Emergence and growth of *huacle* chili seedlings (*Capsicum annuum* L.) with the use of biological formulations in commercial plot soil

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ABSTRACT

Objective: To evaluate the effect of biological control agents (BCA) and two biological formulations (Bio-Terra and Bio-CNPR) against the phytopathogens found in the soil of *huacle* chili (*Capsicum annuum*) plantations, in order to obtain healthy seedlings.

Design/Methodology/Approach: The following design variables were evaluated: germination (%), disease incidence (%), and survival (%). Stem height and root length were also evaluated. The experimental unit was a tray with n=30 seedlings per each treatments (T0, T1, T2, T3, and T4) and four repetitions. An analysis of variance (ANOVA) was used. Tukey's mean test was applied (using the Minitab Statistical program version 20.0) in units that presented differences ($p \leq 0.05$).

Results: The phytopathogenic microorganism *Fusarium* spp. was isolated from the soil of the plots cultivated with *huacle* chili. The T1 treatment recorded the best inhibitory effect against *Fusarium* spp.: it had 92.2% germination, 18.3% incidence of *Fusarium* spp., and 82% surviving seedlings (with an average height of 16 cm and a root length of 7.9 cm). The sterilization of the soil lead to T4 having the best results in germination, incidence, and survival, since *Fusarium* spp. did not damage *huacle* chili seedlings. In contrast, T0 did not prosper neither with BCA nor with biological products, since all the seedlings in non-sterile soil died.

Study Limitations/Implications: A plastic barrier was placed between the trays with the chili seedlings to avoid cross-contamination between treatments.

Findings/Conclusions: T1 (the BCA with nitrogen-fixing bacteria and *B. subtilis*) recorded the best counteracting results against the damage generated by *Fusarium* spp. Their antagonism allowed a high percentage of survival of *huacle* chili seedlings and encouraged plant development through the best root growth and height of the seedlings.

Keywords: *huacle* chili, biological product, *Fusarium*.

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INTRODUCTION

The *huacle* chili (*Capsicum annum* L.) is an endemic plant of the region of San Juan Bautista Cuicatlán, Oaxaca, where it is cultivated in small areas in the open field (Aguilar-Rincón *et al.*, 2010; García-Gaytán *et al.*, 2017). The critical stage of the crop takes place during the seedbed

—between 35 to 45 days (three days for germination, 12 days for emergence, and the rest as seedling) (López-López and Pérez 2015). Common diseases in nurseries or seedbeds can be caused by *Pythium* spp., *Fusarium oxysporum*, *Rhizoctonia* sp., or *Phytophthora capsici* in the preemergence and postemergence stages. The damages observed during germination include wet rot, neck decapitation of the seedling, fall of necrotic cotyledons or necrotic roots, all of which cause the death of the seedling (Barranco, 2016).

One way to counteract these phytosanitary problems is the application of antagonistic microorganisms. Species such as *Rhizobium* —which is obtained from rice (*Oryza sativa* L.) plants— produce high concentrations of indole compounds, polyhydroxybutyrate, and ammonium, and solubilize calcium phosphate (Hernández Forte and Nápoles García, 2019). Fungi of the genus *Trichoderma* and bacteria of the genus *Bacillus* compete for the substrate, parasitize organisms producing lytic enzymes that degrade the cell wall of phytopathogens (particularly, *Fusarium*), and stimulate the growth and development of plant roots, improving the uptake of nutrients and water (Martínez *et al.*, 2013; Vásconez *et al.*, 2022). *Bacillus subtilis* produces siderophores, indole compounds, and jasmonic acid, which promote stem height and a greater number of leaflets (Anguiano Cabello *et al.*, 2019). As for *Trichoderma harzianum*, it can potentially control vascular fusariosis, resulting in a high germination percentage in miahuateco chili (Miguel-Ferrer *et al.*, 2021) and mycoparasitism, through the production of antifungal enzymes with potential antibiotic characteristics. In addition, it increases the pod production (24%), root length (40%), and yield (23%) of chickpea crops (Martínez-Martínez *et al.*, 2020). Consequently, the effect of a set of biological control agents (BCA) and two biological formulations (Bio-Terra and Bio-CNPR) were evaluated, combining substrate with soil from an area cultivated with *huacle* chili, in order to obtain healthy seedlings.

MATERIALS AND METHODS

The experimental phase was carried out at the facilities of the Universidad de la Cañada, located in the municipality of Teotitlán de Flores Magón, Oaxaca, Mexico.

Sample collection

The experimental plot is located at 17° 79' 47.8" N and 96° 96' 49.8" W, and 588 m.a.s.l. Soil samples were collected, duly labeled (NOM-021-RECNAT-2000), and transported to the laboratory. *Huacle* chili plants were found to have been damaged by wilting or strangling at the base of the stem.

Isolation and identification of the causative agent

The samples of local cultivation soil were treated according to AS-01 of the NOM-021-RECNAT-2000. They were mixed until a 1-kg sample was obtained. Subsequently, 1.0 g of soil was extracted and mixed with 9 mL of distilled water in an Erlenmeyer flask and stirred for 20 min. Serial dilutions were made up to 10-4. Under aseptic conditions, 0.1 mL of the dilution were taken and inoculated in a Petri-dish with Bioxon® Potato Dextrose Agar (PDA) culture medium, which was prepared according to the product label (39 g L^{-1}) and sterilized in an All American® pressure cooker for 15 min at 15 Lb. The Petri-dishes

with PDA were inoculated per streak with the serial dilutions of soil and incubated at 27 °C for seven days; the process was repeated until pure cultures were obtained (Samaniego-Fernández *et al.*, 2018).

The sticky tape technique with cotton blue staining (Díaz *et al.*, 1999) was used to identify the causative agent: fragments of mycelium were extracted gently pressing the sticky side of the tape against the pure cultures and placing the tape on the slide, for observation under an optical microscope at 100x with an Euromex® LCD monitor. The morphological characteristics were recognized according to the taxonomic keys proposed by Barnett and Hunter (1998). The purpose of the isolation and identification was to corroborate the presence of *Fusarium* in the soil for future assessments.

Germplasm

Six hundred *huacle* chili seeds were used for the experiment. Each biological product to be evaluated was applied to 120 seeds per treatment. The seeds were subsequently germinated. They were sprayed with a set of antagonists and nitrogen-fixing bacteria (biopolymer 1 g/L, *B. subtilis* 5.5×10^8 UFC/L, and *Rhizobium* spp., 6.0×10^8 UFC/L), Bio-Terra (1 g biopolymer, *B. subtilis* 5.5×10^8 UFC, *B. thuringiensis* 6.0×10^8 UFC, *T. harzanium* 5.0×10^{10} spores, and *Beauveria bassiana* 4.6×10^{10} spores in 500 g of inert wettable powder), and Bio-CNPR (1 g/L biopolymer, *B. subtilis* 5.5×10^8 UFC/L, *Rhizobium* sp. 6.0×10^8 UFC/L, *B. thuringiensis* 6.0×10^8 UFC/L, *T. harzanium* 5.0×10^{10} spores/L, and *Metarhizium anisopliae* 2.08×10^{10} spores/L) (Hernández-Rosas, 2019).

Experimental design

A base mixture of the soil collected from the cultivation area was made with Peatmoss®, at a 1:1 v/v ratio. The resulting mixture was used to carry out the following treatments: T0=non-sterilized base mixture + chili seed, T1=non-sterilized base mixture + chili seed + *Rhizobium* + *B. subtilis*; T2=non-sterile base mix + chili seed + Bio-Terra; T3=non-sterile base mix + chili seed + Bio-CNPR; and T4=sterilized base mixture + chili seed.

The biological products were sprayed once a week with an atomizer to guarantee the inoculation in the substrate of each treatment (Gómez and Cruz, 2016). The following doses were applied: 1 L of BCA and Bio-CNPR in 200 L of water; and 500 g of Bio-Terra in 200 L of water. The treatments were placed at a height of 30 cm, with 50 cm between trays, and divided into blocks by a plastic barrier to avoid possible cross contamination.

Experiment response variables

The measurement of variables began when the 600 seeds were placed inside the trays (emergence). The germination percentage was obtained multiplying the number of germinated seeds/number of seeds sown by 100 (Prado-Urbina *et al.*, 2015). Disease incidence was calculated according to the formula proposed by Van der Plank in 1975 (Navarrete-Maya *et al.*, 2009): number of affected plants/number of total plants multiplied by 100. The percentage of survival was calculated based on the number of surviving plants/number of germinated seeds multiplied by 100 (Cóbar-Carranza *et al.*, 2015). The plant height and root length were measured from the base of the stem to the apex of the seedling

and from the base of the stem to the apex of the root (Candelero *et al*., 2015). Fifteen of the surviving seedlings per treatment were measured with a Vernier® (MetroMex).

Statistical analysis

The response variables were subjected to an analysis of variance (ANOVA) to determine the germination percentage, disease incidence percentage, survival percentage, plant height, and root length. The experimental unit was a tray with 30 seedlings and four repetitions per treatment. The response variables with significant differences were analyzed with Tukey's mean test ($p \leq 0.05$), using with the Minitab statistical software version 20.0.

RESULTS AND DISCUSSION

The soil samples showed white mycelial growth in the PDA culture medium with violet pigmentation, cottony mycelium, typically curved and pointed macroconidia, ellipsoidal (canoe-shaped) with three septa, septate hyphae, and thick-walled chlamydospores (Figure 1A-C). These findings match the description of the genus *Fusarium* spp. made by some authors (Barnett and Hunter, 1998; Rentería-Martínez *et al*., 2019).

The analysis of variance of the germination percentage, disease incidence, survival, plant height, and root length were different ($p \leq 0.05$) (Figure 2).

With the T4 treatment, the *huacle* chili seeds recorded a high germination percentage (99.6%), as a result of the previous management, and no impact by *Fusarium* spp. However,

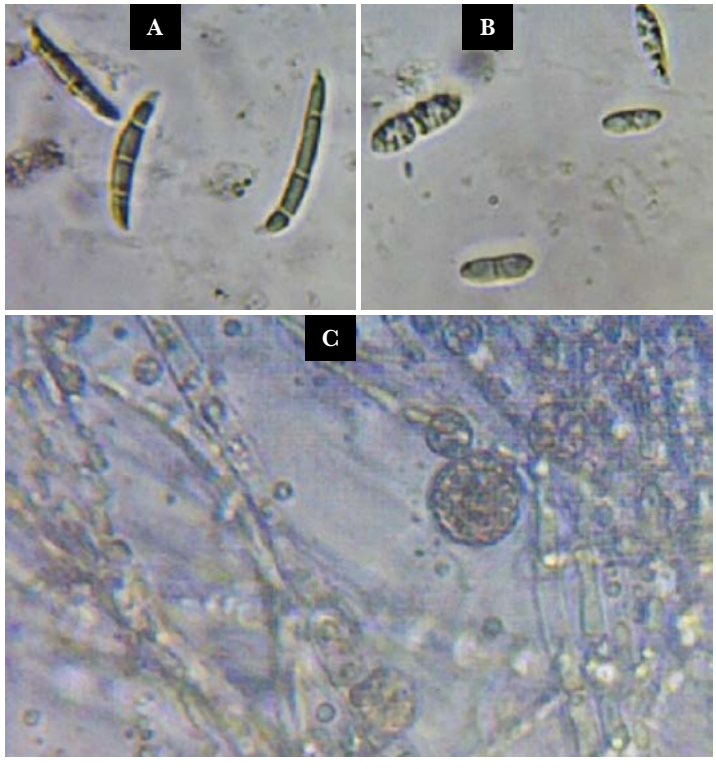

Figure 1. *Fusarium* spp. morphology observed at 100x. A: macroconidia; B: microconidia and C: chlamydospores.

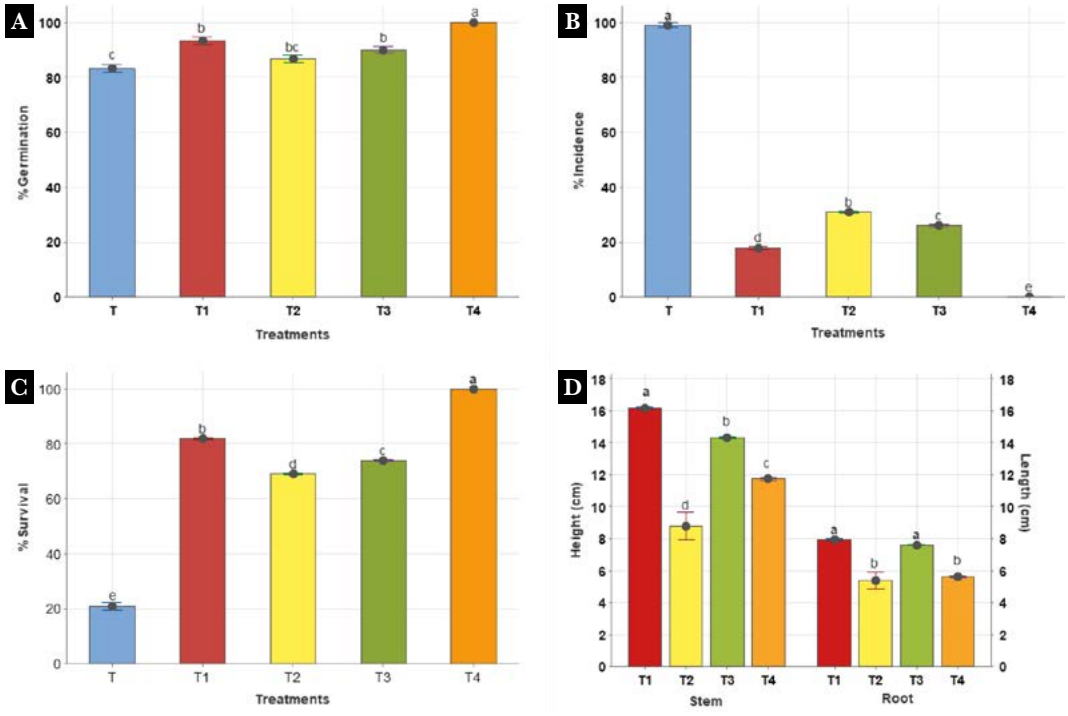

Figure 2. 2A: germination, 2B: incidence, 2C: survival, 2D: stem height and root length (± standard error) of *huacle* chili seedlings subject to different treatments. Letters A-C indicate significant differences (P<0.05) between the treatments, according to Tukey's multiple-comparison test.

treatments T1 and T3 —in which the biological products with *Rhizobium* – *B. subtilis* and Bio-CNPR were applied— had 92.2% and 90.9% germination, respectively (Figure 2A). Therefore, *B. subtillis* and *Rhizobium* encourage germination (Hernández Forte and Nápoles García, 2019; Anguiano Cabello *et al.*, 2019) and, as antagonists of *Fusarium* spp., they protect the *huacle* chili seeds (Martínez *et al.*, 2013; Rojas *et al.*, 2017, Pérez; García-Godos, 2019; Vásconez *et al.*, 2022; Miguel-Ferrer *et al.*, 2021).

Regarding the incidence of the disease (Figure 2B), unlike the other treatments, T4 did not show any symptoms, due to the use of the sterile substrate. Regarding the T1 treatment —which contained the BCAs (*Rhizobium* and *B. subtilis*)— only 18.3% of the seedlings showed signs of disease, because *B. subtilis* acted favorably by activating defense mechanisms (Anguiano Cabello *et al.*, 2019).

For their part, the T3 and T2 treatments had an incidence of 25.7% and 30.7% respectively, resulting from the reduction of the damage caused by *Fusarium*. Although these treatments include both *Trichoderma* and *Bacillus*, their effectiveness is lower than T1. Regarding the control (T0), all the seedlings were damaged by *Fusarium* spp. (Figure 3, A-C).

Regarding the survival rate (Figure 2C), all the *huacle* chili seedlings in T4 survived and reached a height of 11.7 cm and a root system length of 5.6 cm. Meanwhile, the BCA set of the T1 treatment inhibited *Fusarium* spp.; in this case, the survival percentage was 82%, the seedlings grew up to be 16 cm tall and the root system was 7.9 cm long (Figures 2D and 3D). It is likely that the inoculum of both bacteria encouraged synergy

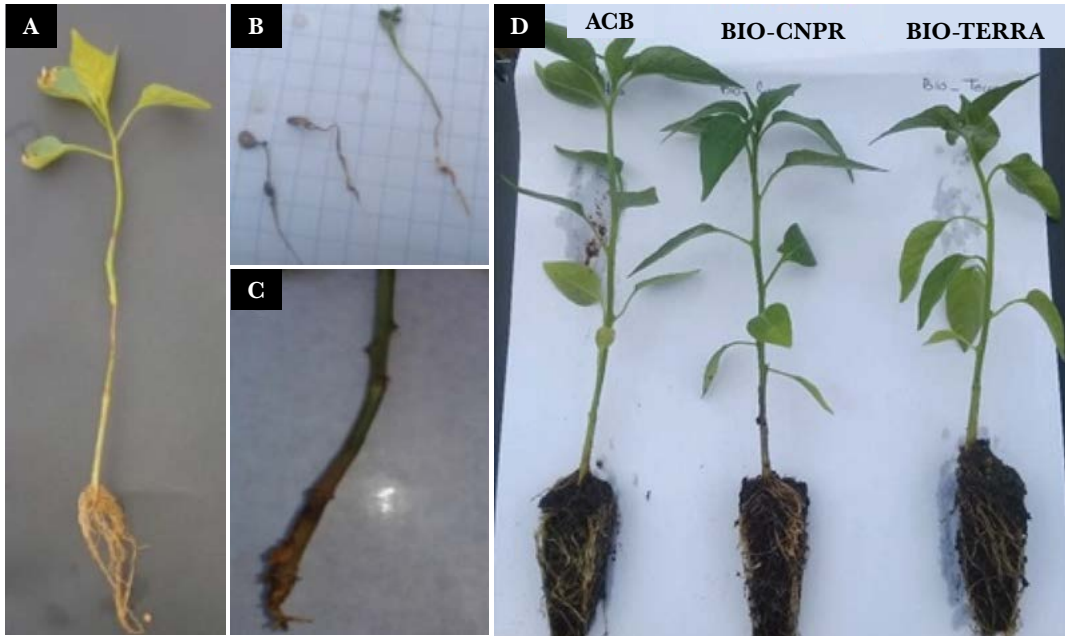

Figure 3. *Huacle* chili seedlings. A-C) damage caused by *Fusarium* spp.; and D) seedlings treated with BCA (ACB) and *Rhizobium* – *B. subtilis*, Bio-CNPR, and Bio-Terra.

among *Rhizobium* and *B. subtilis*, which resulted in healthy plants (Hernández-Forte and Nápoles-García, 2019; Anguiano-Cabello *et al.*, 2019). In T3, 74% of *huacle* chili seedlings survived, reaching a height of 14.3 cm and a root length of 7.6 cm. Despite the complexity of microorganisms found in the content of the biological product (Bio-CNPR), a favorable performance was observed, while all the control chili seedlings (T0) showed symptoms of damage by Fusarium spp. and did not survive (Figure 3A).

CONCLUSIONS

The best treatment regarding germination, incidence, survival, stem height, and root length in *huacle* chili seedlings was recorded with biological control agents (BCA) of the *Rhizobium* + *B. subtilis* bacteria group. Their microbiological duality enabled the antagonistic activity against *Fusarium* spp. in this phase of the growth of the *huacle* chili, promoting the highest root growth and seedling height values. Antagonistic effects could be distinguished even with the survival levels recorded in the different treatments where the biological products were applied.

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REFERENCES

Aguilar-Rincón, V. H., T. Corona Torres, P. López López, L. Latournerie Moreno, M. Ramírez Meraz, H. Villalón Mendoza y J. A. Aguilar Castillo. 2010. Los chiles de México y su distribución. SINAREFI,

Colegio de Postgraduados, INIFAP, IT-Conkal, UANL, UAN. Montecillo, Texcoco, Estado de México. 114 p.

Anguiano Cabello, J. C., Flores Olivas, A., Olalde Portugal, V., Arredondo Valdés, R., Laredo Alcalá, E. I. 2019. Evaluation of *Bacillus subtilis* as promoters of plant growth. *Revista Bio Ciencias* 6: 1-13.

Barnett, H.L. y Hunter, B. B. 1998. Illustrated genera of imperfect fungi (4th ed). Saint Paul Minnesota APS Press, 218p.

Barranco R. T. 2016. Caracterización fenotípica de chile Huacle (*Capsicum annunm* L.) bajo condiciones de agricultura orgánica y agricultura convencional en invernadero. Tesis de Licenciatura. Universidad Autónoma Agraria Antonio Narro, Torreón Coahuila, 75 p.

Candelero, D. J., Cristóbal, A. J., Reyes, R. A., Tun, S. J. M., Gamboa, A. M. M. y Ruiz, S. E. 2015. *Trichoderma* spp. promotoras del crecimiento en plántulas de *Capsicum chinense* Jacq. y antagónicas contra *Meloidogyne incognita*. ΦYTON 84:113-119.

Cóbar-Carranza A. J., García R. A., Pauchard A., Peña E. 2015. Efecto de la alta temperatura en la germinación y supervivencia de semillas de la especie invasora *Pinus contorta* y dos especies nativas del sur de Chile. *Bosque, 36*(1): 53-60.

Díaz, R., Gamazo, C. y Lopez-Goñi I. (1999). Manual Práctico de Microbiología 2º ed. España. Masson SA. 208 p.

García-Gaytán V., Gómez-Merino F. C., Trejo-Téllez L. I., Baca-Castillo G. A., García-Morales S. 2017. The Chilhuacle Chili (*Capsicum annuum* L.) in Mexico: Description of the Variety, its cultivation, and uses. *International Journal of Agronomy*, 5:1-13.

Gómez, T. A. y Cruz, F. C. (2016). Producto biológico para el control de plagas y enfermedades del cultivo de caña de azúcar. Colegio de Postgraduados Campus Córdoba, Veracruz.: U.N.C. 21 p.

Hernández Forte, I., Nápoles García, M.C. 2019. Rhizobia promote rice (*Oryza sativa* L.) growth: First evidence in Cuba. In: Zúñiga-Dávila, D., González-Andrés, F., Ormeño-Orrillo, E. (eds) Microbial Probiotics for Agricultural Systems. *Sustainability in Plant and Crop Protection*. Pp 155-168.

Hernández-Rosas, F. 2019. Bio-CNPR: Formulado de Control biológico para el cultivo de caña de azúcar (*Saccharum* spp.). *Agroproductividad, 12*(11): 105-106.

López-López, P., Pérez B. D. 2015. El chile Huacle (*Capsicum annuum* sp.) en el estado de Oaxaca, México. *Agroproductividad, 8*(1): 35-39.

Martínez, B., Infante, D., y Reyes, Y. 2013. *Trichoderma* spp. y su función en el control de plagas en los cultivos. *Revista de Protección Vegetal, 28*(1): 1-11.

Martínez-Martínez T. O., Guerrero-Aguilar B. Z., Pecina-Quintero V., Rivas-Valencia P., González-Pérez E., Angeles-Núñez J. G. 2020. Antagonismo de *Trichoderma harzianum* contra la fusariosis del garbanzo y su efecto biofertilizante. *Revista Mexicana de Ciencias Agrícolas, 11*(5): 1135-1147.

Miguel-Ferrer L., Romero-Arenas O., Andrade-Hoyos P., Sánchez-Morales P., Rivera-Tapia J.A., Fernández-Pavía S.P. 2021. Antifungal activity of *Trichoderma harzianum* and *T. koningiopsis* against *Fusarium solani* in seed germination and vigor of Miahuateco chili seedlings. *Mexican Journal of Phytopathology 39*(2): 228-247.

Navarrete-Maya R., Trejo-Alabarrán E., Navarrete-Maya J., Prudencio-Sains J. M., Acosta Gallegos J. A. 2009. Reacción de genotipos de frijol a *Fusarium* spp. y *Rhizoctonia solani* Bajo condiciones de campo e invernadero. *Agricultura Técnica en México, 35*(4): 455-466.

NOM-021-RECNAT-2000. Norma Oficial Mexicana NOM-021-RECNAT-2000, Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreos y análisis. 73 p.

Pérez D., García-Godos P. 2019. Identificación del agente causal del marchitamiento en *Caesalpinia spinosa* "tara" y el efecto antagónico de aislados de *Bacillus* spp. y *Trichoderma* sp. *Ecología Aplicada, 18*(1): 51-57.

Prado-Urbina, G., Lagunes-Espinoza, L. C., García-López, E., Bautista-Muñoz, C. C., Camacho-Chiu, W., Mirafuentes G. F., y Aguilar-Rincón. V. H. 2015. Germinación de semillas de chiles silvestres en respuesta a tratamientos pre-germinativos. *Ecosistemas y Recursos Agropecuarios, 2*(5): 139-149.

Rentería-Martínez M.E., Guerra-Camacho M.A., Ochoa-Meza A., Moreno-Salazar S.F., Meza-Moller A.C., Guzmán-Ortíz J.M. 2019. Description and comparison among morphotypes of *Fusarium brachygibbosum*, *F. falciforme* and *F. oxysporum* pathogenic to watermelon in Sonora, México. *Revista Mexicana de Fitopatología, 37*(1): 16-34.

Rojas, B. M. M., Sánchez C. D., Rosales P. K., Lugo M. D. 2017. Antagonismo de *Bacillus* frente a hongos fitopatógenos de cultivos hortícolas. *Revista de Protección Vegetal, 32*(2): 1-9.

Samaniego-Fernández, L. M., Maimouna, H. O. C, Rondon, C. A. J., Placeres-Espinosa, I. 2018. Aislamiento, identificación y evaluación de cepas autóctonas de *Trichoderma* spp. antagonistas de patógenos del suelo. *Revistas de Protección Vegetal, 33*(3): 1-11.

Vásconez G. G., Tórres G. A., Touma H. C., Ezeta F. H. 2022. Incidência de dois antagonistas (*Bacillus subtilis* e *Trichoderma harzianum*) sobre pathogens em culturas pepper (*Capsicum annuum* l.) Na área de Babahoyo, província Los Rios. *Brazilian Journal of Animal and Environmental Research, 5*(3): 2721-2737.