COMBINED USE OF *BRASSICA CARINATA* SEED MEAL, THYME OIL AND A *BACILLUS AMYLOLIQUEFACIENS* STRAIN FOR CONTROLLING THREE SOIL-BORNE FUNGAL PLANT DISEASES

C. Pane, D. Villecco and M. Zaccardelli

Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per l'Orticoltura, via dei Cavalleggeri 25, I-84098 Pontecagnano, SA, Italy

SUMMARY

The effectiveness of non-chemical control methods is a critical point of the research about the development of alternatives to banned and/or unsustainable fungicides. In order to extend the magnitude and/or the spectrum of the control ability of each component, the combination of different tools has been proposed. In this work, glucosinolate-containing *Brassica carinata* seed meal, combined with essential oils from different aromatic plants and the antagonistic bacteria *Bacillus amyloliquefaciens* strain 17S, was assayed for the control of the soil-borne phytopathogens *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani* and *Sclerotinia minor*. Seed meal and essential oils showed significant dose-dependent antifungal effects towards all three pathogens, being able to completely inhibit growth at the highest concentration tested. Moreover, the combination of meal and thyme oil enhanced *in vitro* antagonistic activity of the *B. amyloliquefaciens* strain by 37%, on average. The use of these three components *in planta* experiments revealed that the three-combined treatments proved, in general, high level of biocontrol ability (around 80%), but not better than the treatments on their own in reducing disease levels on *Fusarium oxysporum* f. sp. *lycopersici*/tomato and *Sclerotinia minor*/lettuce pathosystems, except for *Rhizoctonia solani* on bean.

Keywords: *Fusarium* wilt, glucosinolates, plant-derived antifungals, *Rhizoctonia* root rot, *Sclerotinia* drop, soil-borne disease.

Corresponding author: C. Pane
E-mail: catello.pane@crea.gov.it

INTRODUCTION

Soil-borne phytopathogenic fungi can cause significant yield and economic losses in several cropping systems when favorable conditions to epidemics occur. Generally, soil-borne diseases have a dramatic impact on the plants by undermining the functionality of aboveground plant parts, collar, stems and/or vascular tissues. Therefore, particular attention must be paid to the control of these deleterious microorganisms, although it is very difficult because of their high adaptation to soil environments. Soil fumigation by broad-spectrum chemicals has been used over years by growers as the most effective prevention tool against the very feared soil-borne plant pathogens. However, public concerns about health and environmental risks linked to the use of synthetic fungicides, including also fumigants, and losses of their effectiveness due to the occurrence of resistance in plant pathogens to chemicals, incite to find new non-chemical alternatives. The exploitation of natural resources is becoming crucial to consistently address the issue.

Biological control is among the most studied control strategies as an alternative to fungicides for the management of soil-borne plant diseases (Spadaro and Gullino, 2005; Pal and McSpadden Gardener, 2006). It is based on the antagonistic activity of living microorganisms, which are able to contain plant diseases by mechanisms (competition, antibiosis and hyperparasitism) interfering with pathogens and/or plants (defenses elicitation). Bacterial antagonistic strains belonging to the *Bacillus* genus are considered suitable for application as biological control agents (BCAs) due to their favorable ecological and biological characteristics (Cawoy *et al*., 2011). These Gram positive antagonistic bacteria are generally non-pathogenic for humans, form heat- and desiccation-resistant spores and secrete antibiotics, enzymes and other inhibitory molecules (Raaijmakers *et al*., 2010; Cawoy *et al*., 2011).

Lastly, plant-derived products also are receiving more attention, being applicable alone or by combination in integrated approaches with microorganisms exhibiting enhanced control efficacy. Plants are a source of a plethora of bioactive secondary metabolites belonging to different chemical groups, known as phytochemicals (Gurjar *et al*.,

2012; Pane *et al*., 2016), that are exploitable in biological control implementation because of their remarkable antifungal properties (Uppal *et al*., 2008).

A well-known class of phytochemicals consists of sulfur- and nitrogen-containing metabolites stored in the vacuoles of *Brassicaceae*, named glucosinolates. Through a myrosinase-mediated hydrolysis, meals containing these compounds, in presence of water, instantaneously release large amounts of highly toxic volatile isothiocyanates (ITCs). The antimicrobial activity of ITCs is at the basis of soil biofumigation with glucosinolate-containing plant residues for the control of soil-borne plant pathogens (Brown and Morra, 1997). Recently, a new formulation of brassica's glucosinolates is made available from the biodiesel chain in the form of dry meals originated from *Brassica carinata* oil-less seed cakes (Gasol *et al*., 2007). *B. carinata* defatted seed meals (BCSMs) are promising to develop as safe biofumigants (Smolinska *et al*., 2003; Guerrero-Diaz *et al*., 2013). However, their potentiality needs to be further developed in practical applications against soil-borne plant diseases (Pane *et al*., 2012a). Additionally, plant-derived products, such as essential oils (EOs), are also included in eco-friendly control tools exploitable against plant pathogens (Pane *et al*., 2013a). EOs are a complex of many different volatile plant secondary metabolites, primarily terpenes, aromatic compounds and isoprenoids, exhibiting synergistically antifungal effects through the hypothesized mechanisms of cell phospholipid bilayer destabilization and mitochondrial damages (Nazzaro *et al*., 2013). These soil-diffusible bioactive molecules can have promising effects in the control of a wide spectrum of soil-borne fungal pathogens (Pandey and Dubey, 1994) although, to our knowledge, few studies have verified their effective potential in potted-plant applications.

The present work was conducted to evaluate the effects of *B. carinata* seed meal, essential oils and the biocontrol bacterial *B. amyloliquefaciens* strain 17S, *in vitro* and *in planta*, against the following pathogen-plant pathosystems: 1) *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen on tomato, 2) *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* [A.B. Frank] Donk) on bean and 3) *Sclerotinia minor* Jagger on lettuce.

MATERIALS AND METHODS

Fungal pathogens and biocontrol agent strain. The fungal pathogens used in this study were *F. oxysporum* f. sp. *lycopersici* (strain ATTC 16605 from CRA-SCS collection), *R. solani* AG-4 (strain RT10 from CRA-CAT collection) and *S. minor* (our isolate from lettuce) maintained at 4°C on potato dextrose agar medium (PDA, Oxoid). Each strain was preliminarily tested for pathogenicity on inoculated plants (data not showed).

The heat-resistant bacterial antagonistic strain 17S of *Bacillus amyloliquefaciens*, isolated from a suppressive compost amended soil and identified as species according to 16S rRNA gene sequencing analysis (Pane *et al*., 2012b), was used. This BCA was also chosen based on a previous screening carried out for tolerance to BCSM and EOs (Pane *et al*., 2013b).

Evaluation of BCSM *in vitro* for volatile antifungal activity. A commercial defatted BCSM (Biofence, Triumph, Italy) was utilized in this study. The BCSM's *in vitro* volatile antifungal activity was assessed through the method described by Galletti *et al.* (2008) with few modifications. A 5 mm-plug from an actively growing fungal colony was transferred to the centre of 90-mm diameter Petri dishes containing 20 ml of PDA. The inoculated plates were turned upside down, and then BCSM was added into a small vessel installed centrally on the lid. After the meal was wetted with 5 ml mg^{-1} of sterile and distilled water, the dishes were immediately sealed with Parafilm® M. BCSM was tested at doses of 6.25, 12.5, 25, 50 and 100 mg/plate. Dishes without meal were used as control. All the dishes were incubated at 25°C. The experimental plan used was a completely randomized design with three replicates *per* treatment. The colony diameter was measured at the end of the experiment when control plates were fully covered with mycelium. Fungal growth under treatments was expressed as percentage of the untreated control plates according to the following formula: Fungal Growth (%) = (di/dc) × 100, where dc and di are the colony diameter measured in the control and in the *i*-th experimental treatment, respectively.

Evaluation of essential oils *in vitro* for antifungal activity. The antifungal activity of commercial EOs, extracted from caraway, marjoram, melissa, oregano, sage and thyme (Acef, Italy), were determined by an *in vitro* method assay. A 5 mm-plug from an actively growing fungal colony was transferred to the centre of a 90-mm diameter Petri dish containing PDA (20 ml) supplemented with essential oil dissolved in 1% Tween-20. EOs were tested at doses of 0.125, 0.25, 0.5, 1 and 2%. Thyme and oregano EOs were additionally tested at concentrations 0.0125 and 0.0625%. In control plates, instead, PDA medium was supplemented with sterile distilled water. Inoculated dishes were sealed with Parafilm® M and incubated at 25°C. A completely randomized design with three replicates was used. The Fungal Growth (%) was calculated as described above.

***In vitro* performances of BCA in combination with BCSM and selected Eos.** The interactive effects of BCSM and selected thyme and oregano EOs on BCA antifungal activity were evaluated in plate challenge experiments performed according to Boulter *et al.* (2002) with some modifications. A 5-mm plug was transferred from the edge of the fungal colony to the centre of PDA 90-mm diameter plates, while pure culture of the bacterial strain was streaked along the edges. To evaluate the inhibitory

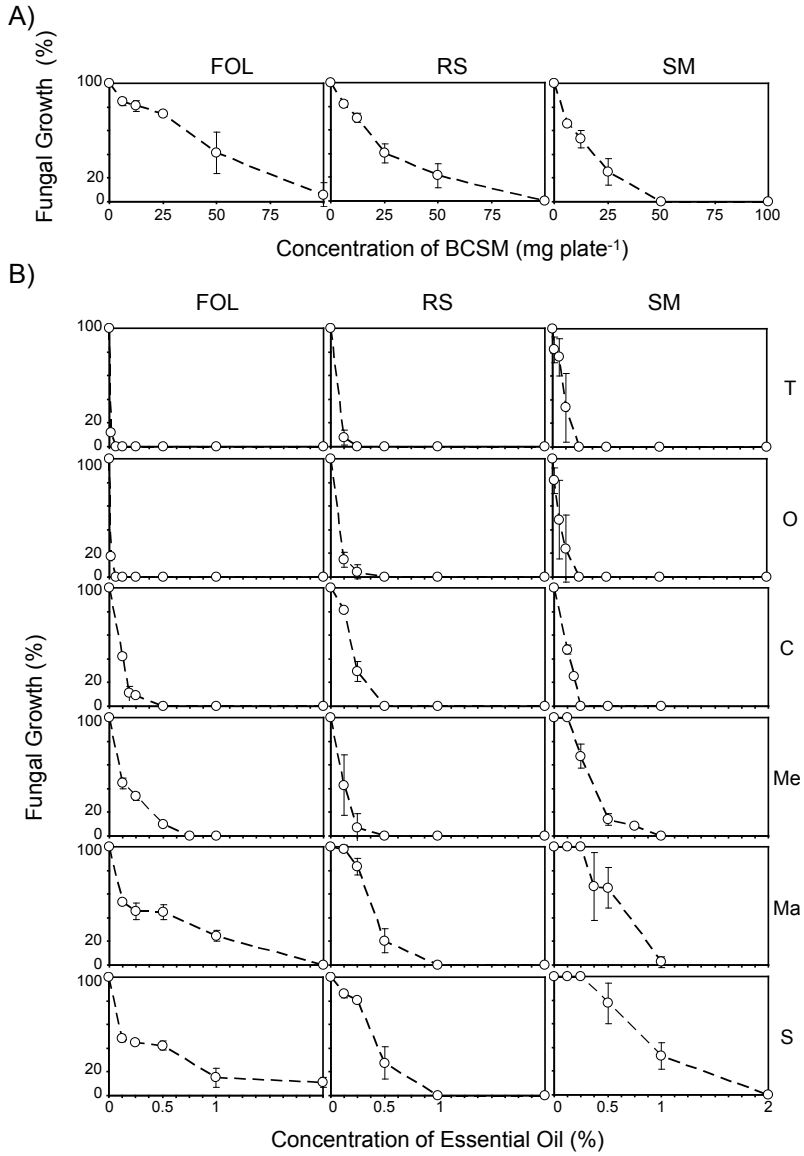

Fig. 1. Effects of *Brassica carinata* seed meal (panel A) and essential oils of thyme (T), oregano (O), caraway (C), melissa (Me), marjoram (Ma) and sage (S) (panel B) on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Rhizoctonia solani* (RS) and *Sclerotinia minor* (SM). Mean value of percentage of fungal growth in treated plates compared to untreated ones (100% of growth), are shown (±SD).

activity of BCSM (20 mg) and EOs (0.125%), used singularly or in conjunction, each substance was tested in Petri dishes prepared as described above. Plates were inoculated in triplicate and incubated at 25°C. The percentage of inhibition was calculated according to the following formula: Fungal Growth Inhibition (%) = [(dc-di)/dc] × 100, where dc and di are the colony diameter measured in the control and in the *i*-th experimental treatment, respectively.

***In planta* assays against soil-borne diseases.** *In planta* experiments were carried out on the following pathosystems: *F. oxysporum* f. sp. *lycopersici*/tomato ecotype Vesuviano, *R. solani*/bean cv. Borlotto and *S. minor*/lettuce cv. Jumper. Inoculum of each pathogen was produced on autoclaved 10% Potato Dextrose Broth (PDB) amended with equal dry weight of millet as recently described by Pane *et al.* (2013c). Then, infected millet was added, at final concentration of 1% w/w, to 12-cm diameter pots (approx. 300 ml vol.) filled with sterilized commercial soil-peat (dark and light peats mixture). The treatments were: B = 0.5 g of BCSM *per* pot (Galletti *et al.*, 2008), A = 50 ml of a water suspension of 5×10^7 living cells ml^{-1} (Yang *et al.*, 2009) of *B. amyloliquefaciens* strain 17S *per* pot and T=25 ml of thyme essential oil water emulsion at 4% (Hashem *et al.*, 2010) *per* pot. Each antifungal component was applied singularly or at double and triple combinations, three days before transplanting. Untreated pots, both inoculated and not-inoculated with the pathogen, were used as reference controls. Then, pots with five one-month-old nursery plants each were moistened to field capacity and placed in a growth chamber (26 ± 2°C) under 14 h light/10 h dark photoperiod in a completely

randomized design with five replicates *per* treatment. After two weeks-incubation, disease assessment was performed, in each pot, giving to the plant a score ranging from 0 to 3 (0 = not infected; 1 = yellowing; 2 = withering and/or wilting; 3 = death). Then, specific indexes were calculated as follows: Disease Incidence (%) = (Number of diseased plants/ Total number of inoculated plants)×100 (Van Beneden *et al*., 2010); Disease Severity (%) = [∑ (Number of diseased plants in this score×disease score)/(Total number of inoculated plants×The highest disease score)]×100 (Yang *et al*., 2009). Moreover, to evaluate the plant biomass, fresh and dry-weight *per* pot of the whole plant were also measured and expressed as percentage of the not inoculated plants. Experiments were carried out twice.

Statistical analysis. The data on the effects of the treatments on the growth of the pathogens and on the plant disease assessments were submitted to analysis of variance (ANOVA) after verification of the assumptions, and treatment means were compared by Duncan's Test ($P \leq 0.05$). Percentage data were angular transformed by arcsine square-root before undergoing statistical analysis, while the not transformed ones were presented.

RESULTS

***In vitro* assays.** *In vitro* assays revealed the capacity of BCSM and EOs to inhibit the mycelial growth of *F. oxysporum* f. sp. *lycopersici*, *R. solani* and *S. minor* (Fig. 1). Volatiles released by the wetted BCSM reduced growth of fungi in a dose-depended manner, reaching the complete growth inhibition for *F. oxysporum* f. sp. *lycopersici* and *R. solani* with 100 mg ml^{-1}, and for *S. minor* with 50 mg ml^{-1}.

Essential oils were supplemented in the growing media and the fungal growth was inhibited according to the dose used. Thyme and oregano oil showed higher inhibitory effects, with a minimum inhibitory concentration at 0.0125%. Sage oil proved the least inhibitory effect.

Overall, *F. oxysporum* f. sp. *lycopersici* was the most sensitive fungus to both plant-derived antifungals as opposed to *S. minor* that slightly tolerated lower concentrations. Thus, thyme and oregano EOs were chosen for testing the effect of their combination with BCSM on the antifungal activity of *B. amyloliquefaciens* strain in dual challenge *in vitro* experiments against the fungi (Fig. 2).

All substances applied in these challenge experiments, enhanced the *in vitro* antagonistic activity of *B. amyloliquefaciens* against fungi. In particular, thyme oil and BCSM combined with the antagonist increased significantly the growth inhibition of *F. oxysporum* f. sp. *lycopersici*, *R. solani* and *S. minor*, up to +34%, +35% and +42%, on average, respectively, in comparison with the bacteria alone.

***In planta* assays.** In the *in planta* experiments performed in climatic room, no phytotoxic symptoms were observed as a consequence of the antifungal agents applied. Results showed the effectiveness of all treatments to control diseases caused by the soil-borne pathogens analysed. The effectiveness reflected the plant biomass yields.

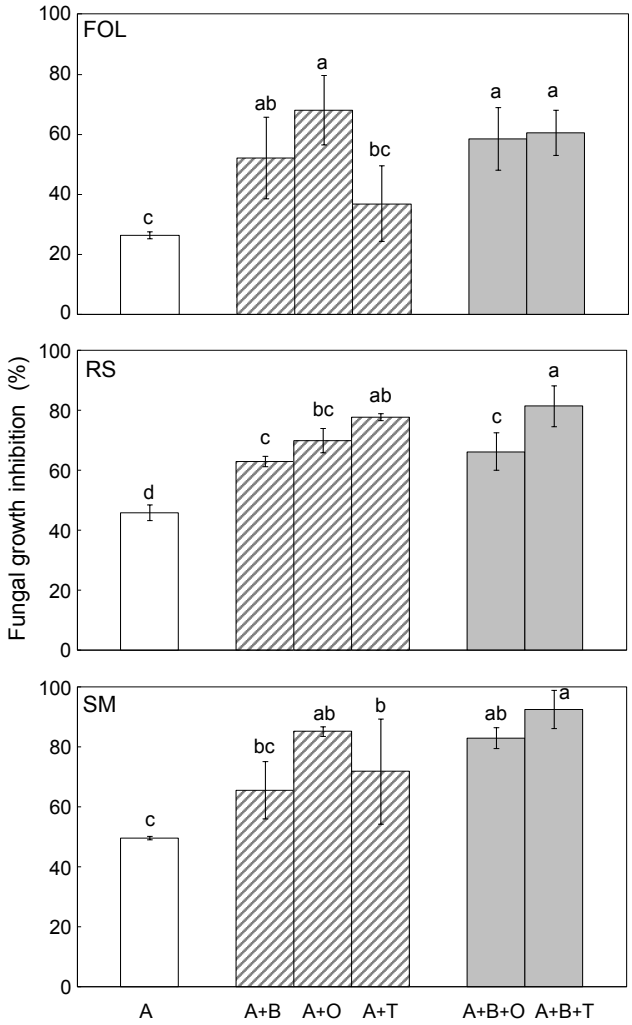

Fig. 2. Effects of *Brassica carinata* seed meal (B), thyme (T) and oregano (O) essential oils on the *in vitro* antagonism of *Bacillus amyloliquefaciens* strain 17S (A) against *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Rhizoctonia solani* (RS) and *Sclerotinia minor* (SM). Mean value of percentage of fungal growth inhibition with respect to the untreated control is shown (±SD). Different lowercase letters indicate significant differences among bars according to Duncan's test ($P \leq 0.05$).

Antifungal agents and their combinations significantly ($P < 0.05$) decreased the incidence and severity of Fusarium wilt of tomato by about 50%, on average, in comparison with the untreated plants, in which the disease incidence was around 96% and severity was about 84% (Fig. 3). However, significant differences ($P < 0.05$) in plant biomass between treatments and infected control were found only when the antagonistic *B. amyloliquefaciens* mixed with BCSM was used.

On the *R. solani*/bean pathosystem, the combination of BCSM with the antagonist reduced disease incidence by 58%, i.e., at statistically comparable levels showed by the

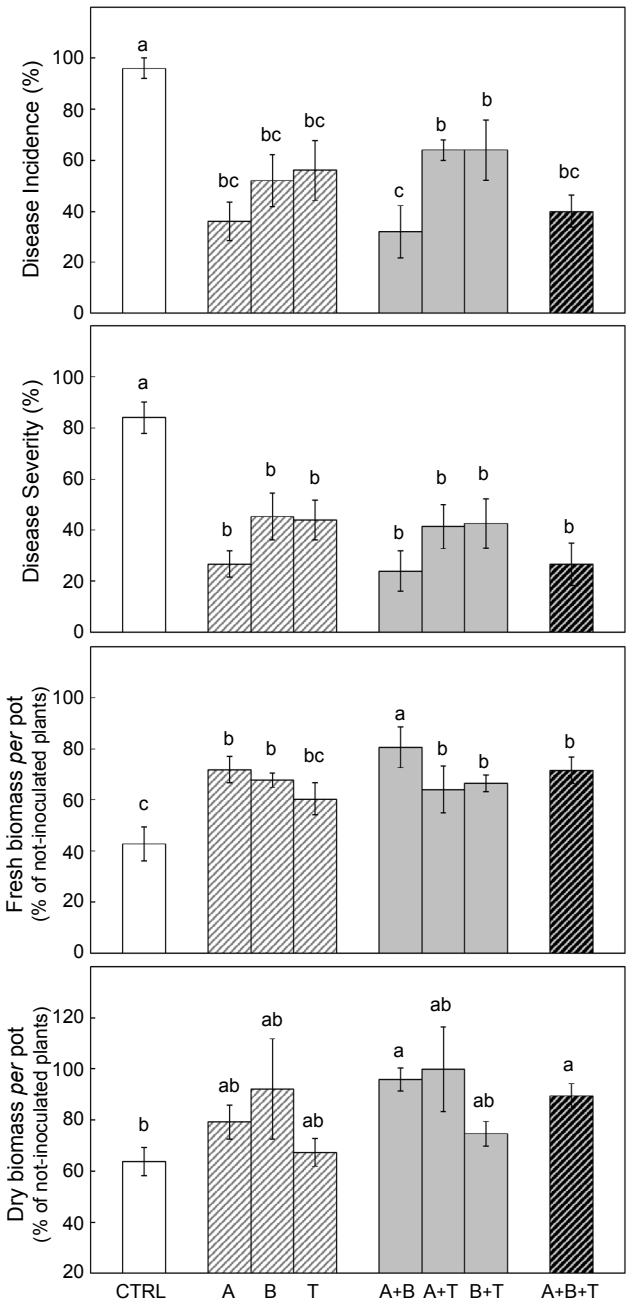

Fig. 3. Means of wilt disease incidence and severity and plant fresh and dry biomass (±SE) for transplanted tomato plants grown in soil contaminated with *Fusarium oxysporum* f. sp. *lycopersici* and treated with single, double and triple combinations of *Bacillus amyloliquefaciens* antagonist (A), BCSM (B) and thyme essential oil (T) in comparison with untreated control. Different letters indicate significant differences ($P \leq 0.05$) among bars according to Duncan's test.

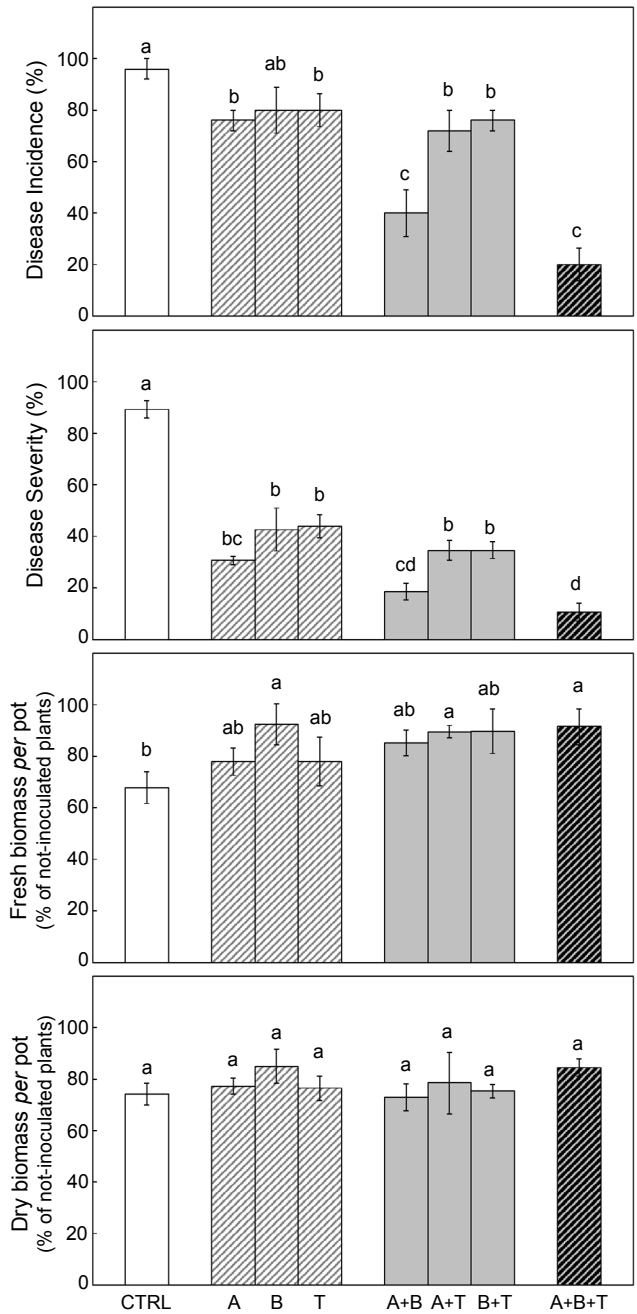

Fig. 4. Means of root rot disease incidence and severity, and plant fresh and dry biomass (±SE) for transplanted bean plants grown in soil contaminated with *Rhizoctonia solani* and treated with single, double and triple combinations of *Bacillus amyloliquefaciens* antagonist (A), BCSM (B) and thyme essential oil (T) in comparison with untreated control. Different letters indicate significant differences ($P \leq 0.05$) among bars according to Duncan's test.

three disease-control treatments mixed (showing a reduction of about 79%) (Fig. 4). Furthermore, this last treatment exhibited significant protective effects on plant biomass yield. The remaining treatments, with the exception of BCSM, significantly decreased the incidence of infected plants by 20%, on average. However, the antifungal agents more markedly contained Rhizoctonia rot severity, which assumed values within the range 10-44% (Fig 4).

Sclerotinia lettuce drop was significantly ($P<0.05$) reduced in terms of disease incidence and severity by treatments with the exception of the individual application of BCSM (Fig. 5). All *Bacillus*-based treatments were effective in controlling lettuce drop disease by 62%, on average. In all treated pots lettuce biomass, in comparison with untreated control was, on average, five-fold increased.

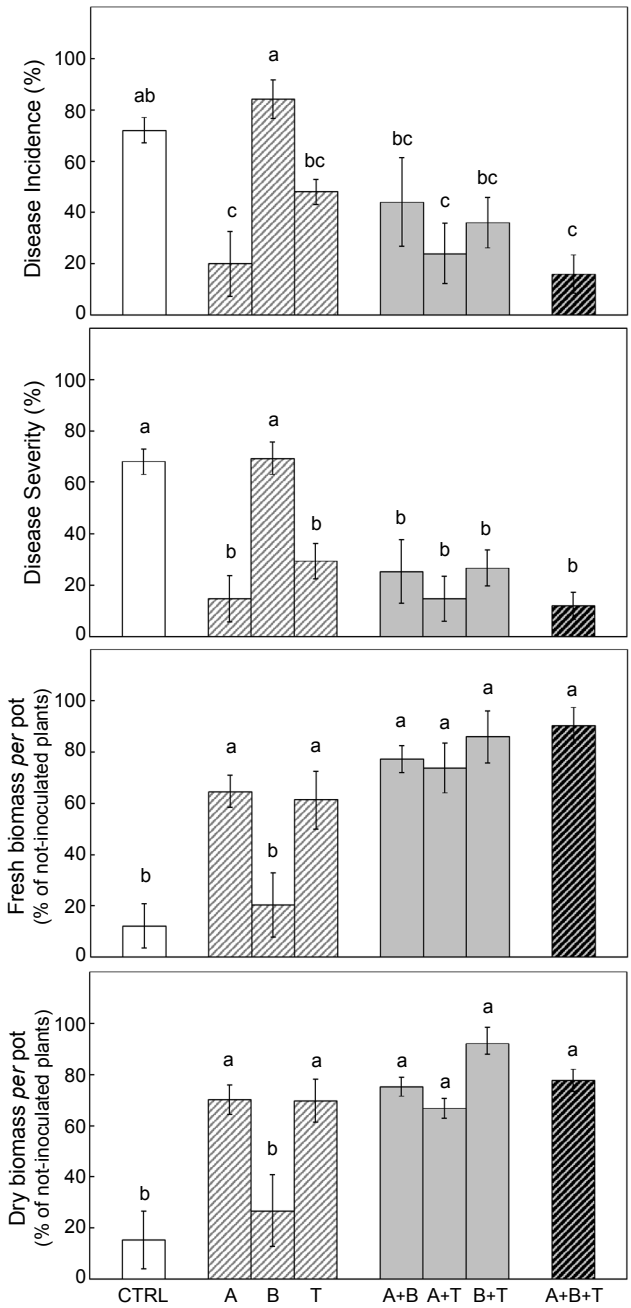

Fig. 5. Means of drop disease incidence and severity and plant fresh and dry biomass (±SE) for transplanted lettuce plants grown in soil contaminated with *Sclerotinia minor* and treated with single, double and triple combinations of *Bacillus amyloliquefaciens* antagonist (A), BCSM (B) and thyme essential oil (T) in comparison with untreated control. Different letters indicate significant differences ($P \leq 0.05$) among bars according to Duncan's test.

DISCUSSION

Efforts are required to implement new non-chemical technologies for the control of soil-borne plant diseases to extend the spectrum of their efficacy, thereby making the transition from chemical fungicides easier. Hence, here we have explored the combination of different non-synthetic low-impacting control tools in a unique treatment to enhance the management of soil-borne plant pathogens. Some studies have focused on the integrated use of biological control agents with other physical, chemical, agronomical and biological methods, just to fortify their overall suppressive action (Jacobsen *et al*., 2004; Spadaro and Gullino, 2005).

Here, soil treatments with a microbial antagonist *Bacillus amyloliquefaciens*, Brassica seed meal containing glucosinolates and thyme essential oil reduced the incidence of diseases caused by *F. oxysporum* f. sp. *lycopersici* on their own and in different combinations in the range between 33 and 66%, on average. Similarly, antifungal agents, with the exception of BCSM alone, reduced Sclerotinia drop disease on lettuce in the range between 33 and 77%, on average. Instead, only in the case of *R. solani*/bean pathosystem, the three-combination gave better plant protection with 79% reduction of disease incidence. The highly effective treatments improved plant biomass yields contrarily to untreated infected controls. Overall, the experiment with potted plants indicated that the *B. amyloliquefaciens* antagonistic strain 17S played a crucial role in determining treatments effectiveness. This microbial antagonist has been previously selected through a stepwise screening strategy for its high specificity against soil-borne fungal pathogens, such as *Fusarium solani*, *R. solani* and *S. minor* (Pane *et al*., 2012b): thus, its significant contribution may be expected. Studies aimed to elucidate mechanisms of *B. amyloliquefaciens* biocontrol against plant pathogens, suggested that rhizosphere competence of these bacteria associated to the ability of synthesizing antimicrobial metabolites (such as surfactants and antibiotics) are crucial for protecting plants (Raaijmakers *et al*., 2010; Chowdhury *et al*., 2015).

Thyme and oregano essential oils were highly active against the *in vitro* development of all tested fungi. Previously, Zaccardelli *et al*. (2007) analyzed the composition in monoterpenes of essential oils used in this study, and their implication on antifungal properties. Carvacrol and thymol, and carvacrol and linalool, for example, have been documented as the main constituents responsible for the antimicrobial activity exhibited by thyme and oregano essential oils, respectively. In this study, thyme oil was selected on the basis of its high *in vitro* antifungal activity, for its ability to enhance BCSM and *B. amyloliquefaciens* strain in the plate challenge experiments and its ability to complement the *in planta* effectiveness of the antagonist.

In agreement with our results, previously it has been reported that the use of lemongrass essential oil in conjunction with the biocontrol agent *Bacillus amyloliquefaciens* PPCB004 increased the post-harvest control of *Botrytis cinerea* Pers., *Penicillium expansum* Link and *Rhizopus stolonifer* (Ehrenb.) Vuill. on peach (Arrebola *et al*., 2010). Hao *et al*. (2011) attributed the complementary properties of *Bacillus amyloliquefaciens* antagonistic strain and tea saponin to the enhanced efficacy of the combination

in the control of *Penicillium italicum* Wehmer, *Penicillium digitatum* (Pers.) Sacc. and *Geotrichum candidum* Link on mandarin fruits under storage conditions.

In this study, BCSM alone slightly reduced tomato wilting, but failed to significantly control lettuce drop and Rhizoctonia rot of bean. Moreover, it did not improve the effects of the other two control tools when used in combination.

B. carinata defatted seed meal is widely reported as sustainable soil bio-fumigant against plant pathogens due to its high antifungal potential (De Corato *et al*., 2015). The composition of defatted seed meal of *B. carinata* has been documented by Zaccardelli *et al*. (2011) who reported that glucosinolates concentration may vary in the range 95-115 mM kg^{-1}. Here, preliminary *in vitro* assays have confirmed a remarkably significant volatile antifungal activity of the *B. carinata* seed meals against all fungal pathogens tested in a dose-depended model. These results were consistent with the inhibitory effects of BCSM-released toxic volatiles on soil-borne fungal pathogens, including *F. oxysporum*, *R. solani* and *S. minor*, according to Sanchi *et al*. (2005) and Galletti *et al*. (2008). However, in *in planta* experiment, the BCSM antifungal effects may have been transient because of the evanescence of toxic volatiles (Manici *et al*., 2004).

Different and complementary biological methods can provide effective alternatives to the fungicides for combating soil-borne pathogens. Moreover, their combinations can be useful to extend the spectrum of diseases to be managed. This study, in particular, reports the performances of a new biological method, based on the association of a microbial agent with plant-derived products, in controlling some soil-borne diseases under pot experiments. Findings indicate that the combined approach is better than the separate treatments in controlling diseases only in few cases. Likely, this may be due to the diverse contribute of each factor in producing the general effect displayed by *in planta* assays, which follow specie-specific behaviours. Actually, fungal pathogens are differentially sensitive to the antifungal agents, and these last may show variable efficacy in counteracting plant diseases because of peculiarity of the mechanisms of pathogenicity to be contrasted (Spadaro and Gullino, 2005). Therefore, the development of a multicomponent technology using natural products to control plant diseases in an integrated management strategy, should be more specifically calibrated on the target system.

Further studies focused on the dynamic of action mechanisms of each component in the complex systems, particularly under field conditions, are still necessary, as well as researches about formulations and application practices of this designed multicomponent biofungicide.

ACKNOWLEDGEMENTS

This research was supported by the Project "Valorizzazione dei sottoprodotti della filiera del biodiesel - Extravalore", funded by Italian Ministry of Agricultural, Food and Forestry Policies.

REFERENCES

Arrebola E., Sivakumar D., Bacigalupo R., Korsten L., 2010. Combined application of antagonistic *Bacillus amyloliquefaciens* and essential oils for the control of peach postharvest diseases. *Crop Protection* **29**: 369-377.

Boulter J.I., Trevors J.T., Boland G.J., 2002. Microbial studies of compost: bacterial identification, and their potential for turfgrass pathogen suppression. *World Journal of Microbiology and Biotechnology* **18**: 661-671.

Brown P.D., Morra M.J., 1997. Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy* **61**: 167-231.

Cawoy H., Bettiol W., Fickers P., Ongena M., 2011. Bacillus-Based Biological Control of Plant Diseases. In: Stoytcheva Rijeka M. (ed.). Pesticides in the Modern World - Pesticides Use and Management, pp. 273-302. InTech, Croatia.

Chowdhury S.P., Hartmann A., Gao X., Borriss R., 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 – a review. *Frontiers in Microbiology* **6**: 780. doi: 10.3389/fmicb.2015.00780

De Corato U., Pane C., Bruno G.L., Cancellara F.A., Zaccardelli M., 2015. Co-products from a biofuel production chain in crop disease management: A review. *Crop Protection* **68**: 12-26.

Galletti S., Sala E., Leoni O., Burzi P.L., Cerato C., 2008. *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biological Control* **45**: 319-327.

Gasol C.M., Gabarrell X., Anton A., Rigola M., Carrasco J., Ciria P., Solano M.L., Rieradevall J., 2007. Life cycle assessment of a *Brassica carinata* bioenergy cropping system in southern Europe. *Biomass and Bioenergy* **31**: 543-555.

Guerrero-Diaz M.M., Lacasa-Martinez C.M., Hernandez-Piñera A., Martinez-Alarcon V., Lacasa Plasencia A., 2013. Evaluation of repeated biodisinfestation using *Brassica carinata* pellets to control *Meloidogyne incognita* in protected pepper crops. *Spanish Journal of Agricultural Research* **11**: 485-493.

Gurjar M., Ali S., Akhtar M., Singh K., 2012. Efficacy of plant extracts in plant disease management. *Agricultural Sciences* **3**: 425-433.

Hao W., Li H., Hu M., Yang L., Rizwan-ul-Haq M., 2011. Integrated control of citrus green and blue mold and sour rot by *Bacillus amyloliquefaciens* in combination with tea saponin. *Postharvest Biology and Technology* **59**: 316-323.

Hashem M., Moharam A.M., Zaied A.A., Saleh F.E.M., 2010. Efficacy of essential oils in the control of cumin root rot disease caused by *Fusarium* spp. *Crop Protection* **29**: 1111-1117.

Jacobsen B.J., Zidack N.K., Larson B.J., 2004. The role of *Bacillus*-based biological control agents in integrated pest management systems: plant diseases. *Phytopathology* **94**: 1272-1275.

Manici L.M., Caputo F., Babini V., 2004. Effect of green manure on *Pythium* spp. population and microbial communities in intensive cropping systems. *Plant Soil* **263**: 133-142.

Nazzaro F., Fratianni F., De Martino L., Coppola R., De Feo V., 2013. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals* **6**: 1451-1474.

Pal K.K., McSpadden Gardener B., 2006. Biological control of plant pathogens. In: The Plant Health Instructor, pp. 1-25, APSnet.

Pandey V.N., Dubey N.K., 1994. Antifungal potential of leaves and essential oils from higher plants against soil phytopathogens. *Soil Biology and Biochemistry* **26**: 1417-1421.

Pane C., Villecco D., Pentangelo A., Lahoz E., Zaccardelli M., 2012a. Integration of soil solarization with *Brassica carinata* seed meals amendment in a greenhouse lettuce production system. *Acta Agriculturae Scandinavica - Section B, Soil and Plant Science* **62**: 291-299.

Pane C., Villecco D., Campanile F., Zaccardelli M., 2012b. Novel strains of *Bacillus*, isolated from compost and compost-amended soils, as biological control agents against soilborne phytopathogenic fungi. *Biocontrol Science and Technology* **22**: 1373-1388.

Pane C., Villecco D., Roscigno G., De Falco E., Zaccardelli M., 2013a. Screening of plant-derived antifungal substances useful for the control of seedborne pathogens. *Archives of Phytopathology and Plant Protection* **46**: 1533-1539.

Pane C., Villecco D., Zaccardelli M., 2013b. *Brassica carinata* tissues containing glucosinolates and essential oils enhance bacillus-based antagonistic activity against soil-borne plant diseases. *Proceedings of the International Congress "Pesticide Use and Risk Reduction for future IPM in Europe", Riva del Garda (TN), Italy,* March 19-21, p. 172.

Pane C., Piccolo A., Spaccini R., Celano G., Villecco D., Zaccardelli M., 2013c. Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi *Rhizoctonia solani* and *Sclerotinia minor*. *Applied Soil Ecology* **65**: 43-51.

Pane C., Fratianni F., Parisi M., Nazzaro F., Zaccardelli M., 2016. Control of Alternaria post-harvest infections on cherry tomato fruits by wild pepper phenolic-rich extracts. *Crop Protection* **84**: 81-87.

Raaijmakers J.M., de Bruijn I., Nybroe O., Ongena M., 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiology Reviews* **34**: 1037-1062.

Sanchi S., Odorizzi S., Lazzeri L., Marciano P., 2005. Effect of *Brassica carinata* seed meal treatment on *Trichoderma harzianum* T39-*Sclerotinia* species interaction. *Acta Horticolturae* **698**: 287-292.

Spadaro D., Gullino M.L., 2005. Improving the efficacy of biocontrol agents against soilborne pathogens. *Crop Protection* **24**: 601-613.

Smolinska U., Morra M.J., Knudsen G.R, James R.L., 2003. Isothiocyanates produced by brassicaceae species as inhibitors of *Fusarium oxysporum*. *Plant Disease* **87**: 407-412.

Uppal A.K., El Hadrami A., Adam L.R., Tenuta M., Daayf F., 2008. Biological control of potato Verticillium wilt under controller and field conditions using selected bacterial antagonists and plant extracts. *Biological Control* **44**: 90-100.

Van Beneden S., Leenknegt I., França S.C., Höfte M., 2010. Improved control of lettuce drop caused by *Sclerotinia sclerotiorum* using Contans combined with lignin or a reduced fungicide application. *Crop Protection* **29**: 168-174.

Yang D., Wang B., Wang J., Chen Y., Zhou M., 2009. Activity and efficacy of *Bacillus subtilis* strain NJ-18 against rice sheath blight and Sclerotinia stem rot of rape. *Biological Control* **51**: 61-65.

Zaccardelli M., Mancini E., Campanile F., De Feo E., De Falco E., 2007. Identification of bio-active compounds in essential oils of medicinal plants toxic for phytopathogenic fungi and bacteria. *Journal of Plant Pathology* **89**: S66.

Zaccardelli M., Pentangelo A., D'Onofrio B., Lazzeri L., 2011. Ammendamento in orticoltura con farine di disoleazione provenienti dalla filiera del biodiesel. *Fertilitas Agrorum* **4**: 38-44.

Received May 24, 2016
Accepted December 1, 2016