Artículo Original /Original Article
DOI:https://doi.org/10.56152/StevianaFacenV14N1A4_2022

Effectiveness of biological, botanical and synthetic products in the control of onion (*Allium cepa*) wilt caused by *Fusarium* sp. Resources for integrated management.

Díaz-Nájera, J. F.[1]; Ayvar-Serna, S.[1]; Vargas-Hernandez, M.[2]; Sanabria-Velázquez, A. D.[3]; Cerezo-Aparicio, C. M.[1]; Enciso-Maldonado, G. A.[3]*

[1]Departamento de Fitotecnia, Centro de Estudios Profesionales del Colegio Superior Agropecuario del Estado de Guerrero, Cocula, México
[2] Departamento de Suelos, Universidad Autónoma Chapingo, Texcoco, México
[3]Centro de Desarrollo e Innovación Tecnológica, Hohenau, Paraguay
* E-mail del autor: gui77eenciso@gmail.com

Efectividad de productos biológicos, botánicos y sintéticos en el control de la marchitez de la cebolla (*Allium cepa*) causada por *Fusarium* sp. Recursos para una gestión integrada.. La marchitez de la cebolla puede reducir los rendimientos hasta un 25-30%. A partir de plantas de cebolla con síntomas de marchitez, se obtuvo un aislado monospórico del hongo *Fusarium* spp. y se verificó su patogenicidad. El hongo fue identificado morfológica y molecularmente mediante la región ITS como *Fusarium* sp. Posteriormente, se determinó la susceptibilidad del patógeno a pesticidas biológicos, botánicos y químicos a través de cuatro bioensayos. En el experimento I, se evaluó la antibiosis de *Trichoderma* spp. *in vitro* mediante la técnica del celofán. En los experimentos II y III, se evaluó el efecto *in vitro* de fungicidas botánicos y químicos mediante la técnica de cultivo en agar papa dextrosa modificada (NeemAcar®). En el experimento IV, se seleccionaron los mejores tratamientos de los ensayos *in vitro* para conocer su efecto en invernadero. *Trichoderma virens* (PHC Root Mate®) inhibió el 33,3% del crecimiento micelial del patógeno. El extracto de canela-neem (NeemAcar®) en dosis de 0,06, 0,08 y 0,10 mL L^{-1} inhibió en un 100% el crecimiento micelial del patógeno. Los fungicidas benomyl, prochloraz y pyraclostrobin suprimieron por completo el desarrollo del patógeno. En condiciones de invernadero, la incidencia de marchitez de las plantas tratadas con *Trichoderma* spp. y los extractos botánicos aplicados individualmente y en combinación no presentaron diferencias significativas con los fungicidas químicos. Estos resultados permitirán desarrollar futuros programas de manejo integrado de enfermedades para la marchitez de la cebolla.
Palabras clave: *Allium cepa* L., *Trichoderma* spp., extractos vegetales, fungicidas químicos

Effectiveness of biological, botanical and synthetic products in the control of onion (*Allium cepa*) wilt caused by *Fusarium* sp. Resources for integrated management. Onion wilt is an endemic disease in farms of horticulture production in Michoacán, Mexico. This disease can reduce yields by up to 25-30%. A monosporic isolate of the fungus *Fusarium* spp. was obtained from onion plants with wilting symptoms, and its pathogenicity was verified. The fungus was morphologically and molecularly identified using the ITS region of reference. Also, *Fusarium* sp. susceptibility to biological,

Steviana, Vol. 14 (1), 2022 pp.32-42
Original recibido el 30/04/2022
Aceptado el 13/11/2022

botanical, and chemical pesticides was assessed through four different bioassays. In experiment I, *Trichoderma* spp. *in vitro* antibiosis was tested using the cellophane technique. In Trials II and III, the *in vitro* effect of botanical and chemical fungicides on the pathogen was evaluated through the amended potato dextrose agar (PDA) culture technique. In Trial IV, the efficacy of the products selected during *in vitro* assays was evaluated under greenhouse conditions. During the antibiosis trials, the commercial strain of *Trichoderma virens* (PHC Root Mate®) inhibited 33.3% of *Fusarium* sp. mycelial growth. Among botanical pesticides, cinnamon-neem extract (NeemAcaⁱ®) at doses of 0.06, 0.08- and 0.10-mL L^{-1} inhibited 100% mycelial growth of *Fusarium* sp. The fungicides benomyl, prochloraz, and pyraclostrobin completely suppressed *Fusarium* sp. development. Under greenhouse conditions, the incidence of wilt of plants treated with *Trichoderma* spp. and botanical extracts applied individually and in combination, did not present significant differences from plants treated with chemical fungicides. These results will help to develop an integrated disease management program for onion wilt.
Keywords: *Allium cepa* L., *Trichoderma* spp., plant extracts, chemical fungicides

INTRODUCTION

In Mexico, onions (*Allium cepa* L.) are part of the popular diet and an important vegetable for consumption, produced in more than 49,311 ha with 1,525,501 t harvested in 2018 (Tirado-Ramírez et al., 2019). Nevertheless, the symptoms of bulb and root rot caused by *Fusarium* spp. significantly affect the quality of the product and reduce crop productivity by up to 25-30%. The disease is caused by different species of the genus *Fusarium*, including *Fusarium oxysporum*, *F. solani*, *F. proliferatum*, *F. acuminatum*, *F. culmorum*, *F. equiseti*, *F. subglutinans*, *F. tricinctum*, and *F. redolens* (Bayraktar and Dolar, 2011; Haapalainen et al., 2016). The fungus can be spreaded by infected seeds causing wilting of young plants as well as bulb rot during pre and postharvest (Dugan et al., 2019).

The management of bulb and root rot caused by *Fusarium* sp. is based on cultural practices and synthetic pesticides. These management approaches are effective in reducing short-term damage. However, their use increases production costs, and misuse threatens human health and the environment (Ghanbarzadeh et al., 2016). An alternative to the use of these synthetic pesticides is the use of biological control agents (BCA). The species of *Trichoderma* are natural inhabitants in the soil and rhizosphere, grow fast, and act as BCA of fungal root diseases caused by several genera such as *Fusarium*, *Rhizoctonia*, *Pythium*, *Sclerotium*, *Macrophomina*, and *Sclerotinia* (Howell, 2003). Furthermore, it promotes the development and natural defense of the host (Yasmeen and Siddiqui, 2017). In several bioassays, the ability of *Trichoderma* species to decrease onion bulb rot by *Fusarium* has been proven to be effective (John et al., 2010). Bulb and foliage treatments with *Trichoderma* decreased the incidence of *Fusarium* spp. wilt and promote plant development (Naguleswaran and Pakeerathan, 2014). An equally promising alternative is the use of botanical extracts against *Fusarium* sp. as these are safer for the environment. Plant extracts contain potent agents against plant pathogenic fungi and are less toxic than synthetic fungicides. The antimicrobial activity of plant extracts has been attributed to many phytochemical components, including coumarins, terpenoids, flavonoids, carotenoids, curcumins, and others (Villa-Martínez et al., 2015).

The aims of this research were (i) to identify the causal agent of the root and bulb rot of onions produced in Michoacán, Mexico, and (ii) to determine the efficacy of biological, botanical, and chemical products applied *in vitro* and under greenhouse conditions.

MATERIALS AND METHODS

Isolation, identification, and pathogenicity of the fungus

The infected bulbs of onions cultivar "Carta Blanca" were collected from the municipality of La Barca, Jalisco, Mexico. Samples were processed, and a monosporic isolate of the fungus was obtained and grown in potato-dextrose-agar (PDA) culture medium. The fungus was morphologically characterized using fungal identification keys and examining conidia under a compound microscope (Leslie and Summerell, 2006). Fungal DNA was extracted using a modification of the cetyl trimethyl ammonium bromide (CTAB) DNA extraction protocol (Murray and Thompson, 1980). The ITS region was amplified by PCR using the primers ITS4/ITS5 as described by Díaz-Nájera et al. (2017). The amplicons of 700 bp were sequenced at Macrogen Inc. (Seoul, Republic of Korea) for the molecular identification of the fungus using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The pathogenicity was tested by inoculating 100 mL of spore suspension with a concentration of 8×10^6 UFC mL^{-1} "in drench" to 20-day-old onion seedlings "Carta Blanca" grown in pots under greenhouse conditions.

Trial I: Efficacy of *Trichoderma* spp. *in vitro* against *Fusarium* sp.

The effect of extracellular metabolites diffused in the medium produced by native (CN) and commercial (CC) strains of *Trichoderma* was tested using the cellophane membrane technique in PDA (Soliman et al., 2016). A completely randomized design with five repetitions was used. The experimental unit was a Petri plate with 20 mL of PDA + metabolites of *Trichoderma* spp. The treatments were: T1= *T. harzianum* (PHC T22®, Plant Health Care, Mexico City, Mexico), T2 = *T. virens* (PHC RootMate®, Plant HealthCare, Mexico City, Mexico), T3 = *T. fasciculatum* (Fithan®, Nafex, Mexico City, Mexico), T4 = *T. asperellum* (Santa Teresa, Mexico native strain), T5 = *T. asperellum* (Cocula, Mexico native strain), T6 = *T. asperellum* (Chilapa, Mexico native strain), in addition to the T7 = Control (Petri plate inoculated only with *Fusarium* sp.). A five-day-old *Fusarium* sp. strain mycelial plug was placed in the center of the Petri plates. The amended plates were incubated in the laboratory at room temperature (28 ±2 °C) and 12 hs photoperiod for nine days. The diameter of the fungus colony was measured every 24 hours for nine days, and the percentage of mycelial growth inhibition was calculated (Sanabria-Velazquez, 2020).

Trials II and III: Efficacy of botanical extracts and chemical fungicides

Two tests were carried out. Trial II compared the effect of three commercial botanical extracts in three different doses and one control (10 treatments, Figure 4). In Trial III, eight chemical fungicides were tested (Figure 5). In both trials, PDA culture medium was amended with each treatment (Kumar and Mane, 2017) and the experimental units were completely randomized with five repetitions per treatment. The pathogen was placed in the center of the plates and then incubated at room temperature (30 ±2 oC) during 12 hs photoperiod. The diameter of the pathogen colony (cm) was measured every 24 hours. Mycelial growth was measured, and the inhibition percentage was calculated (Patil et al., 2014).

Trial IV: Integrated management of *Fusarium* sp. under greenhouse conditions

Thirty-day-old onion plants grown in polystyrene pots (11.5×7.5×16.8 cm) with 750 g of sand + clay mixture (1:1 v/v) were used. Fertilizer amendments, irrigation, and pest management were provided throughout the duration of the experiments. Pure cultures of *Fusarium* sp.

were transferred to Petri plates with PDA and incubated for ten days at room temperature. A suspension of spores was prepared from the pure cultures of *Fusarium* sp. with a concentration of (4.6×106 UFC). The strains of *T. virens* and *T. harzianum* were grown in PDA, and suspensions of 5×10^6 and 5.6×10^6 UFC mL^{-1} were prepared, respectively. The most effective treatments against *Fusarium* sp. from the previous trials were selected (Figure 6). For the inoculation of *Fusarium* sp., 60 mL pot-1 of the fungus inoculum suspension (4.6×10^6 UFC mL^{-1}) was applied "in drench" to the base of the plant 30 days after transplanting.

For the application of treatments, organic and chemical products, alone or in the mixture, were dissolved in 30 mL of sterile distilled water and applied to the base of the plant 30 days after transplanting. The treatments *T. virens* and *T. harzianum* were applied with concentrations of 5×10^6 and 5.6×10^6 UFC mL^{-1}, respectively. A completely randomized design with ten repetitions was used. Each experimental unit was a pot with a plant totalizing 300 plants in total.

Disease incidence was evaluated when 100% of control treatment plants had visible symptoms of the disease ten days after inoculation. A binomial scale was used in which healthy plants were assigned a value of 0 (zero), and a value of 1 (one) to plants with symptoms of yellowing and wilting (disease plants).

Data analysis

For all studies, normality of distributions was checked before analysis, and data were transformed using the arcsine square root if appropriate. The analysis of variance was performed with Statistics Analysis System (SAS) version 9.4 (SAS Institute Inc., Cary, NC). Tukey's significance test with a family-wise error rate of 5% was used for the comparison of means.

RESULTS AND DISCUSSION

Identification and pathogenicity characterization of *Fusarium* sp.

The fungal isolate obtained from infected onion bulbs was identified as *Fusarium* sp. with cotton white, hyaline mycelium, which over time turned to purple (Figure 1), having an average daily growth of 0.93 cm under 28 °C. The isolate produced abundant microconidia, showing one to two cells, and usually occurring in short chains and with false heads. Macroconidia were also seen consisting of three to five cells, gradually thin and hunched towards both ends (Figure 1). The products obtained by PCR from the ITS region were 544 pairs of bases and the sequences deposited with accession number KX757772.1 in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). Our sequence showed 100% concordance with *F. proliferatum* accessions MN481208.1, MN481207.1, MN481206.1, MN481205.1, MN481204.1, MN481203.1, and MN461565.1 available in the database using BLAST. These BLAST results confirmed the findings of the morphological characterization.

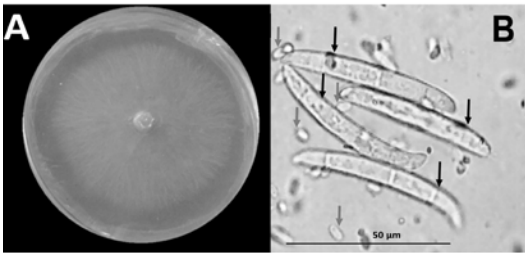

Figure 1. Morphological characterization of *Fusarium* sp. (A) Colony of *Fusarium* sp. on PDA medium. (B) Microconidia (grey arrows) and macroconidia (black arrows).

The isolate of *Fusarium* sp. was catalogued as pathogenic after fulfilling Koch's postulates.

Symptoms of yellowing, withering of the leaves, and root infection were observed when the pathogen was inoculated in healthy onion plants. Ten days after the inoculation of the monoconidial isolate of *Fusarium* sp., reduction in growth, yellowing, descending leaf death, root rot, and necrosis were observed on inoculated samples, while control plants remained healthy (Figure 2). In the USA, onion wilt caused by *F. proliferatum* has been reported to affect both young and adult onion plants causing rot of the base and the bulb (Dugan et al., 2019). The disease is typically attributed to *F. oxysporum* f. sp. *cepae* (Swift et al., 2002). However, rot of the onion bulb is most often caused by *F. proliferatum* which can be responsible for losses of up to 20% in the field and storage (du-Toit et al., 2003). In this work, we only confirmed that the pathogen belongs to the genus *Fusarium* because, for the identification of *Fusarium* at the species level, it is necessary to use molecular sequencing of multiple genes such as the ITS (internal transcribed spacer), EF1α (elongation factor) genes. 1 alpha), RPB1 (largest subunit of RNA polymerase) and RPB2 (second largest subunit of RNA polymerase) (MYCOBANK Database, https://fusarium.mycobank.org/), actions that were not possible during this experimentation. The identification of fungal pathogens to species level is necessary as different species may differ in their biology, which ultimately, may have an effect the management of the disease (Bhunjun et al., 2021). Therefore, more work focusing on the characterization of the pathogen is still needed.

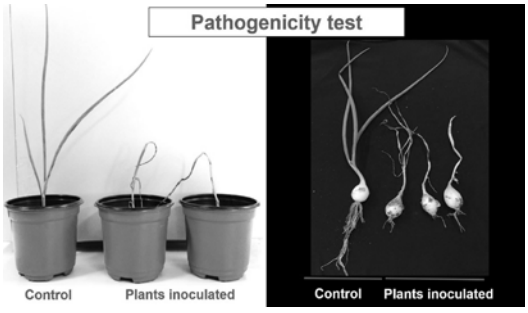

Figure 2. *Fusarium* sp. pathogenicity test in 30-day-old onion plants cultivar "Carta Blanca." Control plant (left) and inoculated plants (right). Antibiosis *Trichoderma* spp. against *Fusarium* sp.

Antibiosis of *Trichoderma* spp. *in vitro* against *Fusarium* sp.

The inhibition of *Fusarium* sp. colonies due to the antibiosis exerted by extracellular metabolites of *Trichoderma* spp. after 48 hours of incubation was statistically significant ($P < 0.0001$). After nine days of incubation, the inhibition of *Fusarium* sp. was higher in treatments with *T. virens* (PHC RootMate®) and *T. harzianum* (PHC T22®) with values of 33.3 and 29.8%, respectively. Mexican native isolates of *Trichoderma* inhibited the mycelial growth of *Fusarium* sp. up to 21.4%, and they were not statically different from the commercial *Trichoderma* isolates (Figure 3). Similarly, Ayvar-Serna et al. (2021) and Quiroz et al. (2008) reported that two Mexican native strains of *Trichoderma* sp. inhibited 100% of the mycelial growth of *Fusarium* sp. isolated from tomato and garlic. In bioassays made with species other than *Fusarium* sp., Suárez et al. (2008) found that *T. harzianum* inhibited up to 50% of radial growth of *Fusarium solani* isolated from papaya. It is well documented that the antibiosis that the genus *Trichoderma* exerts on phytopathogenic fungi is due to the ability to produce hydrolytic enzymes, including cellulases, chitinases, and glucanases (Cubilla-Rios et al., 2019). These results *in vitro* confirm the antagonistic potential of the strains to compete for space and nutrition in the substrate and inhibit the growth of pathogenic *Fusarium* sp.

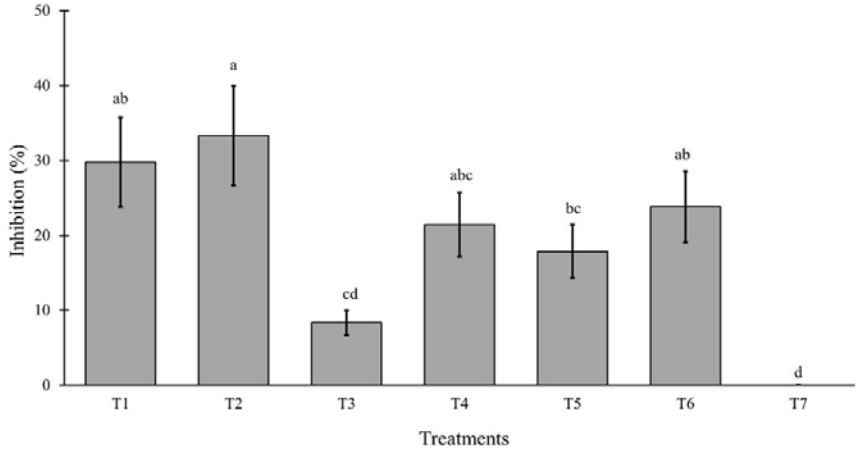

Figure 3. Mycelial inhibition of *Fusarium* sp. grown on PDA medium amended with secondary metabolites of *Trichoderma* spp. The mean values with the same letter are not statistically different (Tukey 0.05). The treatments were: T1 = *Trichoderma harzianum* (PHC T22®, Plant Health Care, Mexico City, Mexico), T2 = *T. virens* (PHC RootMate®, Plant HealthCare, Mexico City, Mexico), T3 = *T. fasciculatum* (Fithan®, Nafex, Mexico City, Mexico), T4 = *T. asperellum* (Santa Teresa, Mexico native strain), T5 = *T. asperellum* (Cocula, Mexico native strain), T6 = *T. asperellum* (Chilapa, Mexico native strain), and the T7 = Control

Effect of botanical extracts against *Fusarium* sp.

Botanical extract treatments significantly inhibited *Fusarium* sp. mycelial growth (P <0.0001). We observed that *Azadirachta extract indica* + *Cinnamomum zeylanicum* (NeemAcar®) inhibited 100% mycelial growth of the pathogen on amended PDA after 17 days of incubation regardless of the dose. The extracts of Regalia Maxx® (*Reynoutria sachalinensis*) and Bio-Canela® (Oils of *Cinnamomum verum*, *Cassia graveolens*, *Lippia berlandieri*, and *Ricinus communis* + *Allium* spp.) were less effective inhibiting

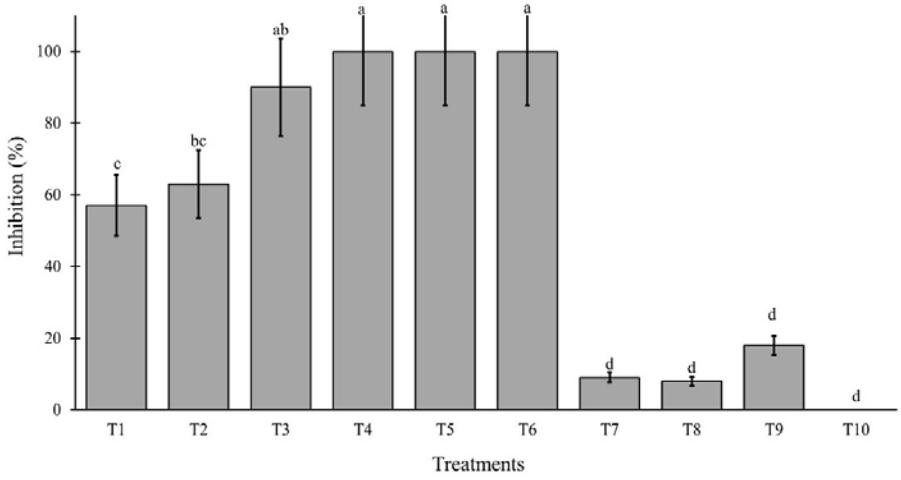

Figure 4. Mycelial inhibition of *Fusarium* sp., after 17 days of incubation in PDA amended with botanical extracts. Mean values with the same letters above the bar are not statistically different (Tukey α = 0.05). The treatments were: T1 = Regalia Maxx® 0.02 mL, T2 = Regalia Maxx® 0.03 mL, T3 = Regalia Maxx® 0.04 mL, T4 = NeemAcar® 0.06mL, T5 = NeemAcar® 0.08 mL, T6 = NeemAcar® 0.1 mL, T7 = Bio-Canela® 0.02 mL, T8 = Bio-Canela® 0.04 mL, T9 = Bio-Canela® 0.06 mL, T10 = Control

the mycelial growth of *Fusarium* sp. except for T3 = Regalia Maxx® 0.04 mL (Figure 4). Various phytochemicals, such as flavonoids, phenols, terpenes, essential oils, alkaloids, lecithin, and polypeptides, have shown antifungal effects (Chouhan et al., 2017). The efficacy of 100% obtained with *Azadirachta indica* (NeemAcar®) in the present experiment is greater than 50% obtained by Díaz-Nájera et al. (2017), with this extract applied in vitro against *F. oxysporum* f. sp. *cepae.*

Effect of chemical pesticides against *Fusarium* sp.

Treatments with benomyl (Promyl®), prochloraz (Sportak®), and pyraclostrobin (Headline®) inhibited the growth of the pathogen by 100% after 12 days of incubation on amended PDA. The fungicides iprodione (Rovral®), methyl thiophanate (Cercobin®), quintozene (Pentaclor®), cyprodinil + fludioxonil (Switch®), and azoxystrobin + difenoconazole (Amistar®), reduced the growth of the pathogen colonies by 71, 76, 68, 69 and 45%, respectively (Figure 5). These results may suggest that *Fusarium* sp. started to develop some resistance to these fungicides, explaining the differential sensitivity to these products. The fungicides benomyl (Promyl®), prochloraz (Sportak®), and pyraclostrobin (Headline®) can be rotated in an integrated management program for onion wilt because their active ingredients have different modes of action. While benomyl interferes with cell division and intracellular transport (Fishel and Dewdney, 2012), pyraclostrobin prevents the germination of spores and the formation of appressoria, and Procloraz interrupts the synthesis of ergosterol in the membrane. Programs implementing the fungicide prochloraz (Mirage® 50 WP) for seed treatment and bulb immersion against basal rot in onions caused by *F. oxysporum* f. sp. cepae reported lower disease incidence and higher yield (Sintayehu et al., 2011).

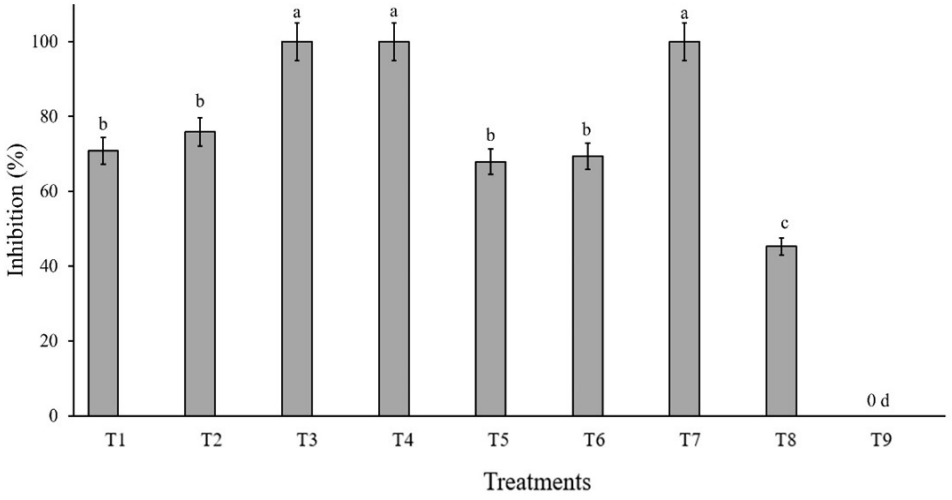

Figure 5. Percentages of inhibition of the growth of *Fusarium* sp. after 12 days of incubation in PDA medium amended with chemical fungicides. The mean values with the same letter above the bar are not statistically different (Tukey α = 0.05). The treatments were: T1 = Iprodione (Rovral®), T2 = Thiophanate methyl (Cercobin®), T3 = Benomyl (Promyl®), T4 = Prochloraz (Sportak®), T5 = Quintozene (Pentaclor®), T6 = Cyprodinil + Fludioxonil (Switch®), T7 = Pyraclostrobin (Headline®), T8 = (Azoxystrobin + Difenoconazole) Amistar®, T9 = Control

Integrated management of *Fusarium* sp. in greenhouse conditions

Onion plants treated with *Trichoderma* (PHC RootMate® and PHC T22®) were highly effective in controlling the disease in greenhouse conditions. However, there were no significant differences among biological, botanical, and chemical treatments, with disease incidence ranging from 0 to 20%. Nevertheless, all treatments were significantly effective compared to the control treatment with 100% incidence (Figure 6). The greenhouse experiment results are supported by our *in vitro* assays, in which *Trichoderma* showed antibiosis as one of the mechanisms to antagonize *Fusarium* sp. Furthermore, it has also been reported that *Trichoderma* spp., combined with other chemical fungicides can improve the control of *Fusarium* sp. (Omar et al., 2006). In this research, neem (*Azadirachta indica*) extract was highly effective and can be recommended as a preventive treatment for this disease. Aqueous extracts of neem and willow (*Salix babylonica*) had been used successfully in another integrated management program for *Fusarium wilt* (Hanaa et al., 2011). The combination with fungicides was equally successful in reducing the disease incidence, suggesting that rotation of different active ingredients can be proposed to growers to manage onion wilt.

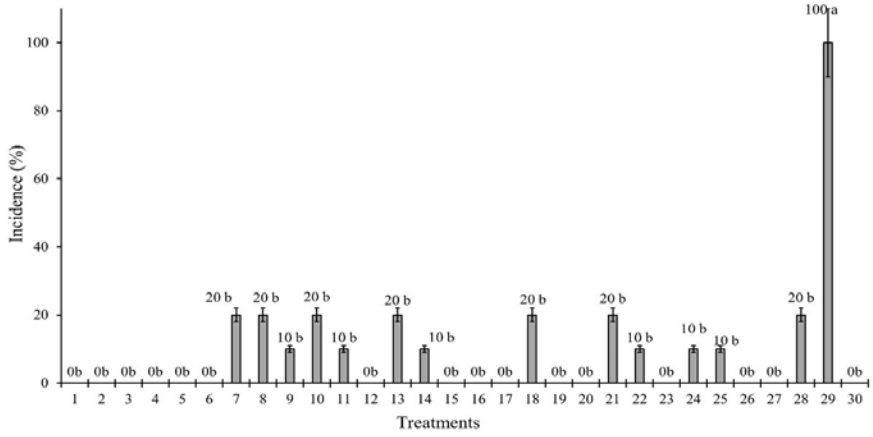

Figure 6. Disease incidence of onion wilt caused by *Fusarium* sp. in greenhouse conditions integrating biological, organic, and chemical products. The treatments were: T1 = PHC T22® (5.6-106 UFC mL-1), T2 = PHC RootMate® (5-106 UFC mL), T3 = PHC T22® + Cercobin® DB (5.6-106 UFC mL-1 + 0.008 g), T4 = PHC RootMate® + Cercobin® DB (5-106 UFC mL + 0.008 g), T5 = PHC® (T22) + Cercobin® DA (5.6-106 UFC mL-1+ 0.011 g), T6 = PHC RootMate® + Cercobin® DA (5-106 UFC mL + 0.011 g), T7 = NeemAcar® DB (0.045mL), T8 = NeemAcar® DM (0.06 mL), T9 = NeemAcar® DA (0.062 mL), T10 = Regalia Maxx ® (0.03 mL), T11 = Regalia Maxx®+ NeemAcar® DB (0.03 mL + 0.045 mL), T12 = Regalia Maxx® + NeemAcar® DA (0.03 mL + 0.062 mL), T13 = Promyl® DB (0.006 mL), T14 = Promyl® DA (0.0075 mL), T15 =Sportak® DB (0.015 mL), T16 = Sportak® DA (0.0225 mL), T17 = Headline® DB (0.015 mL), T18 = Headline® DA (0.045 mL), T19 = Cercobin® DB (0.008 g), T20 = Cercobin® DA (0.011 g), T21 = NeemAcar® DB + Promyl® DB (0.045 mL + 0.006 mL), T22 = NeemAcar® DB + Promyl® DA (0.045 mL + 0.0075 mL), T23 = NeemAcar® DB + Sportak® DB (0.045 mL + 0.015 mL), T24 = NeemAcar® DA + Cercobin® DB (0.062 mL + 0.008 g), T25 = Regalia Maxx® + Promyl® DA (0.03 mL + 0.0075 mL), T26 = Regalia Maxx® + Sportak® DA (0.03 mL + 0.0225 mL), T27 = Regalia Maxx® + Headline® DA (0.03 mL + 0.045 mL), T28 = Regalia® + Cercobin® DA (0.03 mL + 0.011 g), T29 = Control (inoculated with *Fusarium* sp. and non-treated), T30 = Absolute control. *DB: low dose, DM: medium dose. DA: high dose. Commercial dose recommended by the manufacturer of each product was used.

CONCLUSIONS

In conclusion, we verified by morphological and molecular identification that *Fusarium* sp. was the causal agent of wilting and root rot of onion. This isolate reproduced root infection symptoms when inoculated in healthy onion plants in the variety "Carta Blanca." The commercial strains of *T. harzianum* (PHC T22®), *T. virens* (PHC RootMate®), *T. fasciculatum* (Fithan®), and the Mexican native strains of *T. asperellum* exhibited antagonistic activity on the mycelial growth of the pathogen. The botanical extract of *A. indica* + C. *zeylanicum* had a 100% inhibitory effect on the pathogen growth regardless of the dose used. The chemical fungicides benomyl, procloraz, and pyraclostrobin were 100% effective in inhibiting the mycelial growth of *Fusarium* sp. The incidence of onion wilt caused by *Fusarium* sp. was significantly reduced by biological, organic, and chemical products applied individually and in combination in greenhouse conditions. To conclude, in the conditions specified on this research, the results indicates that an effective control of the fungus *Fusarium* sp. can be accounted by the biological, botanical and synthetic products assessed in this trial. The variety of products used in this research can be taken into account for a rotational scheme of application at the time of controlling this disease.

JFDN and SAS conceptualized the general research objectives and were responsible for supervision for the planning and execution of the research activity. MVH developed the methodology design and verified the overall reproducibility of results and experiments. ADSV applied statistical, mathematical, and computational techniques to analyze the study. CMCA carried out the experiments and data collection. GAEM prepared the original draft of the published work. All authors have approved the submission of this manuscript.

AUTHOR CONTRIBUTIONS

JFDN and SAS conceptualized the general research objectives and were responsible for supervision for the planning and execution of the research activity. MVH developed the methodology design and verified the overall reproducibility of results and experiments. ADSV applied statistical, mathematical, and computational techniques to analyze the study. CMCA carried out the experiments and data collection. GAEM prepared the original draft of the published work. All authors have approved the submission of this manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors do not endorse any products, services, or companies mentioned in this paper. Commercial names are provided just to ensure reproducibility of the methods and results reported in this paper.

BIBLIOGRAPHIC REFERENCES

Ayvar-Serna, S., Díaz-Nájera, J. F., Vargas-Hernández, M., Enciso-Maldonado, G. A., Alvarado-Gómez, O. G., y Ortíz-Martínez, A. I. (2021). Actividad antifúngica de pesticidas biológicos, botánicos y químicos sobre el agente causal de la marchitez vascular del jitomate. *Revista Fitotecnia Mexicana, 44*(4), 617-617. https://revfitotecnia.mx/index.php/RFM/article/view/896/852

Bayraktar, H. & Dolar, F. S. (2011). Molecular identification and genetic diversity of *Fusa-*

rium species associated with onion fields in Turkey. *Journal of Phytopathology, 159*(1): 28-34.

Bhunjun, C.S., Phillips, A.J., Jayawardena, R.S., Promputtha, I. & Hyde, K.D. (2021). Importance of molecular data to identify fungal plant pathogens and guidelines for pathogenicity testing based on Koch's Postulates. *Pathogens, 10*(9), p.1096.

Chouhan, S., Sharma, K. & Guleria, S. (2017). Antimicrobial activity of some essential oils-present status and future perspectives. *Medicines, 4*(3): 58.

Cubilla-Ríos, A. A., Ruíz-Díaz-Mendoza, D. D., Romero-Rodríguez, M. C., Flores-Giubi, M. E. & Barúa-Chamorro, J. E. (2019). Antibiosis of proteins and metabolites of three species of Trichoderma against paraguayan isolates of *Macrophomina phaseolina*. *Agronomía Mesoamericana, 30*(1): 63-77.

Díaz-Nájera, J. F., Ayvar-Serna, S., Vargas-Hernández, M., Damián-Flores, M., Alvarado-Gómez, O. G, Mena-Bahena, A. & Acosta-Ramos, M. (2017). Identification and *in vitro* control of the causal agent of wilt in onion. *Pakistan Journal of Agricultural Science, 54*(3): 497-502.

Dugan, F. M., Lupien, S. L., & Hellier, B. C. (2019). Infection by Fusarium proliferatum in aerial garlic bulbils is strongly reduced compared to rates in seed cloves when both originate from infected bulbs. *Crop Protection, 116*, 43-48.

du-Toit, L. D., Inglis, D. A. & Pelter, G. Q. (2003). *Fusarium proliferatum* pathogenic on onion bulbs in Washington. *Plant Disease, 87*(6): 750-750.

Fishel, F. M. & Dewdney, M. M. (2012). Fungicide Resistance Action Committees (FRAC) Classification scheme of fungicides according to mode of action. PI94. University of Florida. 7 p. https://edis.ifas.ufl.edu/pdffiles/PI/PI13100.pdf

Ghanbarzadeh, B., Safaie, N., Goltapeh, E. M., Danesh, Y. R. & Khelghatibana, F. (2016). Biological control of Fusarium basal rot of onion using *Trichoderma harzianum* and *Glomus mosseae*. Journal of *Crop Protection, 5*(3): 359-368.

Hanaa, R. F., Abdou, Z. A., Salama, D. A., Ibrahim, M. A. & Sror, H. A. M. (2011). Effect of neem and willow aqueous extracts on *Fusarium* wilt disease in tomato seedlings: Induction of antioxidant defensive enzymes. *Annals of Agricultural Sciences, 56*(1): 17.

Haapalainen, M., Latvala, S., Kuivainen, E., Qiu, Y., Segerstedt, M. & Hannukkala, A. O. (2016). *Fusarium oxysporum*, *F. proliferatum* and *F. redolens* associated with basal rot of onion in Finland. *Plant Pathology, 65*(8): 1310–1320.

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant disease, 87*(1): 4-10. doi: 10.1094/PDIS.2003.87.1.4

John, R. P., Tyagi, R., Prévost, D., Brar, S. K., Pouleur, S. & Surampalli, R. (2010). Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes* and as a growth promoter of soybean. *Crop Protection, 29*(12): 1452-1459.

Kumar, P. & Mane, S. S. 2017. Studies on the compatibility of biocontrol agents with certain fungicides. *International Journal of Current Microbiology and Applied Sciences, 6*(3): 1639-1644.

Leslie, J. F. & Sumerell, B. A. (2006). The *Fusarium* laboratory manual. Blackwell Publishing. Iowa, USA. pp: 80-110. 388 p.

Murray, M. G. & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic acids research, 8*(19): 4321-4326.

Naguleswaran, V. & Pakeerathan, K. (2014). Biological control: A promising tool for bulb-rot and leaf twisting fungal diseases in red onion (*Allium cepa* L.). Jaffna District. World *Applied Sciences Journal, 31*(6):1090-1095.

Omar, I., O'Neill, T. M. & Rossall, S. (2006). Biological control of *Fusarium* crown and root rot of tomato with antagonistic bacteria and integrated control when combined with the fungicide carbendazim. *Plant Pathology, 55*(1): 92-99.

Patil, N. N., Waghmode, M. S., Gaikwad, P. S., Gajbhiye, M. H., Gunjal, A. B., Nawani, N. & Kapadnis, B. P. (2014). Potential of *Microbispora* sp. V2 as biocontrol agent against *Sclerotium rolfsii*, the causative agent of southern blight of *Zea mays* L. (Baby corn) *in vitro* studies. *Indian Journal of Experimental Biology*, *52*(1): 1147-1151.

Quiroz-Sarmiento, V. F., Ferrera-Cerrato, R., Alarcón, A. & Lara-Hernández, M. E. (2008). Antagonismo *in vitro* de cepas de *Aspergillus* y *Trichoderma* hacia hongos filamentosos que afectan al cultivo del ajo. *Revista Mexicana de Micología*, *26*, 27-34.

Sanabria-Velázquez, A. D. (2020). Evaluación de aislados de *Trichoderma* spp. nativos del Paraguay para el control de *Colletotrichum* spp. causante de la antracnosis en frutilla. *Investigación Agraria*, *22*(1): 53-62.

Sintayehu, A., Sakhuja, P. K., Fininsa, C. & Ahmed, S. (2011). Management of *Fusarium* basal rot (*Fusarium oxysporum* f. sp. *cepae*) on shallot through fungicidal bulb treatment. *Crop Protection*, *30*(5), 560–565.

Soliman, H. M., Abdel-Fattah, G. & Metwally, E. (2016). Antagonistic interactions between the foliar pathogen *Botrytis fabae* Sard. and *Trichoderma harzianum* Rifai. *Asian Journal of Plant Pathology*, *10*(3): 21-28.

Suárez, M. C. L., Fernández, B. R. J. & Osvaldo, V. N. (2008). Antagonismo *in vitro* de *Trichoderma harzianum* Rifai sobre *Fusarium solani* (Mart.) Sacc., asociado a la marchitez en maracuyá. *Revista Colombiana de Biotecnología 2*: 35-43.

Swift, C. E., Wickliffe, E. R. & Schwartz, H. F. (2002). Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cepae* from onion in Colorado. *Plant Disease*, *86*(6): 606-610.

Tirado-Ramírez, M. A., López-Orona, C. A., Díaz-Valdés, T., Velarde-Félix, S., Martínez-Campos, A. R., Romero-Gómez, S. J. & Retes-Manjarrez, J. E. (2019). First Report of Basal Rot of Onion Caused by *Fusarium brachygibbosum* in Sinaloa, Mexico. *Plant Disease*, *103*(3): 582-582.

Villa-Martínez, A., Pérez-Leal, R., Morales-Morales, H. A., Basurto-Sotelo, M., Soto-Parra, J. M. y Martínez-Escudero, E. (2015). Situación actual en el control de *Fusarium* spp. y evaluación de la actividad antifúngica de extractos vegetales. *Acta Agronómica*, *64*(2):194 – 205.

Yasmeen, R. & Siddiqui, Z. (2017). Physiological responses of crop plants against *Trichoderma harzianum* in saline environment. *Acta Botanica Croatica*, *76*(2): 154–162.