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Chemical and biological control of *Fusarium* species involved in garlic dry rot at early crop stages

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Abstract The aim of the study was to test in vitro and in vivo the efficacy of triazoles and biocontrol agents (BCAs) against *Fusarium proliferatum* and *F. oxysporum*, the former signaled as the main causal agent of garlic dry rot and the latter also involved. In vitro trials were organized using potato dextrose agar with added chemicals or BCAs inoculated with selected *F. proliferatum* and *F. oxysporum*. Garlic cloves were dipped before sowing in suspensions prepared with the fungicides showing the best performances in vitro; then they were dipped in Fusaria suspension before sowing. In in vitro trials, the maximum Fusaria growth inhibition was performed by Propiconazole + Prochloraz (100%), followed by Tebuconazole (88.9%). BCAs showed great capacity to control Fusaria, with a maximum growth inhibition of 80% (*Trichoderma harzianum* + *T. gamsii*). In vivo bacterial BCAs showed a similar capacity to control *F. proliferatum* and *F. oxysporum* compared to chemical products (mean of severity index 18.6% and 11.7%, respectively). In vivo results confirmed the in vitro performances, except for *Trichoderma*, which had the worst performances in vivo. Therefore, the results are preliminary but promising for future field application.

Keywords *Allium sativum* · *Fusarium proliferatum* · *Fusarium oxysporum* · Seed coating · Biocontrol agent · Triazoles

Highlights
- Chemicals and biocontrol agents to control *Fusarium* in early garlic stages were tested
- *In vitro* the best performing chemical was Propiconazole+Procloraz
- *Trichoderma harzianum*+*T. gamsii* showed great capacity to control *Fusaria in vitro*
- *In vivo* bacteria and triazoles showed the best capacity to control *Fusarium*
- Clove-seed coating was promising to control garlic *Fusarium* spp. at early growth stages

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Introduction

Garlic (*Allium sativum* L.) is cultivated worldwide in temperate regions and is used both for feed and for medical purposes. At a global level, 1.5 million hectares were occupied by this crop in 2018 and Italy was the sixth producer in Europe with 3573 ha (FAO-STAT 2018) characterized by high production values as PDO (Protected Designation of Origin) (Spagnoli 2014). In Piacenza province, an average of 328 tons of white garlic is produced annually, but up to 18% of the production is lost due to dry rot, an emerging disease, with local growers facing huge economic losses (COPAP, Cooperative of Piacenza garlic producers, personal communication). Worldwide, dry rot disease of garlic is a postharvest problem that results in the case of the most severe attacks of up to 30% of the garlic bulbs being completely emptied and softened. The disease symptoms are generally visible during the drying process of garlic bulbs as necrotic spots, centrally

depressed, sometimes with white mycelium developed. With careful observation, they can also be detected during the growing season, mainly on roots and basal plates. Severe attacks, extending to bulbs, can lead to the death of garlic plants (Mondani et al. 2021). *Fusarium proliferatum* (T. Matsushima) Nirenberg is reported as the main causal agent of garlic dry rot. This fungus has been identified in the past few years as a garlic dry rot agent in Germany (Seefelder et al. 2002), North America (Dugan et al. 2003), Serbia (Stankovic et al. 2007), Spain (Palmero et al. 2010), Italy (Tonti et al. 2012), India (Sankar and Babu 2012), Egypt (Moharam et al. 2013; Elshahawy et al. 2017), Argentina (Salvalaggio and Ridao 2013), and France (Leyronas et al. 2018). Further, *F. oxysporum* has been reported as a causal agent of basal rot (Schwartz and Krishna Mohan 2016). In recent studies conducted in Piacenza province, *F. proliferatum* was reported in garlic bulbs from the crop's early growth stages, suggesting potential seed transmission as a source of this fungal pathogen, and it was the dominant fungus in infected bulbs postharvest, confirmed as the causal agent of dry rot (Mondani et al. 2020; Mondani et al. 2021).

Garlic is commonly propagated via cloves; therefore, the selection of healthy plant material is crucial to reduce pathogen dissemination. In a recent study, Dugan et al. observed that up to 77% of visibly healthy bulbs at harvest developed symptoms after 9–16 months storage, suggesting that cloves used as seeds, even if apparently healthy, can be infected by Fusaria (Dugan et al. 2019).

Garlic dry rot control is not established; only a few studies on the effectiveness of chemical and physical treatments against *Fusarium* spp. in garlic are available. Dugan et al. (2007) demonstrated the efficacy of Benomyl in eradicating *Fusarium* spp. from wounded bulbs, but this active ingredient is now banned in Europe. Elshahawy et al. (2017) reported that in in vitro assays, Carbendazim had a strong inhibition effect on fungal growth when followed by Metalaxyl + Mancozeb (8% + 64%) and thiophanate-methyl. Recently, Gálvez et al. (2017) investigated the ability of three commercial fungicides to control *F. proliferatum* in vitro with promising results, but field treatments failed to control garlic rot through the commercial stage. Physical methods, such as thermotherapy as a seed sanitization system, were also studied by Palmero Llamas et al. (2013), who demonstrated that thermotherapy at 50 °C for one minute greatly decreases *F. proliferatum* conidia viability. However, this method should be carefully evaluated for its effect on clove germination. Finally, regarding BCAs, a few studies have reported their ability to control *Fusarium* spp. in vitro, but their application in garlic crop has not been tested yet (Evangelista-Martínez 2014; Ghanbarzadeh et al. 2014; Ju et al. 2014; Kavitha et al. 2013; Samsudin and Magan 2016). *Trichoderma* spp. and *Bacillus subtilis* seem to be the most promising active ingredients available commercially. Elshahawy et al. (2017) demonstrated the efficacy of *Trichoderma* spp. against *F. proliferatum* isolated from garlic in dual culture assays, while Bjelić et al. (2018) tested in vitro at 25 °C for seven days the capacity of indigenous Serbian *B. subtilis* to limit the growth of *Fusarium* spp. isolated from garlic crops. In both cases, the results were promising, but no information is available regarding their efficacy.

As the disease was detected from early crop stages in field, sowing healthy cloves is crucial to grow healthy plants, but due to the high incidence of *Fusarium* spp. in soil and in symptomless cloves, it is vital to protect the plantlet during the early growth stages. Therefore, the aim of this study was to verify the efficacy of chemicals and biological fungicides towards *F. proliferatum* and *F. oxysporum*: i) grown in vitro on artificial media; ii) naturally occurring or artificially inoculated in cloves sown in pots, as possible seed coating treatments.

Materials and methods

Media

Water Agar (WA): 20 g of agar (2%; Oxoid®, Basingstoke, UK), 1 L of bi-distilled water, amended with 250 ppm of Streptomycin sulfate.

Potato Dextrose Agar (PDA): 15 g of agar (2%; Oxoid®), 10 g of dextrose, 1 L of potato broth (200 g of potato/L of water).

Fungal strains

Fungal strains were selected to represent the main target fungus of garlic dry rot. One strain of *F. oxysporum* and two strains of *F. proliferatum* were included, isolated from garlic and maize—the crop of interest and that prevalent in the Po valley and frequently included in crop rotation, respectively. Two strains isolated from garlic cloves showing dry rot symptoms at field stages—one *F. oxysporum* ITEM18686 (FO) and one *F. proliferatum* ITEM18687 (FPg), confirmed by

molecular identification (Mbofung and Pryor 2010; Nicolaisen et al. 2009), and one isolated from maize, *F. proliferatum* ITEM7595 (FPm), were included in the study. Strains are stored in the fungal collection of ISPA-CNR (Institute of Science of Food Production, National Research Centre; http://server.ispa.cnr.it/ITEM/Collection/). Five mm tassels were collected from the margin of five-day-old colonies, grown on WA incubated at 25 °C, and used as inoculum.

In vitro test

Chemicals

Six commercial chemical fungicides were considered, all including triazoles, the active ingredients effective against Fusaria (Table 1). Before putting PDA into Petri dishes, fungicides were added in five different concentrations of the active ingredients: 0, 1, 10, 100, 1000 ppm.

Amended PDA was centrally inoculated with a 5 mm disc of each of the above-motioned strains (Gálvez et al. 2017).

Biocontrol agents

Four commercially available BCAs were selected based on their mode of action (Table 1); fungi are competitors, while bacteria and *Actinomycetes* produce antifungal compounds. The selected antagonists were tested in dual culture experiments.

Forty μl of cell suspension of *Bacillus subtilis* and *Streptomyces griseoviridis* (1 g of Serenade or Mycostop dissolved in 10 ml of sterile potato broth, 200 g potato/L) were streaked in four lines on a half Petri dish containing PDA. After a five-day incubation at 25 °C, the 5 mm agar disc of the Fusaria strains was inoculated at a 20 mm distance on the other side of the dish (Sangdee et al. 2016). A 5 mm mycelial disc from the margin of a 7-day old colony of *F. oxysporum* and *Trichoderma harzianum* + *T. gamsii* and the Fusaria were inoculated on the opposite side of 90 mm Petri dishes with PDA (approximately 40 mm from each other).

Growth reduction

Both for chemicals and BCAs, control dishes were centrally inoculated with the FPg or FPm and FO (Gálvez Patón et al. 2017). All the plates were incubated at three different temperatures (T; °C): 10 °C, 15 °C, and 25 °C with a 12 h photoperiod. The trials were managed with four replicates. The radial growth of the FPg, FPm and FO and the antagonist was measured after 21 days of incubation. The trials were repeated twice.

Table 1 Fungicides and biocontrol agents used for in vitro and in vivo trials aimed to reduce the growth of *Fusarium proliferatum* and *F. oxysporum* and the resulting disease severity on garlic; active ingredients dosage in commercial products and companies are reported

COMMERCIAL PRODUCT	ACTIVE INGREDIENT	COMPANY
Chemicals		
OPINION ECNA	Propiconazole 250 g/L	ADAMA Italia, Grassobio, Italy
MYSTIC 430SC	Tebuconazole 250 g/L	Nufarm Italia, Bologna, Italy
CUSTODIA	Tebuconazole 200 g/L+ Azoxystrobin 120 g/L	ADAMA Italia, Grassobio, Italy
LUNA EXPERIENCE	Fluopyram 200 g/L+ Tebuconazole 200 g/L	Bayer Crop Science, Milan, Italy
QUILT XCEL	Propiconazole 122.4 g/L+ Azoxystrobin 141.4 g/L	Syngenta Italia, Milan, Italy
BUMPER P	Propiconazole 90 g/L+ Prochloraz 400 g/L	ADAMA Italia, Grassobio, Italy
Biological		
Serenade Max	*Bacillus subtilis* ($5.13*10^{10}$ UFC/g)	Bayer Crop Science, Milan, Italy
IF23	*Fusarium oxysporum* IF23 ($1*10^{7}$ UFC/g)	Xeda Italia, Forlì, Italy
Mycostop	*Streptomyces* griseoviridis K61 ($5*10^{8}$ UFC/g)	Verdera Oy, Kurjenkellontie, Finland
Remedier	*Trichoderma harzianum*+*T. gamsii* ($3*10^{7}$ UFC/g)	Isagro, Milan, Italy

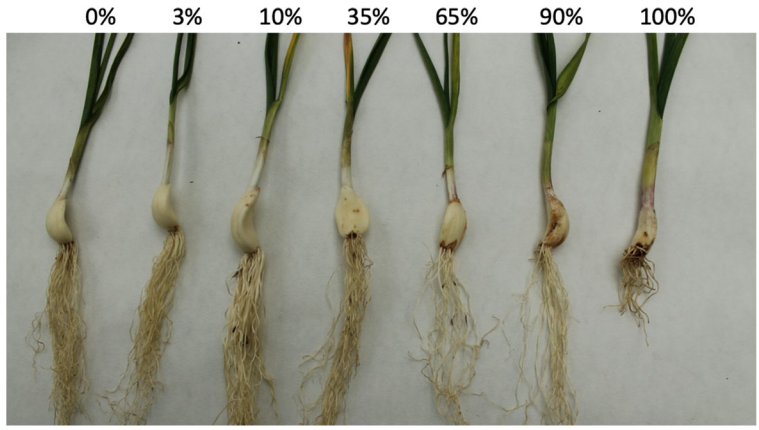

Fig. 1 Severity classes to assess disease severity caused by Fusarium oxysporum and Fusarium proliferatum in garlic. 0 = asymptomatic; 3% = small lesions either on the bulb, the crown or the radicles; 10% = small lesions on two of the considered organs, 35% = small lesions on all the three organs considered; 65% = extended lesions either on the bulb, the crown or the radicles; 90% = extended lesions on two of the considered organs; 100% = extended lesions on all the three organs considered with very limited root growth

At the end of the trials, the percentage of mycelial growth reduction (GR%) was calculated using the formula:

$$\mathrm{GR}\ (\%) = \mathrm{RC{-}RA/RC} \times 100.$$

RC: colony radius (mm) of the control *Fusarium* strain.

RA: colony radius (mm) of the *Fusarium* strain in dual culture with the antagonist or in amended PDA.

In vivo test

Five active ingredients—two chemicals (Propiconazole + Prochloraz and Tebuconazole) and three BCAs (*T. harzianum* + *T. gamsii*, *B. subtilis* and *S. griseoviridis*)–were tested in in vivo trials. The choice was based on in vitro trial results.

Garlic cloves were sown in plastic pots (14 × 14 × 16 cm) containing sterile potting soil and pasteurized field clay soil (2:1). Before sowing, the cloves were washed for 20 min in running tap water, their surfaces disinfected for three minutes in 1% NaOCl solution and rinsed in sterile distilled water three times. Then they were soaked for 15 min in the solutions of the above-mentioned products and dried. Finally, the cloves were inoculated by dipping them in 10^5 conidia/ml suspension of each of the three Fusaria strains. Each treatment consisted of eight cloves per pot, replicated four times. Active ingredients were paired with fungal strains so that each treatment was the combination of one active ingredient and one fungal strain. All possible combinations were tested—60 pots in total. The untreated and uninoculated control was prepared by planting cloves without any previous treatment, either with FPg, FPm, and FO or with active ingredients (negative control). In addition, the inoculated control was made by dipping the cloves in conidial suspensions, but not treating them with active ingredients (positive control). The pots were placed in natural open conditions between October 2, 2018 and January 7, 2019. The experiment was arranged with a complete randomized block design. Meteorological data were collected daily using a data logger. The parameters measured were T (°C), relative humidity (RH; %), and rainfall (R; mm).

At the end of the trial, plants were classified in seven severity classes of *Fusarium* symptoms (Fig. 10% = asymptomatic; 3% = small lesions either on the bulb, the crown or the radicles; 10% = small lesions on two of the considered organs, 35% = small lesions on all the three organs considered; 65% = extended lesions either on the bulb, the crown or the radicles; 90% = extended lesions on two of the considered organs; 100% = extended lesions on all the three organs considered with very limited root growth), root length was measured, and isolation of *Fusarium* was managed on symptomatic plants. Portions of symptomatic tissues were surface sterilized for one minute in NaOCl, rinsed three times in sterile water, dried in sterile conditions, and plated in Petri plates containing WA. Plates were incubated for seven days at 25 °C with a 12-h photoperiod. Emerging colonies were transferred to PDA for identification at

microscope (Nikon, Eclipse 50, Japan, 500x), according to Leslie and Summerell (2006).

Data analysis

GR and severity data were arcsine transformed in order to homogenize variance (Clewer and Scarisbrick 2001), the analysis of variance (ANOVA) was applied and then Tukey's test was used to compare means, using the statistical package PASW statistics (ver.19, SPSS Inc., Chicago, USA, 2009).

Results

In vitro test

Chemicals

All factors investigated in the study, including fungal strains, active ingredients, their concentration, and incubation T, as well as their interaction, significantly affected fungal growth ($P < 0.01$). No statistical differences were observed between repeated trials.

The fungal strains included in the study behaved differently depending on the chemicals applied. The efficacy in decreasing fungal growth was very good, between 88.6% and 81.3% GR impact detected on FPm and FO, respectively. Regarding the active ingredients, the mixture of Propiconazole + Prochloraz showed the greatest growth inhibition, around 100%, followed by Tebuconazole (88.9%), while Propiconazole, alone or in combination with Azoxystrobin, performed the worst. A significant decrease in efficacy in reducing fungal growth, from 99% to 63%, was observed moving from the highest to the lowest active ingredient concentration. In addition, chemicals worked optimally at 10 °C and their efficacy decreased with T increase (89.1% GR at 10 °C versus 76.8% at 25 °C; Table 2). The interaction between the active ingredient and fungal strain tested was significant (Fig. 2). Propiconazole and Propiconazole + Azoxystrobin had the lowest impact on FO, while all the products containing Tebuconazole were more effective on FPm; the best performance on FPg, FPm, and FO was confirmed by Propiconazole + Prochloraz.

Biocontrol agents

In this study, BCA efficacy was significantly affected by the main factors, T and active ingredient ($P < 0.01$). No statistical differences were observed between repeated trials.

All the *Fusarium* strains showed a GR around 64%. BCAs demonstrated varying capacity to reduce *F. proliferatum* and *F. oxysporum* growth; the most effective compound was based on *T. harzianum* + *T. gamsii* (80.0% GR), followed by *B. subtilis* (70.8% GR), *S. griseoviridis* (59.7% GR), and *F. oxysporum* (47.7% GR) (Table 3). Regarding T, the best BCA performance was observed at 15 °C with 68.2% GR, while the worst was at 25 °C, with 61.1% GR (Table 3, Fig. 3). However, the efficacy of *B. subtilis* suffers at the highest T (25 °C), while *F. oxysporum* as BCA performed the worst at 10 °C (Fig. 3).

Table 2 Analysis of variance of the percentage of growth reduction (GR%) of three *Fusarium* spp. strains (one *F. oxysporum* isolated from garlic and two *F. proliferatum*, one of each isolated respectively from garlic and maize) grown on Potato Dextrose Agar added with six chemical products, incubated at three different temperatures (T = 10–15-25 °C) for 21 days

Factors	GR%	
1. Fungal strain	**	
F. oxysporum ITEM18686 (FO)	81.3	*c*
F. proliferatum ITEM18687 (FPg)	84.9	*b*
F. proliferatum ITEM7595 (FPm)	88.6	*a*
2. Active ingredient	**	
Propiconazole	76.4	*d*
Propiconazole+Azoxystrobin	73.2	*e*
Propiconazole+Prochloraz	99.9	*a*
Tebuconazole	88.9	*b*
Tebuconazole+Azoxystrobin	85.7	*c*
Tebuconazole+Fluopyram	85.7	*c*
3. Concentration	**	
1	62.8	*d*
10	84.0	*c*
100	93.7	*b*
1000	99.1	*a*
4. Temperature °C	**	
10	89.1	*a*
15	88.8	*b*
25	76.8	*c*

**(P < 0.01); Different letters indicate significant differences according to the Tukey test ($P < 0.01$)

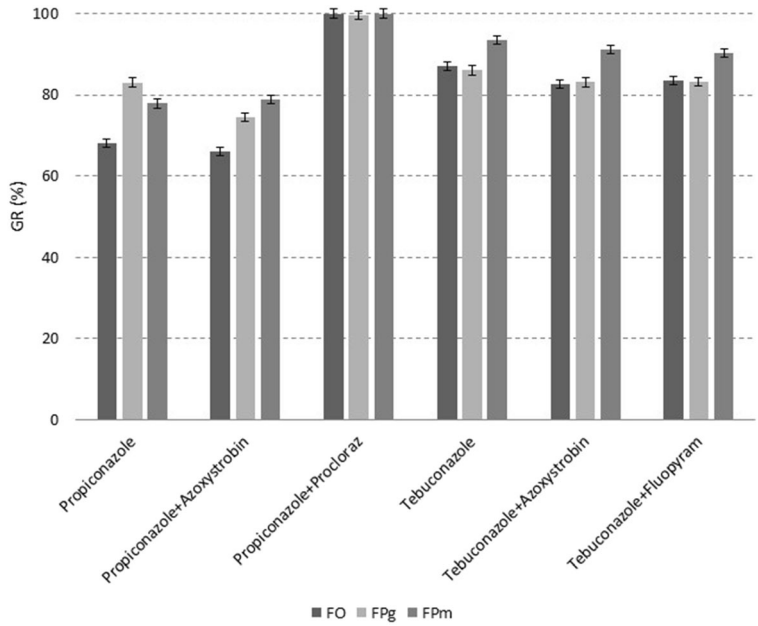

Fig. 2 Growth reduction (GR%) of the three fungal strains, *F. oxysporum* ITEM18686 (FO), *F. proliferatum* ITEM18687 (FPg) and *F. proliferatum* ITEM7595 (FPm), grown in vitro on spiked Potato Dextrose Agar medium with six commercial fungicides added (see Table 1 for details). ($P < 0.05$. Standard error = 1.064)

In vivo test

Garlic cloves sown in October and harvested in January were exposed to natural conditions similar to those occurring in normal garlic cultivation. The trial was stopped at the plant growth stage BBCH 14 (fourth leaf visible). Meteorological data collected during the trial are reported in Fig. 4. The minimum and maximum hourly T were respectively −6.3 °C and 28.7 °C. The calculated sum of degree-day was 884.1 and the mean RH was 87.3. The total R was 277 mm during the three months considered in the study.

Fungal strain and active ingredient applied had a significant impact ($P < 0.01$) on disease severity and root length, as did the interaction between the fungal strain and active ingredient. *F. oxysporum* and *F. proliferatum* were isolated from symptomatic plants inoculated, respectively, with those species; emerging colonies, cultured on PDA, were morphologically identical in color and shape to the original colonies used as inoculum source.

Cloves neither treated nor inoculated (negative control) showed a mean disease severity of 16.7% and mean radicle length of 23.3 cm. Of the fungal strains, FPg produced the highest mean disease severity per plant (33.1%), but plants had a similar radicle length with FO (mean 21.5 cm). FPm caused the lowest disease severity (18.1%), and the radicle reached the maximum length (23.2 cm). All the treatments applied significantly reduced disease severity compared to the inoculated control (11.1%–24.8% versus 65.6%). Chemical products resulted in the lowest disease severity, 11.1% for Propiconazole + Prochloraz and 12.3% for Tebuconazole, significantly lower than *T. harzianum* +

Table 3 Analysis of variance of the percentage of growth inhibition (GR%) of three *Fusarium* spp. strains (one *F. oxysporum* isolated from garlic and two *F. proliferatum*, one of each isolated respectively from garlic and maize) grown on Potato Dextrose Agar added with four different biocontrol agents, incubated at three different temperatures (T = 10–15-25 °C) for 21 days

Factors	GR%	
1. Fungal strain	*n.s.*	
F. oxysporum ITEM18686 (FO)	64.1	
F. proliferatum ITEM18687 (FPg)	64.9	
F. proliferatum ITEM7595 (FPm)	64.6	
2. Active ingredient	**	
S. griseoviridis	59.7	*c*
B. subtilis	70.8	*b*
T. harzianum + *T. gamsii*	80.0	*a*
F. oxysporum IF23	47.7	*d*
3. Temperature	**	
10	64.3	*b*
15	68.2	*a*
25	61.1	*c*

n.s. = not significant; ** ($P < 0.01$); Different letters indicate significant differences according to the Tukey test ($P < 0.01$)

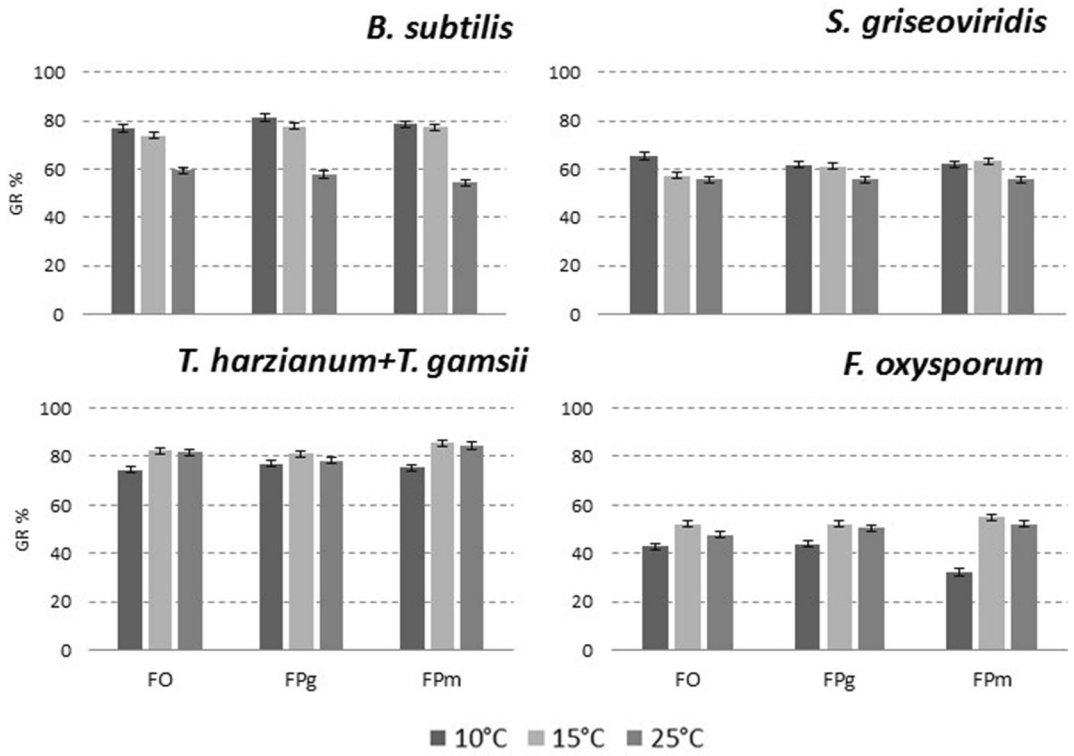

Fig. 3 Growth reduction (GR%) of the three fungal strains, *F. oxysporum* ITEM18686 (FO), *F. proliferatum* ITEM18687 (FPg) and *F. proliferatum* ITEM7595 (FPm), grown in Petri dishes on Potato Dextrose Agar with four biocontrol agents added (see Table 1 for details) in different temperature regimes. ($P < 0.05$. Standard error = 1.382)

T. gamsii (24.8%). In terms of radicle length, the control registered the shortest radicles (18.9 cm) whereas the longest radicles were measured in plants treated with Tebuconazole (+37%; Table 4).

The interaction between disease severity and the different treatments applied is shown in Fig. 5. Disease severity of positive control was higher than all the treated cloves and BCAs were generally less effective in controlling FPg compared to FPm and FO. Also, chemical products showed differences in controlling FPg and FPm and FO, with Propiconazole + Prochloraz being more effective against isolates from garlic

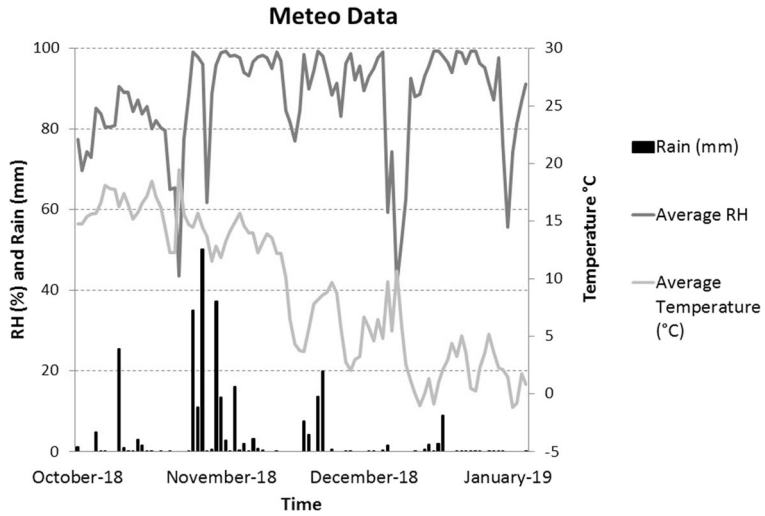

Fig. 4 Meteorological data collected between October 2018 and January 2019 using a data logger placed close to the pots used for the in vivo trial included in this study. (Temperature °C, relative humidity RH and millimeters of rain mm)

Table 4 Analysis of variance of disease severity (assessed according to the scale reported in Fig. 1) and radicle length of garlic plants grown in pots in open space from October 2, 2018 and January 7, 2019. Cloves were artificially inoculated before sowing by dipping in suspensions of five active ingredients, two chemicals (Propiconazole + Prochloraz and Tebuconazole) and three BCAs (*T. harzianum* + *T. gamsii*, *B. subtilis*, *S. griseoviridis*) and then in the conidial suspensions of three *Fusarium* spp. strains (one *F. oxysporum* isolated from garlic and two *F. proliferatum*, one of each isolated respectively from garlic and maize)

Factors	Severity		Radicle Length(cm)	
1. Strain	**		*	
***F. oxysporum* ITEM18686 (FO)**	24.2	*b*	21.5	*b*
***F. proliferatum* ITEM18687 (FPg)**	18.1	*a*	22.3	*ab*
***F. proliferatum* ITEM7595 (FPm)**	33.1	*c*	23.2	*a*
2. Treatment	**		**	
Positive Control [a]	65.6	*a*	18.9	*d*
Propiconazole+Prochloraz	11.1	*c*	21.1	*c*
Tebuconazole	12.3	*c*	25.9	*a*
S. griseoviridis	20.1	*bc*	22.3	*bc*
B. subtilis	17.0	*bc*	23.6	*b*
T. harzianum+T. gamsii	24.8	*b*	22.3	*bc*

[a] Positive control = cloves artificially inoculated with *Fusarium* spp., no fungicide application

** ($P < 0.01$), * ($P < 0.05$); Different letters indicate significant differences according to the Tukey test

compared to FPm isolated from maize (11.3–10.2% disease severity versus 11.8%). Radicle length was greatly affected by the interaction between the *Fusarium* strain-inoculated cloves and those treated with fungicide. The radicles were shorter in cloves inoculated with FO, except when treated with Tebuconazole, whereas they were the longest when the cloves were inoculated with FPm, irrespective of the fungicide applied.

Discussion

Garlic dry rot is mainly reported in literature as a post-harvest disease, but garlic infection starts in the field, and the first growth stages of the crop are crucial (Mondani et al. 2020; Mondani et al. 2021; Palmero et al. 2012; Stankovic et al. 2007). We also observed that apparently healthy cloves used in this study as negative control (neither treated nor inoculated) can develop symptoms during the early stages of garlic growth. This confirms the natural occurrence of Fusaria inoculum in visibly healthy cloves and the relevance of finding out effective seed treatments. Therefore, scoring the efficacy of seed treatment with fungicides as a preventive action against garlic dry rot in the first stages of crop growing was the aim of this study.

Triazoles are known for their capacity to reduce Fusaria growth, with LD 50 ranging from 0.24 to 6.5 mg/L and LD 90 of 10 mg/L (Marín et al. 2013; Müllenborn et al. 2008), by inhibiting the demethylation step in sterol biosynthesis in the cell membrane (Osborne and Scott 2018). They were considered in this study, aimed at controlling *F. proliferatum* and *F. oxysporum,* the former mentioned as the main causal agent of garlic rot and the latter also reported by some authors as involved in the disease (Gálvez Patón et al. 2017; Matuo et al. 1986; Tonti et al. 2012). BCAs were also selected in order to test the widest range of mechanisms of action against pathogens, so as to minimize the impact on the environment.

B. subtilis produces active compounds that inhibit plant pathogen growth and stimulate systemic responses in crops (Stein 2005) and has shown the capacity to reduce mycelial growth and sporulation in *F. graminearum* and *F. oxysporum* (Kim and Knudsen 2013; Zhao et al. 2014). *S. griseoviridis* stimulates plant systemic response, but it is also able to produce antibiotics and hydrolytic enzymes that attack cell membranes, as shown for *F. oxysporum* and *F. proliferatum* isolated from cucurbit plants (Zhao et al. 2013). Finally, fungal BCAs, *Trichoderma* spp. and *F. oxysporum,* compete for space and nutrients and can parasitize the pathogens (Kubicek et al. 2001). For example, Postma and Rattink (1992) were able to isolate non-pathogenic *F. oxysporum,* inoculated in the soil, in carnation stems up to 60 cm, while on the other hand *Trichoderma* spp. showed a capacity to parasitize isolates of *F. oxysporum* even 48 h after the inoculation in dual culture (Sharma 2011).

In this study, triazoles, in both in vitro and in vivo trials, showed the best performances in reducing Fusaria growth when compared to other chemicals or biological control agents. Tebuconazole was more effective than Propiconazole (GR 88.9% versus 76.4%), but the formulation Propiconazole + Prochloraz reduced the growth of Fusaria in vitro up to 100%. A relevant role was apparently played by Prochloraz; in fact, all the other products containing Triazoles in a mixture with other active ingredients, Prochloraz excluded, were less effective compared to active ingredient applied alone. Further, Ivic et al. (2011) applied Prochloraz in in vitro

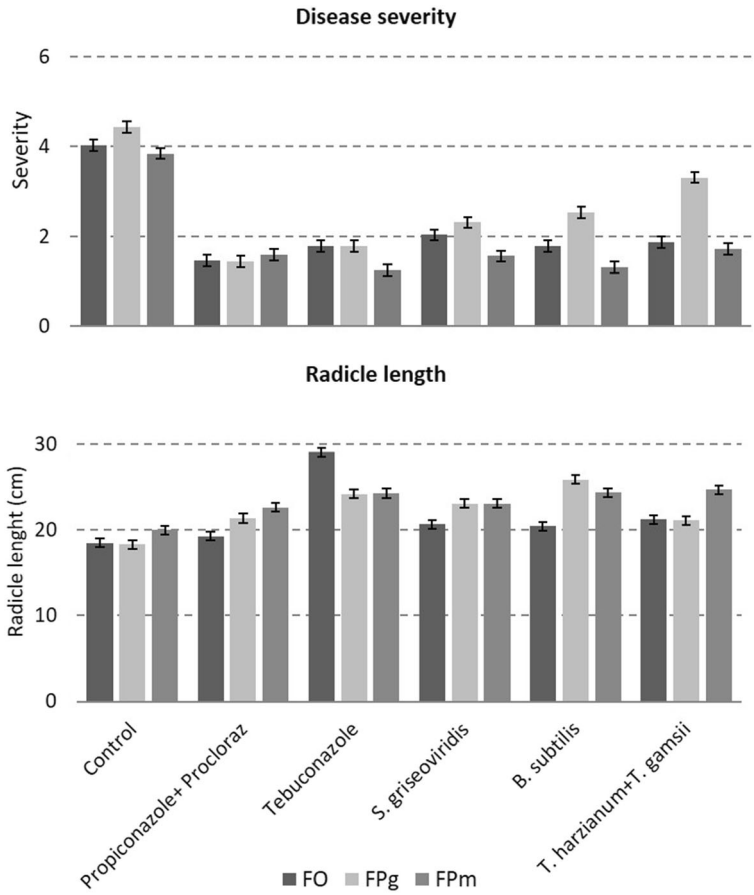

Fig. 5 Disease severity and radicle length (cm) of the three fungal strains, *F. oxysporum* ITEM18686 (FO), *F. proliferatum* ITEM18687 (FPg) and *F. proliferatum* ITEM7595 (FPm), tested in in vivo trial with six seed coating treatments. (Standard error disease severity = 1.791. Standard error radicle length = 0.511)

studies, and *F. verticilloides* growth inhibition was up to 96%.

Azoxystrobin was reported to be almost ineffective in inhibiting *Fusarium* growth in vitro (Müllenborn et al. 2008). The results collected in this in vitro trial confirm this statement; in fact, the mixture of Propiconazole and Azoxystrobin showed the worst performance in inhibiting Fusaria growth among the chemicals tested. The reduced efficacy of products containing Azoxystrobin applied to the cloves could be explained by its translaminar mobility, compared to other compounds, systemically moving in the plant (Ravichandra 2018).

The results obtained in vitro with BCAs are in accordance with literature reports (Elshahawy et al. 2017; Bjelić et al. 2018). A mean growth inhibition of 80% for *T. harzianum* + *T. gamsii* and 70% for *B. subtilis* is very promising, especially if we consider their thermal needs; in fact, those microorganisms can successfully overwinter and continue their action in the later stages of crop growth.

A step forward was achieved by sowing cloves, treated with the most promising fungicides, in pots in the open air. The chemicals performed better in terms of reduction of disease severity compared to BCAs (11.7% versus 18.6% disease severity, respectively with chemical and biological control), but Propiconazole + Prochloraz showed a side effect on radicle lengths, shorter compared to those measured in plants treated with the other chemical products (mean 21 cm versus mean 26 cm, with Propiconazole + Prochloraz and Tebuconazole respectively). Plants with a weak radical apparatus normally are more susceptible to diseases (Fageria 2012); therefore, this could be relevant in the field where the growing season is much longer than in this trial, around 270 versus 90 days, respectively.

BCAs involved in this study showed a similar capacity to control *F. proliferatum* in terms of the severity of symptoms on cloves, and they did not show negative effects on radicle length. *B. subtilis* and *S. griseoviridis* performances were comparable to those observed for chemicals, in terms of reduced disease severity, with mean values of 74% and 69% respectively. In vivo results seem in contrast with those obtained in vitro for

T. harzianum + *T. gamsii*: Fusaria growth was reduced by up to 85% in vitro, the best performance of the BCAs, but only 62% in pot trials, with final disease severity scored at 24.8%. During the pot experiment, T was below 10 °C for a long period and *T. harzianum* + *T. gamsii* showed the worst performance with this T in in vitro studies; this can justify the loss of performance. In addition, artificial culture media such as PDA are rich in sugar and nutrients, which are easily consumed by *Trichoderma* species; on the other hand, soil contained in pots was poorer in nutrients and organic matter, not giving BCAs a competitive advantage over the pathogen (Klein and Eveleigh 1998).

During the early stages of garlic development, water supply and mild T are required for rapid clove germination, but these conditions are also conducive for Fusaria. During our pot trial, T was higher than 10 °C until mid-November and a water supply was guaranteed by periodic rainfall. The rainiest period was between mid-October and the beginning of November, when the plants showed 3–4 visible leaves (BBCH13-BBCH14) and T was around 15 °C. In vitro, BCAs were more effective against *Fusarium* at 15 °C than at 10 °C and 25 °C; all the antagonistic species used in the study are known as mesophilic. *Trichoderma* has its optimum growth with T between 25 and 30 °C (Klein and Eveleigh 1998) and at 5–10 °C, its growth and secondary metabolite activity is reduced (Tronsmo and Dennis 1978). Bacterial BCAs showed a capacity to grow and multiply at a T of 4 °C (Berzina et al. 2016; Isnawati and Trimulyono 2018). The rapidity of bacterial cells to reproduce in favorable conditions could explain their effectiveness against *Fusarium* in the early stages of plant growth when T are around 10–15 °C. On the other hand, *Trichoderma* species are more sensible to low T and their activity is reduced in the early crop stages (Tronsmo and Dennis 1978).

In the present study, all the Fusaria strains caused symptoms on garlic seedlings, but FPg showed the highest severity index in in vivo trials, with symptoms on bulbs, supporting *F. proliferatum* as the main causal agent of dry rot in garlic in Europe (Stankovic et al. 2007; Palmero et al. 2010; Tonti et al. 2012; Mondani et al. 2020; Mondani et al. 2021). However, FPm, which was originally isolated from maize, was capable of infecting garlic plants, scoring a severity index of 18.1% versus 33.1%, confirming the non-host specificity of Fusaria, but also a certain adaptation to the crop (Proctor et al. 2010). Palmero et al. (2012) also demonstrated the capability of *F. proliferatum* strains isolated from garlic to cause disease in other *Liliaceae*. Onion registered the highest disease severity, followed by garlic, leek, chives, and scallion in artificial inoculation trials conducted by dipping the seedlings in conidial suspension before planting them in pots. This observation is highly relevant in the perspective of defining the best crop rotation and minimizing Fusaria inoculum in field soil.

Conclusions

Both BCAs and chemicals showed the capacity to greatly reduce *Fusarium* spp. growth in in vitro trials and the results were confirmed, even with reduced impact, in in vivo experiments. Moreover, the study demonstrated the efficacy of coating products in reducing *Fusarium* symptom severity in the early crop stages. As dry rot is mainly a matter of concern postharvest, it will be of great interest to investigate whether the effectiveness of these treatments lasts long enough to protect garlic until storage and market stage. This is a preliminary study, but it is mandatory to select the most promising active ingredients for garlic dry rot control. Further studies are ongoing to check the efficacy of fungicides used for seed coating during garlic cultivation and storage; if promising results are confirmed, they will hopefully contribute to support farmers in the management of garlic dry rot.

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Code availability Not applicable.

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Declarations

Ethics approval Not applicable, no humans or animals were used in this research.

Consent to participate Not applicable.

Consent for publication Not applicable.

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