Tech Science Press

Control Alternatives for Damping-Off in Tomato Seedling Production

A. C. Michel-Aceves[1], J. F. Díaz-Nájera[1], R. Ariza-Flores[2], M. A. Otero-Sánchez[1], R. Escobar-Martínez[1] and C. H. Avendaño-Arrazate[3,*]

[1]Centro de Estudios Profesionales del Colegio Superior Agropecuario del Estado de Guerrero, Av. Vicente Guerrero No. 81, Colonia Centro, Iguala, Guerrero, C.P. 40000, México.

[2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Iguala, Carr. Iguala-Tuxpan, Km. 2.5 Iguala, Guerrero, México.

[3]INIFAP-Campo Experimental Rosario Izapa. Carr. Tapachula-Cacahoatán, Tuxtla chico, Chiapas, C.P. 30870, México.

[*]Corresponding Author: C. H. Avendaño-Arrazate. Email: fitogeneticarlos@hotmail.com.

Abstract: In two tomato genotypes, we assessed control alternatives for damping-off with combinations of chemical fungicides and native/commercial strains of biological agents. Forty treatments consisting of 19 levels of mixing products, chemical fungicides, native strains and commercial products from biological control agents, and untreated treatment were used onto Ramsés and Toro hybrids. They were distributed on an incomplete block design in divided plots arrangement, where genotypes constitute the larger ones and the 8-repetition mixed products, the smaller ones. Putting 180 mL of fungal complexes, made of spores and mycellium Fusarium-solani (2 × 106 UFC), Rhizoctonia-solani (1 × 106 UFC), Phytophthora-capsici (1 × 105 UFC) and Sclerotium-rolfsii (mycellium-sclerotia) on each seedling trays, made inoculation possible. The mixtures of (Bacillus spp. + Streptomyces spp. + Trichoderma spp.) + (Propamocarb + Fosetyl); (Bacillus spp. + Streptomyces spp. + Trichoderma spp.) + (Metalaxyl + Chlorothalonil); Pseudomonas fluorescens + Streptomyces + Micromonospore + Sporideamium + Aminoacidos, Péptidos, Carbohidratos) + (Propamocarb + Fosetyl); the native strain of Trichoderma asperellum + (Propamocarb + fosetil) and the native strains Trichoderma asperellum + Bacillus subtilis, diminished damping-off, prevented its appearance and had most significant agronomic characteristics. In contrast to this, combination of (Mancozeb) + (Free iodine) + (Metalaxyl + Chlorothalonil) + (Methyl thiophanate) produced some toxicity in the plant. In addition, Ramsés presented the best agronomic parameters, while Toro had the utmost fresh and dry weight in root.

Keywords: Trichoderma harzianum; T. lignorum; biological control

1 Introduction

If we consider the number of hectares sown and consequently its production value, tomato (*Solanum esculentum* L.) is the most cultivated and economically important vegetable worldwide [1]. It occupies a land surface of 5.023.814 ha that contributes to a global production of 177.042.359 t; from which Mexico provides 388.435 t [2]. In recent years, annual production has grown because of an increase in the yield rate and cultivable land [3].

This crop faces numerous phytosanitary issues from early to advanced stages of the production system; the main limiting factors for tomato production are diseases caused by fungi. Seedling seedbeds must be kept protected until they are transplanted into definitive soil. In the southern region, like in other parts of Mexico, the production of tomato seedlings deals with some sanitation difficulties given by these fungi genera: *Phytophthora, Sclerotium, Fusarium, Pythium* and *Rhizoctonia*, among others [4]. They are the main soil-borne pathogens that end up causing root/collar rotting and more than 50% of severe damage [5].

Today, most diseases are being controlled with synthetic fungicides, but their indiscriminate usage has had environmental impacts, caused toxicity in men, and induced pathogens' resistance. Consequently, their efficiency lessen due to phytopathogens constant evolution; they increase production costs. Seeking for more efficient and low-cost alternatives for fungi control are being suggested [6].

The tendency to disease control without using chemical products has increased, due to capacity of parasites compete for nutrients or produce compounds opposing to a great variety of fungi genera *Rhizoctonia, Fusarium* and *Pythium* [7,8]; for example: the use of beneficial microorganisms as a way to biocontrol diseases [9], especially fungi: *Trichoderma, Gliocladium, Coniothyrium* and *Candida,* and bacteria: *Bacillus, Streptomyces, Pseudomonas* and *Agrobacterium* [10,11]. The use of isolated strategies had low impact, for that reason, it is very important and necessary to implement integrated management strategies [12]. Based on this, it was put forward to evaluate the effectiveness of damping-off disease management when utilizing, in two commercial tomatoes hybrids, sole third-generation chemical fungicides and a mixing of native/commercial strains from biological control agents.

2 Material and Methods

2.1 Site of Study

The investigation was established in a greenhouse dedicated to seedling crops. It is located in the district of Tonatico, Estate of Mexico, that has subtropical climate, an average temperature of 20.5°C a year, and rainfall from June to September with 1199 mm in the year, Latitud 18°80 28" Longitud 99°.67 00" [13].

2.2 Genetic Material

Two hybrid genotypes of tomatoes were used: 1. Ramsés F1 (Harris Moran Seed Compañy), is of indefinite growth, energetic, adapts to both indoor and outdoor production with excellent quality and resistance throughput. It gives Saladette fruit-like, XL, firmness with high resistance to *Fusarium oxysporum f*. sp *lycopersici* races 1, 2 and 3, to powdery-mildew (*Leveillula taurica*), to tomato mosaic virus, root-knot (*Meloidogyne* spp.), and verticillium wilt (*Verticillum albo-atrum, V. dahlie*). 2. Toro (Harris Moran Seed Compañy) is of determined growth, has great reproductive ability, and early maturing (it harvests begin after 100 days of sowing). Produced big and oblong fruit (120 g of weigth) with pronounced firmness and bright red color. It shows good tolerance to heat, bacterial stain *(Xanthomonas vesicatoria)* and to early smut *(Alternaria solani)*. It is highly resistant to *Fusarium oxysporum* f. sp *lycopersici* races 1, 2, 3; root-knot nematode (*Meloidogyne* spp.) and verticillium wilt (*Verticillum albo-atrum, V. dahlie*) [14].

2.3 Factors and Study of Treatments

Two factors were studied: genetic material with the hybrids (Ramsés and Toro) and disease management with chemical fungicides, native *strains* of fungi or bacteria, and commercial products from biological control agents resulting in 40 treatments (Tab. 1).

2.4 Design and Experimental Unit

In 200 cell trays, treatments were distributed on an incomplete block design in split plot arrangement. In big plots, genotypes were arranged as randomized blocks, but in smaller plots, disease management were onto a complete 8-repetition randomized design. The experimental unit consisted of 20 plants per replicate, five of them were randomly selected for variables data. Eight seedling trays were used for each genotype, so that the 19 mixing products and the absolute control with eight replicates could be applied (Tab. 1).

Table 1: Treatments by genotype used in this investigation, including active ingredient and doses

No.	Active ingredient	Dose
T1	(*Trichoderma virens* strain GL-21) + (Propamocarb + Fosetyl) + (Quintozeno)	(17.5 g + 5.6 mL + 32.5 mL)
T2	(*Trichoderma virens* strain GL-21) + (Metalaxyl + Chlorothalonil) + (Quintozeno)	(17.5 g + 3.5 g + 32.5 mL)
T3	(*Trichoderma virens* strain GL-21) + (Benomil) + (Quintozeno)	(17.5 g + 0.5 g + 32.5 mL)
T4	(*Trichoderma virens* strain G-41) + (Propamocarb + Fosetyl) + (Quintozeno)	(0.5 g + 5.6 mL + 32.5 mL)
T5	(*Trichoderma virens* strain G-41) + (Metalaxyl + Chlorothalonil) + (Quintozeno)	(0.5 g + 3.5 g + 32.5 mL)
T6	(*Trichoderma virens* strain G-41) + (Benomil) + (Quintozeno)	(0.5 g + 0.5 g + 32.5 mL)
T7	(Mancozeb) + (free iodine) + (Propamocarb + Fosetyl) + (Methyl thiophanate) + (*Bacillus subtilis* strain ULTAQ-07).	(6.25 g + 1.5 mL + 5.6 mL + 2.12 g + 7.5 mL)
T8	(Mancozeb) + (free iodine) + (Propamocarb + Fosetyl) + (Methyl thiophanate) + (*Bacillus subtilis* strain QST 713)	(6.25 g + 1.5 mL + 5.6 mL + 2.12 g + 3.0 g)
T9	(Mancozeb) + (free iodine) + (Metalaxyl + Chlorothalonil) + (Methyl thiophanate)	(6.25 g + 1.5 mL + 3.5 g + 2.12 g)
T10	(Azoxistrobin) + (Methyl thiophanate) + (*Bacillus subtilis* strain ULTAQ-07)	(2.0 g + 2.12 g + 7.5 mL)
T11	(Azoxistrobin) + (Methyl thiophanate) + (*Bacillus subtilis* strain QST 713)	(2.0 g + 2.12 g + 7.5 mL)
T12	(*Bacillus* spp.+*Streptomyces* spp. + *Trichoderma* spp.) + (Propamocarb + fosetil)	(1.88 g + 5.6 mL)
T13	(*Bacillus* spp.+*Streptomyces* spp. + *Trichoderma* spp.) + (Metalaxyl + Chlorothalonil)	(1.88 g + 3.5 g);
T14	*(Pseudomonas fluorescens* + *Streptomyces* + *Micromonospore* + *Sporideamium* + Amino Acids, Peptides, Carbohydrates) + (Propamocarb + Fosetyl)	(0.5 mL + 5.6 mL);
T15	*(Pseudomonas fluorescens* + *Streptomyces* + *Micromonospore* + *Sporideamium* + Amino Acids, Peptides, Carbohydrate) + (Cymoxanil)	(0.5 mL + 6.25 g)
T16	(Native strain of *Trichoderma* asperellum) + Native strain of *Bacillus subtilis*	(1×10^8 UFC + 1×10^8 UFC)
T17	(Native strains of *Trichoderma* asperellum) + (Propamocarb + fosetil)	(1×10^8 UFC + 5.6 mL)
T18	(Native strain of *Trichoderma* asperellum) + (Cymoxanil)	(1×10^8 UFC + 6.25 mL);
T19	(Native strains of *Bacillus subtilis*) + (Propamocarb+Fosetyl) + (Methyl thiophanate)	(1×10^8 UFC + 5.6 mL + 2.12 g)
T20	Absolute control	without application of products

2.5 Experiment Setup

Seedling trays were immersed in 0.1% of sodium hypochlorite commercial solution for their sterilization and then put to dry. Subsequently, the substrate used to fill them in consisted of: Sunshine Mix 3, a mix containing 27.2 kg of peat that integrates a uniform combination of moss (*Sphagnum canadiense)* and added with carbonates to adjust pH (5.9 a 6.2). It was mixed with 13 kg of Vermiculite or peat moss. Finally, it was mixed with 10 kg of Humibac, an organic fertilizer made of bovine manure. And subsequently, trays'cells were completely filled.

Seeds were deposited at a depth of 1.5 cm and covered with vermiculite to keep humidity and allow emergence. Trays were placed in a dark room for four days at 25 ± 1°C. But at the time of emerging, they were moved to a greenhouse for carrying out seedlings management. The growing conditions of the greenhouse were 25 ± 1°C and 70% of relative humidity. Plants were daily irrigated using a finely watering can and fertilized with Hakaphos Violeta in doses of 0.5 g L^{-1} mixed with irrigation water.

2.6 Application in the Management and Inoculation of Treatments for Phytopathogens

After ten days, treatments on seedlings with true leaves, for this, we used the recommended average dose (Tab. 1). Two days after the mixing of products was applied, a suspension of spores was inoculated with concentrations of Fusarium *solani:* 1.8×10^6 UFC, *Rhizoctonia solani*: 1.0×10^6 UFC, *Phytophthora capsici*: 1×10^5 UFC. But to *Sclerotium rolfsii*, a mycelium suspension and one thousand sclerotia was prepared. We put on each tray, 180 mL of the fungi complex directly on plants' stalks. Biological commercial products are formulated as powders and the natives strains spores are developed in rice grain.

2.7 Variables of Study

Were quantified twenty days after phytopathogens inoculated: seedlings height, number of leaves, dry weight of root, disease severity, disease necrosis, and presence of damping-off. For the evaluation of severity and necrosis, the scale proposed by St-Arnaud et al. [15], was used. The values of the scale range from 0 to 5.

Statistical procedures were performed using the statistical software SAS (version 9.1; SAS Institute, Cary, NC), to analysis of variance (ANOVA) and Tukey followed by a least significant difference (LSD) test ($p = 0.05$).

3 Results and Discussion

3.1 Plant Height

Ramsés hybrid showed to have the most plant height with 9.15 cm ($P > 0.0002$). Average values within the disease management factor ranged from 4.0 to 14.6 cm; Tukey test separate by levels ($P > 0.0001$), where combinations of (*Bacillus* spp.+ *Streptomyces* spp. + *Trichoderma* spp.) + (Metalaxyl + Chlorothalonil) (T13) showed to have higher efficiency (Fig. 1), and surpassed the whitness by 34.9%. Meanwhile, the mixture of (Mancozeb) + (Free idoine) + (Metalaxyl + Chlorothalonil) + (Methyl thiophanate) (T9) reflected the lowest value that was surpassed by the whitness.

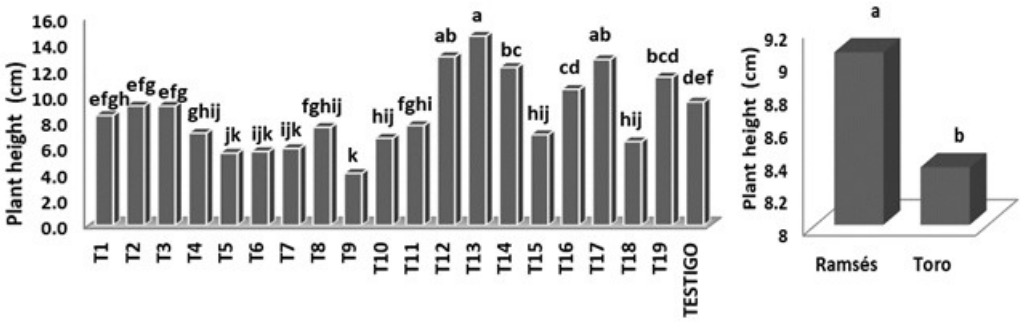

Figure 1: Plant height (cm) based on management and genotypes

In a greenhouse [16,17], assessed *Trichoderma in vitro* strains as bio-controller agents of *R. solani* and *S. rolfsii*. *T. asperellum* species exercised better control over phytopathogens. In the same way [18,19], studied the bio-controlling effect of various species of *Trichoderma,* and reported that *T. asperellum* was the most effective in controlling *R. solani,* as against RIDOMIL GOLD® (Metalaxyl+Chlorothalonil) fungicide. Hu et al. [20], point out that Propamocarb and Fosetyl have good curative and protective effects against oomycetes, as found in *P. capsici*, that's being back up in the current investigation. In synthetic fungicides with the best disease management efficiency were (RIDOMIL GOLD® (Metalaxyl + Chlorothalonil) + PREVICUR®ENERGY (Propamocarb + Fosetyl)), Matheron & Porchas [21] proved that Fosetyl suppress sporangium formation, motility, zoospores and clamidospores germination; and has a very high efficacy activity, just like with Metalaxyl [22]. It also reduces *Phytophthora* spp destructive ability. *T. asperellum* species have been utilized with success in managing soil-borne phytopathogens (*R. solani*, *Fusarium* spp. *P. capsici* & *S. rolfsii*), either in greenhouses or in the field [23,7,24]. Besides, the *T. asperulum* native strain represents an alternative to control diseases.

Ming et al. [25] state that *Bacillus subtilis* holds great potential as bio-controller because of the production of secondary metabolites with antifungal activity. Just the same, Zhu et al. [26] mention that *Streptomyces* species produce prime chitinolytic enzymes that are responsible for the excellent control

over phytopathogens of fungi. In this trial, the PHC® BIOPAK-F® commercial product, formed with different strains of *Bacillus* and *Streptomyces,* exerted good greenhouse control. It has been reported that Fosetil-al holds a great systematic activity and their efficacy against oomycetes is highly superior which generates great control; and Metalaxyl too [20].

3.2 Dry Weight of Root

Toro hybrid showed the average of dry weight in root with 0.64 g (Fig. 2), which was superior to the one in Ramsés ($P > 0.0001$). Average values ranged from 9 to 88 mg ($P > 0.0001$). The mix of T12 (*Bacillus* spp. + *Streptomyces* spp. + *Trichoderma* spp.) + (Propamocarb + Fosetyl) had the greater biomass of dehydrated root exceeding the withness by 50%.

The microorganisms included in PHC BIOPACK-F® are efficient due to bacteria as they exceptionally suppressed the development of phytopathogenic fungi. The species belonging to the *Bacillus* genus form endospores tolerant to external climate changes [27]. Just the same, *Streptomyces* have proven that the production of antifungal compounds, both *in vitro* and field trials, are capable of inhibit *S. rolfsii* and improve vegetative growth [28]. Moreover, the potential of *Trichoderma* spp. provides protection against *Phytophthora* spp and induce resistance in plants as a defense mechanism to *Trichoderma* colonization [29].

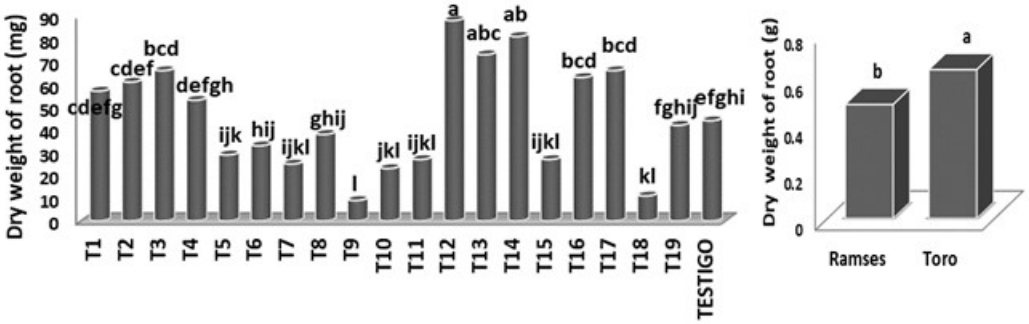

Figure 2: Dry weight of root based on management factor and genotype

3.3 Disease Severity

Genotypes presented similar severities, for instance; Ramsés hybrid has in average of 1.55 to the utilized scale ($P > 0.2144$) and Toro with 1.46 (Fig. 3). In the management, the mix of (Mancozeb) + (Free iodine) + (Metalaxyl + Chlorothalonil) + (Methyl thiophanate) (T9) exhibit a valor to the 4.0 of the utilized scale ($P > 0.0001$); which indicated the severe damages done to the stalk by fungi action. We found, low severity in eleven combinations with values of 1.1 having good protection.

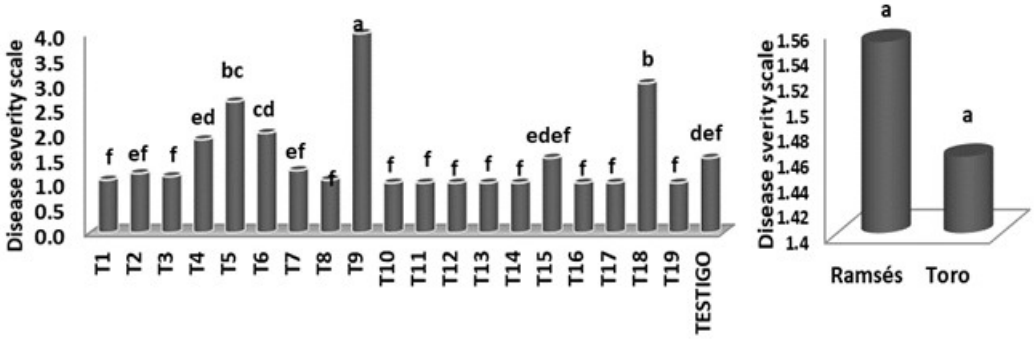

Figure 3: Disease severity based on management factor and genotypes

We support the efficiencies of the commercial biological product PHC BIOPACK-F, for containing gram positive bacteria (*Bacillus* and *Streptomyces* spp.), which produce spores resistant to heat and desiccation that can be made as powder, and are compatible to other products [30]. *Trichoderma* spp., secrete various compounds that induce systematic resistance in plants, among them there's ethylene and jasmonic/salicylic acids [31].

3.4 Disease Necrosis

Ramsés hybrid show higher average of necrosis with 2.08 to the utilized scale ($P > 0.0059$), than Toro hybrid (Fig. 4). As a result of the mixtures and their interactions, Tukey test separated by levels, from which (Mancozeb) + (Free iodine) + (Metalaxyl+Chlorothalonil) + (Methyl thiophanate) (T9) and native strain of *Trichoderma asperellum* + Cymoxanil (T18) were not efficient since they presented greater necrotic lesions with a valor of 4.5 to the utilized scale. Therefore, mixtures with biological agents were efficient with values of 1.0 (Fig. 4), from which the control achieved 1.5 with some damage in the root 1 to 3%.

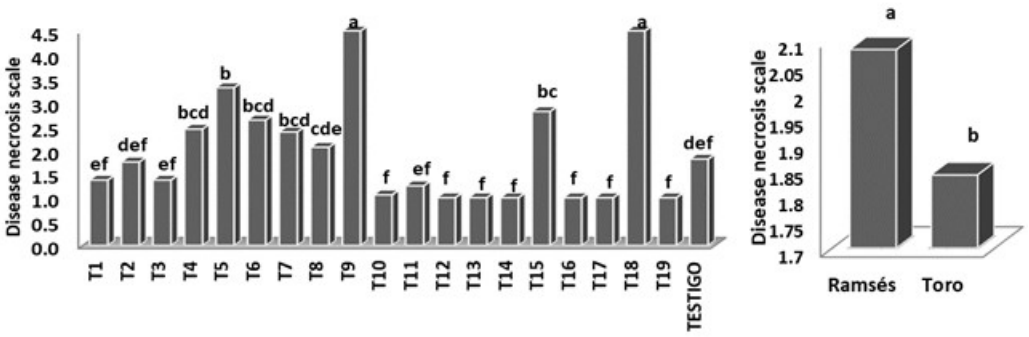

Figure 4: Disease necrosis based on management and genotype factors

In this study, the commercial product of PREVICUR® ENERGY (Propamocarb+Fosetyl), exerted better control with healthy roots. These results coincide to what was found in other studies; they mention this product can efficiently control *Phytophthora* spp. [32]. *Streptomyces* spp., are a group of microorganisms being taken advantage of in the production of secondary metabolites and enzymes of commercially importance for biological control of diseases and for other agricultural purposes [33]. It has been described that species of *Trichoderma* secrete extracellular compounds like gliotoxins, antibiotics, and enzymes for being effective in pathogens elimination [34].

3.5 Damping-off Presence

Ramsés hybrid was the most susceptible because the disease appeared after 0.87 days ($P > 0.0002$), while Toro delay it after 1.25 days (Fig. 5). In regards to management, it should be said that the combinations of products in treatments T12, T13, T14, T16, T17, and T19 were effective because there was no presence of the disease ($P > 0.0001$). From those that did present disease, Tukey test separated the levels, where the mixture of (Azoxistrobin) + (Methyl thiophanate) + (*Bacillus subtilis* strain ULTAQ-07) (T10) exhibited earlier symptoms after 0.19 days. But the *Trichoderma* asperellum + Cymoxanil (T18) native strain was the most tardive with an average of 2.69 days after fungi inoculation (Fig. 5).

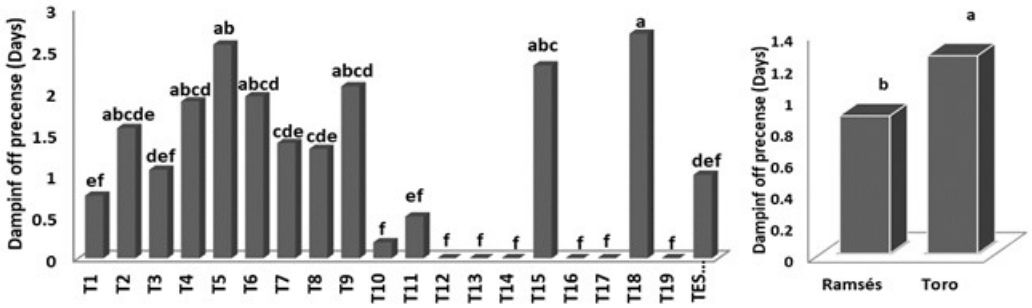

Figure 5: Damping-off presence based on management factor and genotype

The β-glucanase and cellulase of *B. subtilis* and *B. licheniformis* are substances of deterrent activation, as they hydrolized *P. capsici* cell wall. Both strains produce siderophore iron chelators in the soil, resulting in the depletion of iron, which blocks pathogens to grow [35]. *B. subtilis* have proven their effectiveness in controlling *Rhizoctonia* and *Fusarium* [36]. There are several isolated antifungal actives known that have been characterized from *Streptomyces* spp. [37]. This backs up the efficient usage of *Streptomyces* in the phytopathogens biocontrol [32]. All previous characteristics, make these biocontrol agents reliable and their efficacy is maximized if they are used as mixture. A commercial alternative is PHC BIOPACK-F and TRAZAK, as well as the *T. asperellum* native strain. These were used for this trial conducted in Cocula, Gro., with efficient results, achieving seedlings vigorius and healthy [38].

4 Conclusions

Some commercial biological control agents and native strains mixed with synthetic fungicides have efficient results. Mixtures of (*Bacillus* spp. + *Streptomyces* spp. + *Trichoderma* spp.) + (Propamocarb + Fosetyl) (T12), (*Bacillus* spp. + *Streptomyces* spp. + *Trichoderma* spp.) + (Metalaxyl+Chlorothalonil) (T13), *Pseudomonas fluorescens* + *Streptomyces* + *Micromonospore* + *Sporideamium* + Aminoacidos, Péptidos, Carbohidratos) + (Propamocarb+Fosetil) (T14) the native strain *Trichoderma asperellum* + (Propamocarb+Fosetyl) (T17) and the *Trichoderma asperellum* + *Bacillus subtilis* native strains (T16) achieved better management for damping-off, as they prevent its presence with protruding agronomical characteristics. The mixture of (Mancozeb) + (Iodo libre) + (Metalaxyl+Chlorothalonil) + (Methyl thiophanate) generated phytotoxicity.

Ramsés hybrid presented better plant height, number of leaves, and collar diameter; but Toro achieved greater values of dry weight in root. As compared to Toro, Ramsés showed the highest rates for severity and necrosis, however; the disease appeared first in the Toro hybrid.

References

1. Balcha, K., Belew, D., Nego, J. (2015). Evaluation of tomato (*Lycopersicon esculentum* Mill.) varieties for seed yield and yield components under Jimma condition, South Western Ethiopia. *Journal of Agronomy 14(4),* 292-297.
2. FAOSTAT (2018). Datos estadísticos de la producción mundial de tomate. Obtenido de la red. http://www.fao.org/faostat/en/#data/QC.
3. González, A. A., González, C. A., Del Pozo, N. E., Galban, P. B., Domínguez, B. C. et al. (2009). Alternativas para el manejo de *Bemisia* spp., en *Solanum melongena* (l), en el Valle de Culiacán, Sinaloa. *Revista UDO Agrícola, 9(3),* 571-578.
4. Velásquez-Valle, R., Amador-Ramírez, M. D., Medina-Aguilar, M. M., Lara-Victoriano, F. (2007). Presencia de patógenos en almácigos y semilla de chile (Capsicum annuum L.) en Aguascalientes y Zacatecas, México. *Revista Mexicana de Fitopatología, 25,* 75-79.

5. Cruz, O. J., García, E. R. S., Carillo, F. A. (1998). Enfermedades de las hortalizas. Universidad Autónoma de Sinaloa. Culiacán Rosales, Sinaloa, México.
6. Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M. et al. (2002). The strobilurin fungicides. *Pest Management Science, 58(7),* 649-662.
7. Michel, A. A. C., Otero, S. M. A., Solano, P. L. Y., Ariza, F. R., Barrios, A. A. et al. (2009). Biocontrol *in vitro* con *Trichoderma* spp. de *Fusarium subglutinans* (Wollenweb. y Reinking) Nelson, Toussoun y Marasas y *F. oxysporum* Schlecht., agentes causales de la "escoba de bruja" del mango (*Mangifera indica* L.). *Revista Mexicana de Fitopatología, 27,* 18-26.
8. Michel-Aceves, A. C., Otero-Sanchez, M. A., Díaz-Castro, A., Martínez-Rojero, R. D., Ariza-Flores, R. et al. (2013). Biocontrol de la "Escoba de bruja" del mango, con *Trichoderma* spp en condiciones de campo. *Revista Mexicana de Fitopatología, 31(1),* 1-12.
9. Yang, M. M., Xu, L. P., Xue, Q. Y., Yang, J. H., Xu, Q. et al. (2012). Screening potential bacterial biocontrol agents towards *Phytophthora capsici* in pepper. *European Journal of Plant Pathology, 134,* 811-820.
10. Harman, G. E., Obregón, M. A., Samuels, G. J., Lorrito, M. (2010). Changing models for commercialization and implementation of biocontrol in the developing and the developed world. *Plant Disease, 94(8),* 928-939.
11. Pliego, C., Ramos, C., Vicente, A., Cazorla, F. M. (2011). Screening for candidate bacterial biocontrol agents against soilborne fungal plant pathogens. *Plant Soil, 340,* 505-520.
12. Bi, Y., Jiang, H., Hausbeck, M. K., Hao, J. J. (2012). Inhibitory effects of essential oils for controlling *Phythophthora capsici. Plant Disease Journal, 96(6),* 797-803.
13. Anonymous (2018). Datos estadísticos del clima de Tonatico, Estado de México. Obtenido de la red. https://es.climate-data.org/america-del-norte/mexico//tonatico-213735.
14. Anonymous (2017). Harris Moran Seed Company, Inc. Híbridos de tomate utilizados. Obtenido en la red. http://www.ahernseeds.com/?s=harris+moran&post_type=products&x=0&y=0&gclid=EAIaIQobChMIxZ7b0tLb4wIVEVYMCh1RLgM8EAAYASAAEgINuPD_BwE&lang=es.
15. St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. (1994). Incidence of *Pythium ultimum* in roots and growth substrace of mycorrhizal *Tagets patula* colonized with *Glomus intraradices*. *Plant Pathology, 16,* 187-194.
16. Correa, S., Mello, M., Ávila, Z. R., Minaré, B. L., Pádua, R. R. et al. (2007). Cepas de *Trichoderma* spp., para el control biológico de *Sclerotium rolfsii* Sacc. *Fitosanidad, 11(1),* 3-9.
17. Vargas, H. H. A, Rueda, L. E. A., Gilchrist, R. E. (2012). Actividad antagónica de *Trichoderma asperellum* (fungi: ascomycota) a diferentes temperaturas. *Actualidades Biológicas, 34(96),* 103-112.
18. Infante, D., Martínez, B., Peteira, B., Reyes, Y., Herrera, A. (2013). Identificación molecular y evaluación patogénica de trece aislamientos de *Trichoderma* spp. frente a *Rhizoctonia solani* Kühn. *Biotecnología Aplicada, 30,* 17-22.
19. De França S. K. S., Cardoso, A. F., Castro, D. L., Soares, R. E. M. L., Corsi, D. M. C. et al. (2014). Biocontrol of sheath blight by *Trichoderma asperellum* in tropical lowland rice. *Agronomy for Sustainable Development*, vol. 35, no. 1, pp. 317-324.
20. Hu, J., Hong, C., Stromberg, E. L., Moorman, G. W. (2007). Effects of propamocarb hydrochloride on mycelial growth, sporulation, and infection by *Phytophthora nicotianae* isolates from Virginia nurseries. *Plant Disease, 91,* 414-420.
21. Matheron, M. E., Porchas, M. (2000). Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. *Plant Disease, 84,* 454-458.
22. Gent, D. H., Ocamb, C. M., Farnsworth, J. L. (2010). Forecasting and management of hop downy mildew. *Plant Disease, 94,* 425-431.
23. Reyes, Y., Martínez, B., Infante, D. (2008). Evaluación de la actividad antagónica de trece aislamientos de *Trichoderma* spp. sobre *Rhizoctonia* sp. *Revista. Protección Vegetal, 23(2),* 112-117.
24. Infante, D., González, N., Reyes, Y., Martínez, B. (2011). Evaluación de la efectividad de doce cepas de *Trichoderma asperellum* Samuels sobre tres fitopatógenos en condiciones de campo. *Revista. Protección Vegetal, 26(3),* 194-197.
25. Ming, M. Y., Liu, P. X., Qing, Y. X., Jing, H. Y., Quan, X. et al. (2012). Screening potential bacterial biocontrol agents towards *Phytophthora capsici* in pepper. *European Journal of Plant Pathology, 134,* 811-820.

26. Zhu, Y., Pan, J., Qiu, J., Guan, X. (2008). Isolation and characterization of a chitinase gene from entomopathogenic fungus *Verticillium lecanii*. *Brazilian Journal of Microbiology, 39,* 314-320.
27. Chung, S., Lim, H. M., Kim, S. D. (2007). Formulation of stable *Bacillus subtilis* AH18 against temperature fluctuation with highly heat-resistant endospores and micropore inorganic carriers. *Applied Microbiology and Biotechnology, 76,* 217-224.
28. Errakhi, R., Bouteau, F., Lebrihi, A., Barakate, M. (2007). Evidences of biological control capacities of *Streptomyces* spp. against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (Beta vulgaris L.). *World Journal Microbiology Biotechnology, 23,* 1503-1509.
29. Bae, H., Roberts, D. P., Lim, H. S., Strem, M. D., Park, S. C. et al. (2011). Endophytic *Trichoderma* Isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in Hot Pepper using multiple mechanisms. *Molecular Plant Microbe Interactions, 24(3),* 336-351.
30. Poleatewich, A. M., Ngugi, H. K., Backman, P. A. (2012). Assessment of application timing of *Bacillus* spp. suppress pre- and postharvest diseases of apple. *Plant Disease, 96,* 211-220.
31. Miklis, M., Consonni, C., Bhat, R. A., Lipka, V., Schulze-Lefert, P. et al. (2007). Barley MLO modulates actin-dependent and actin-independent antifungal defense pathways at the cell periphery. *Plant Physiology, 144,* 1132-1143.
32. Johnson, D. A., Cummings, T. F., Geary, B. (2000). Post infection activity of selected late blight fungicides. *Plant Disease, 84,* 1116-1120.
33. Narayana, K. J. P., Vijayalakshmi, M. (2009). Chitinase production by *Streptomyces* sp. Anu 6277. *Brazilian Journal of Microbiology, 40,* 725-733.
34. Montealegre, J., Valderrama, L., Sánchez, S., Herrera, R., Besoain, X. et al. (2010). Biological control of *Rhizoctonia solani* in tomatoes with *Trichoderma harzianum* mutants. Electronic *Journal of Biotechnology, 13(2),* 1-11.
35. Kloepper, J. W., Ryu, C. M., Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology, 94,* 1259-1266.
36. Zhang, J. X., Xue, A. G., Tambong, J. T. (2009). Evaluation of seed and soil treatments with novel *Bacillus subtilis* strains for control of soybean root rot caused by *Fusarium oxysporum* and *F. graminearum*. *Plant Disease, 93,* 1317-1323.
37. Rodríguez, L., Aguirrezabalaga, I., Allende, N., Braña, A. F., Méndez, C. et al. (2002). Engineering deoxysugar biosynthetic pathways from antibiotic-producing microorganisms: a tool to produce novel glycosylated bioactive compounds. *Chemistry and Biology, 9,* 721-729.
38. Díaz-Nájera, J. F. (2013). Etiología y manejo de hongos causantes de la pudrición de frutos en calabaza pipiana (*Cucurbita argyrosperma* Huber). Tesis de Maestría en Ciencias. Departamento de Parasitología. Universidad Autónoma Chapingo, Chapingo, Edo. de México.