eggplants and Fusarium wilt on ornamental plants by means of acibenzolar-S-methyl have already been reported by Elmer (2004; 2006) and by Bubici (2006).

The good fungicidal activity of the phosphite-based product, coupled with the positive effect on plant biomass, is of special interest. In the present study, the phosphite-based products have generally caused a consistent disease control of *F. oxysporum* f. sp. *lactucae,* that is, from 61 to 69%, and of *F. oxysporum* f. sp. *raphani,* from 54 to 65%, and have shown statistically similar results to those of azoxystrobin, which has been used as a reference (59 to 65% efficacy), with a significant effect on the yield. A previous study carried out on lettuce reported conflicting data on plant growth for the use of phosphite-phosphate as a fertilizer. However, this may have been due to the different cultivars that were examined, which could have different capabilities of absorbing phosphite (Thao et al., 2009). The rate and timing of application of resistance inducers are considered critical factors that can affect both the level of disease control and the yield (Walters, 2012).

The effectiveness of soil applications of the patented formulation of *Brassica carinata* defatted seed meals has shown conflicting results against the Fusarium wilt of lettuce and rocket. In the present study, *Brassica carinata* pellets have provided a consistent control, when applied 14 days before transplanting the rocket and lettuce into the infested soil, with an efficacy that ranged from 56 to 60%. The present results are consistent with previous research, because organic amendments need protracted periods of time to become effective, since their activity is due to decomposition and the release of volatiles. (Mazzola et al., 2007; Bonanomi et al. 2010). The combination of *Brassica carinata* pellets with solarization can be effective, as already observed, in infested soils, against the Fusarium wilt of basil, lettuce and rocket (Garibaldi et al., 2011; Gilardi et al., 2014b).

Although the Fusarium wilt control provided by such products was not complete in the present experimental conditions, it should be considered that disease severity is generally lower in the field. Since the BCA treatments have not offered a complete Fusarium control, they could be integrated with other strategies, and their contribution to disease management could be interesting, because they can be used to complement other control measures. For instance, in the case of lettuce, the use of resistant or partially resistant commercial cultivars (Garibaldi et al., 2004; Matheron et al., 2005; Scott et al., 2010b; Gilardi et al., 2014b) could be combined with pre-plant treatments.

The four application of *Bacillus subtilis* Qs713 and the microbial *Glomus* spp. + *B. megaterium* + *Trichoderma* complex, as well as the three phosphite-based commercial products, which may be technically effective, especially at the nursery level, as well as economically feasible. The positive effect of these products against leaf pathogens should also be considered. *Bacillus* spp., from the rizhosphere of different vegetable plants, has resulted in induced resistance to *B. cinerea* (Levy et al., 2015). In a preventative application, phosphite and acibenzolar-S-methyl induced resistance to downy mildew on basil (Gilardi et al., 2013).

Such products are easy and safe to apply under nursery conditions, and could represent an effective tool for the management of foliar and soil-borne pathogens. Moreover, the use of these products before they are applied at a farm level could help to standardize their applications in an integrated pest management model.

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489 Tables

490 Table 1. General information on the trials and timing of the operations carried out on lettuce and rocket.

| Protocol, (trial) | Sowing | Tray treatment (Day) | Artificial inoculation | Transplanting | Plot Treatment | End of the trial |
|---|---|---|---|---|---|---|
| 1(1) | 6/04/2012 | 11/04 (T0)[a]; 17/04 (T7); 24/04 (T14) | 17/04//2012 | 25/04/2012 | - | 6/6/2012 |
| 1(2) | 14/06/2012 | 21/06(T0); 28/06(T7); 5/07 (T14) | 28/06/2012 | 6/07/2012 | - | 4/09/2012 |
| 1(3) | 10/01/2013 | 21/01(T0); 28/01(T7); 4/02(T14) | 28/01/2013 | 5/02/2013 | - | 18/03/2013 |
| 2 (4) | 2/04/2013 | 10/04(T0); 17/04(T7); 24/04 (T14) | 17/04/2013 | 25/04/2013 | 30/04/2013 | 28/05/2013 |
| 2 (5) | 7/06/2013 | 14/06 (T0);21/06 (T7); 27/06 (T14) | 21/06/2013 | 28/06/2013 | 4/07 /2013 | 23/07/2013 |
| 2 (6) | 25/07/2013 | 1/08 (T0); 8/08 (T7); 12/08 (T14) | 12/08/2/2013 | 13/08/2013 | 19/08/2013 | 17/09/2013 |
| 2 (7) | 9/09/2013 | 20/09 (T0); 27/09 (T7); 3/10 (T14) | 27/09/2013 | 4/10/2013 | 10/10/2013 | 28/10/2013 |

491 [a]T0 corresponding to the first treatment carried out as leaf spraying at the development stage of 1-2 true leaves and soil
492 mixing application.

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494 Table 2. List of the tested products and of the experimental protocol.

| BCA or active ingredient | Commercial formulation | Dosage a. i. g $L^{-1}$ | Time (days) of application in tray conditions and type of application | Time (days) of application in plastic pot (2-L and 12-L) and type of application |
|---|---|---|---|---|
| *Bacillus subtilis* QST713 | Serenade Max | 0.58 | T0[a],T7,T14 (T21), leaf spray | - |
| *Bacillus velezensis* | Cilus Plus[b] | 0.4[b] | T0,T7,T14, (T21)leaf spray | - |
| *T. asperellum + T. gamsii* | Remedier | 0.04 | T0,T7,T14, (T21)leaf spray | - |
| Acibenzolar-S-methyl | Bion 50 WG | 0.025<br>0.0125 | T0,T7,T14, (T21) leaf spray | - |
| Phosethyl-Al | Aliette | 1.6 | T0,T7,T14, (T21)leaf spray | - |
| *Glomus* spp. +*Bacillus megaterium* +*Trichoderma* | Rizocore[b] | 0.08[b] | T0,T7,T14, (T21) leaf spray | - |
| Potassium phosphite P:K 52:42 | Alexin | 1.3+1.06 | T0,T7,T14, (T21) leaf spray | - |
| Phosphite based glucohumate N:P 4:18 | Glucohumate complex | 1.6+0.72 | T0,T7,T14, (T21)leaf spray | - |
| Azoxystrobin | Ortiva | 0.19 | T14, (T21)<br>leaf spray | - |
| *Brassica carinata* pellet<br>N:P:K: C organic | Biofence | 0.15+0.055+0.05+1.13 | - | T0 - T7,<br>soil mixing[c] |

495 [a]T0 corresponds to the leaf spraying carried out, starting at the second true leaf stage, and at seven-day intervals (T7
496 and T14).

497 [b] Corresponds to the dosage (g $L^{-1}$) of the commercial formulation.

498 [c] Corresponds to the treatment carried out at 14 day before transplanting (T0) and 7 days before transplanting the crops.

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Figure 1a. Effect of three tray-treatments on the Fusarium wilt of lettuce (cv. Crispilla). The data are expressed as DS at the end of the trials and compared with azoxystrobin and *Brassica carinata* pellets applied once.

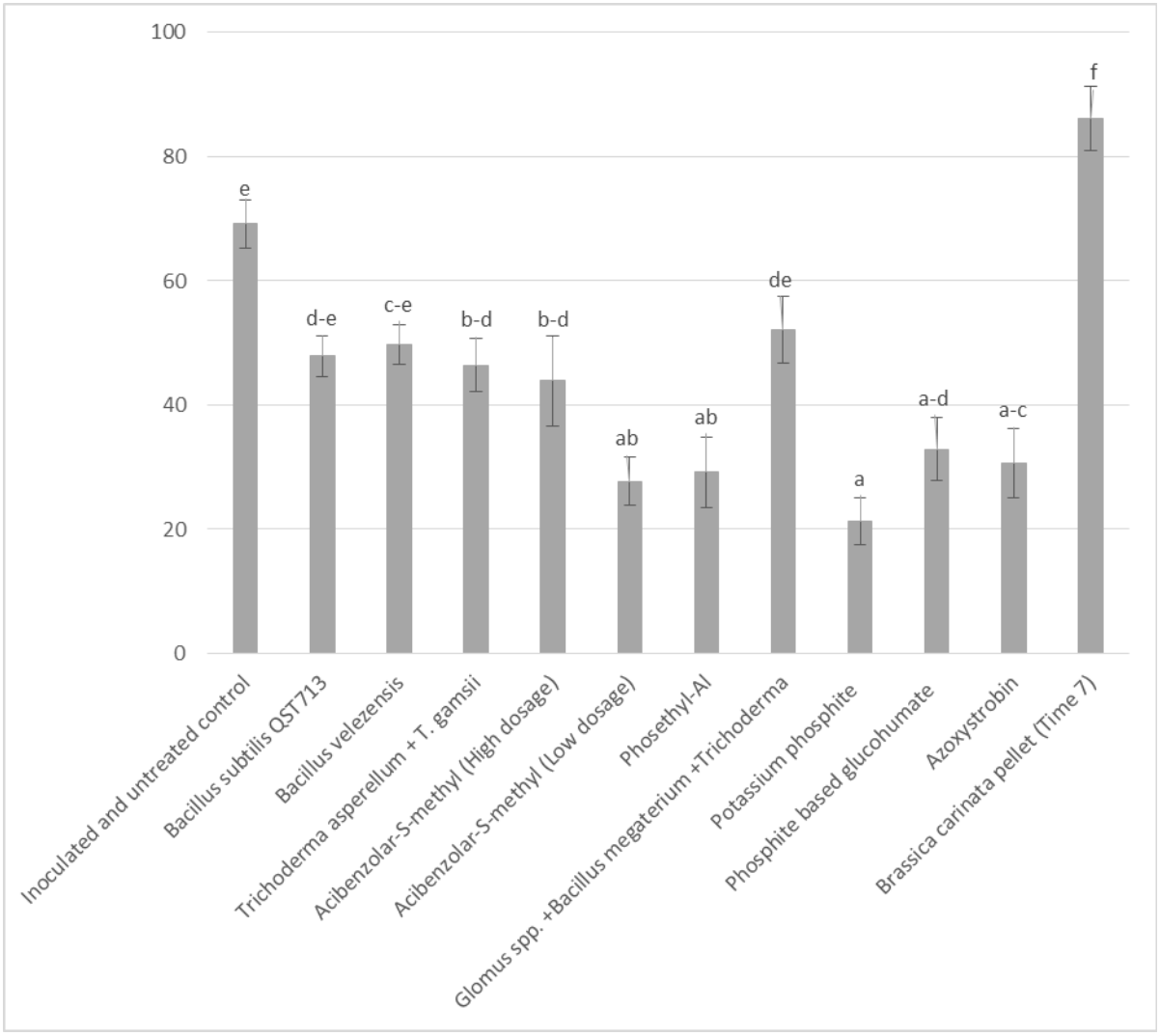

Figure 1b. Effect of three tray-treatments on the yield of lettuce plants in the presence of *F. oxysporum* f. sp. *lactucae*. The data are expressed as fresh weight and compared with azoxystrobin and *Brassica carinata* pellets applied once (g).

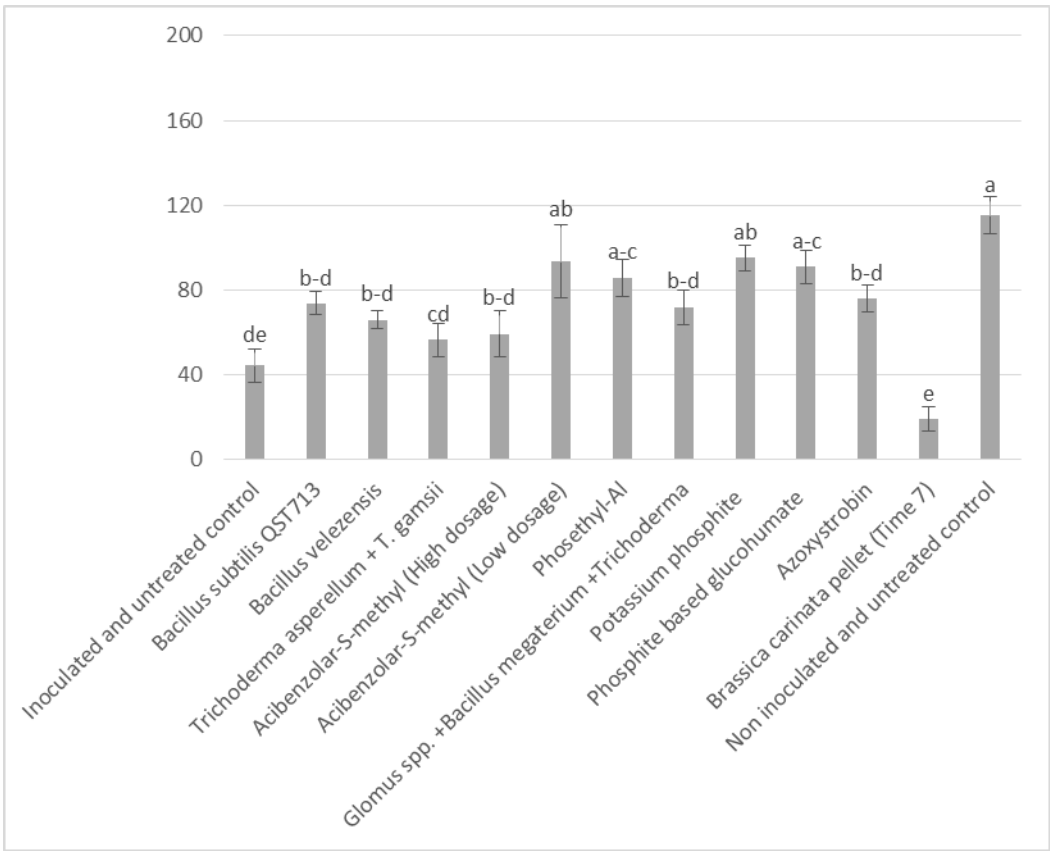

Figure 2a. Effect of four treatments on Fusarium wilt of lettuce (cv. Crispilla). The data are expressed as DS at the end of the trials and compared with azoxystrobin and *Brassica carinata* pellets applied at transplanting (T7) and 14 days before transplanting the lettuce (T0).

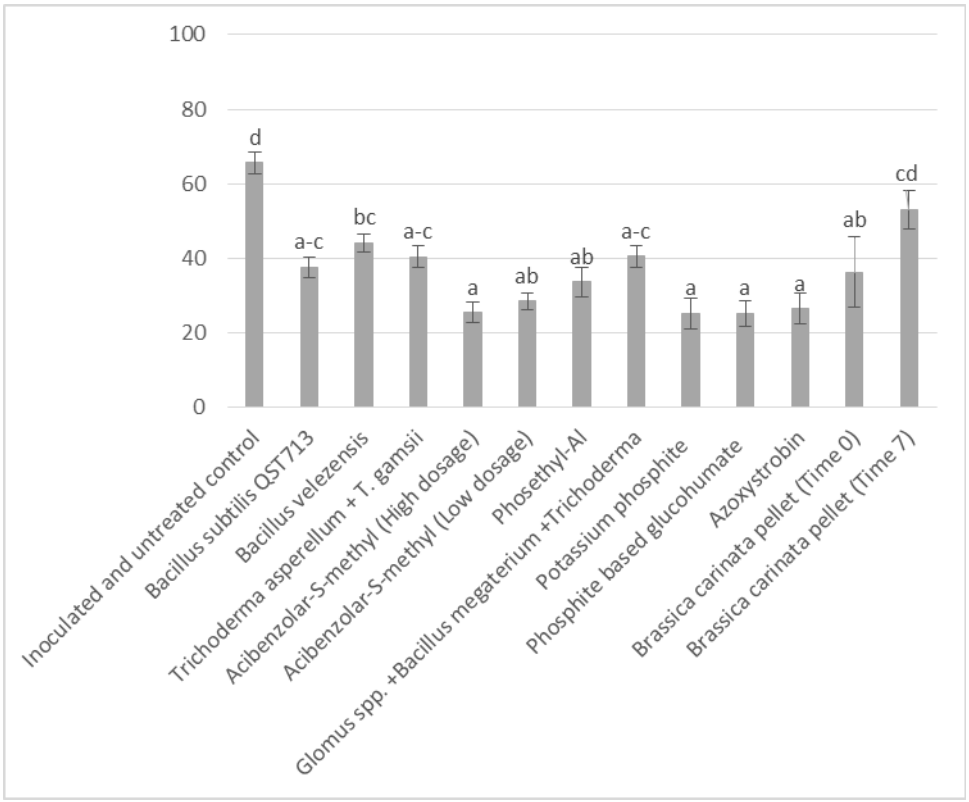

Figure 2b. Effect of four treatments on the yield of lettuce plants in the presence of *F. oxysporum* f. sp. *lactucae*. The data are expressed as fresh weight and compared with azoxystrobin and *Brassica carinata* pellets applied once (g).

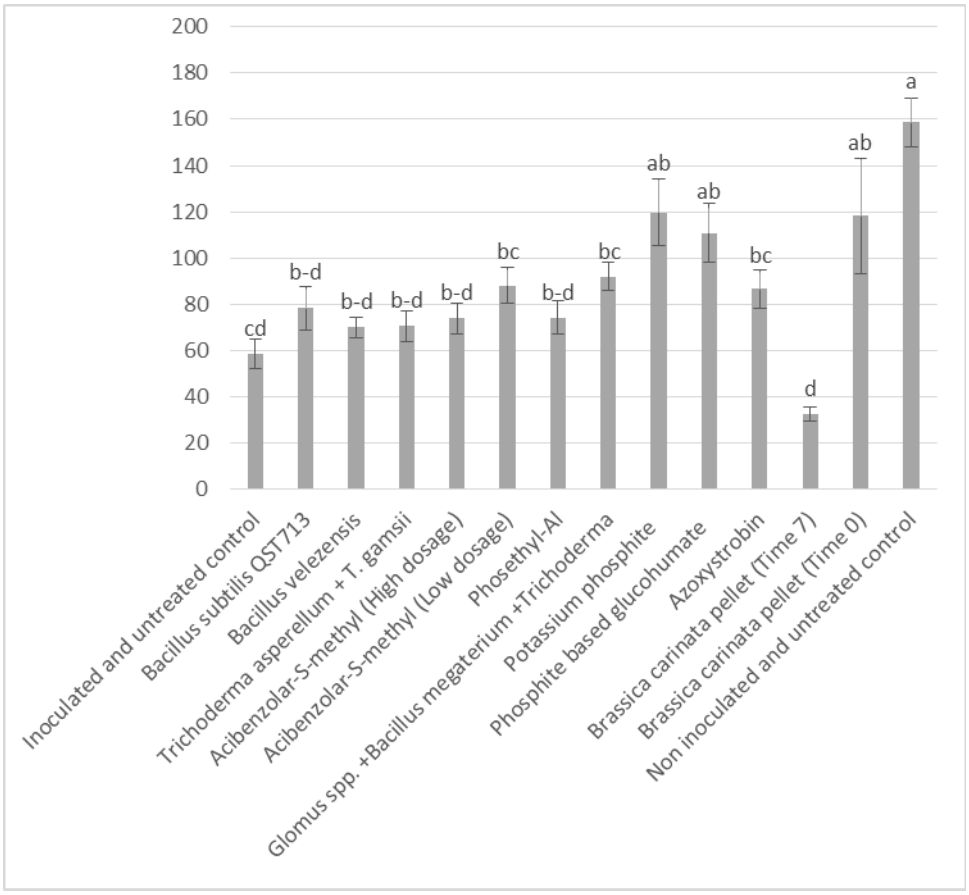

Figure 3a. Effect of three tray-treatments against the Fusarium wilt of rocket (cv. Coltivata). The data are expressed as DS at the end of the trials and compared with azoxystrobin and *Brassica carinata* pellets applied at transplanting (T7).

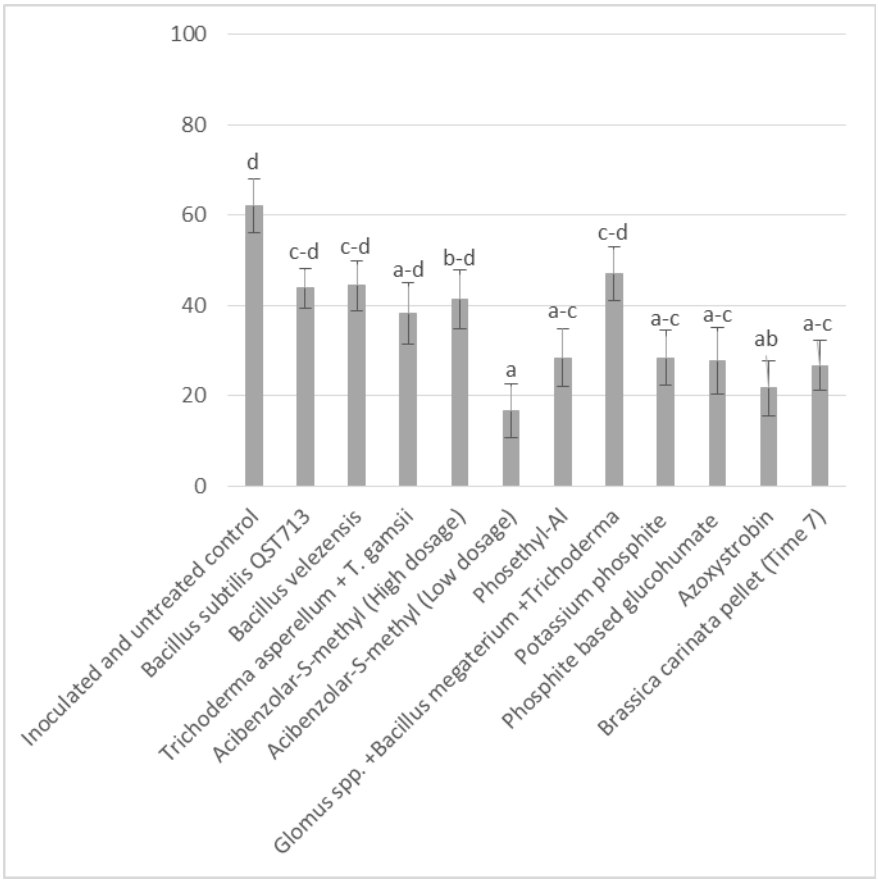

Figure 3b. Effect of three treatments on the yield of rocket plants in the presence of *F. oxysporum* f. sp. *raphani*. The data are expressed as fresh weight and compared with azoxystrobin and *Brassica carinata* pellets applied once (g).

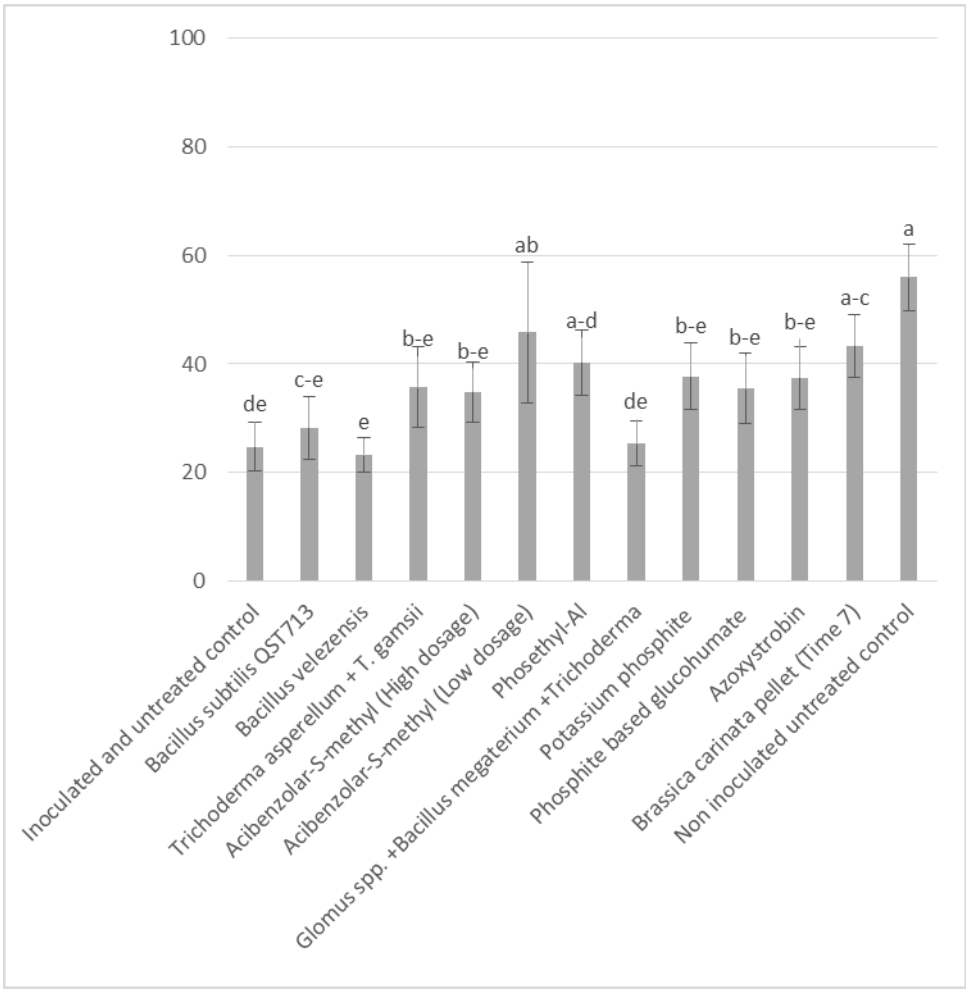

Figure 4a. Effect of four treatments on the Fusarium wilt of rocket. The data are expressed as DS at the end of the trials and compared with azoxystrobin and *Brassica carinata* pellets applied at transplanting (T7) and 14 days before transplanting rocket, at the same time as the artificial infestation (T0).

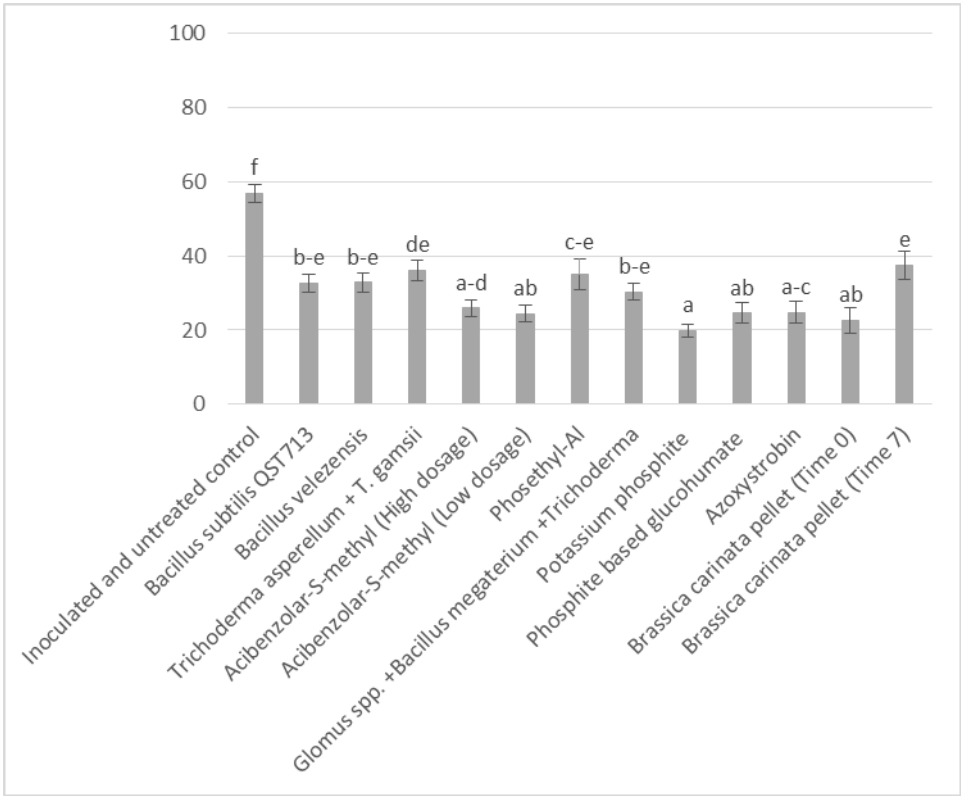

Figure 4b. Effect of four treatments on the yield of rocket plants in the presence of *F. oxysporum* f. sp. *raphani*. The data are expressed as fresh weight and compared with azoxystrobin and *Brassica carinata* pellets applied once (g).