ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

In vitro biocontrol of tomato pathogens using antagonists isolated from chicken-manure vermicompost

Biocontrol *in vitro* de fitopatógenos de tomate mediante antagonistas aislados de vermicomposta de gallinaza

Barocio-Ceja NB[1], LF Ceja-Torres[1*], JL Morales-García[2], HV Silva-Rojas[3], R Flores-Magallón[1], S Ochoa-Estrada[1]

Abstract. The objectives of this study were to (1) isolate and identify pathogenic fungi from vegetative material with wilt symptoms in tomato plantations belonging to the Cienega of Chapala, Michoacán, Mexico, and (2) determine the antagonistic capacity of *Trichoderma* sp. and *Aspergillus* spp. isolated from chicken-manure vermicompost. Pathogens were isolated by means of a completely randomized sampling in 6 locations; 9 plantations were inspected and 45 plants with symptoms of the disease were selected. Portions of root and stem were disinfected and placed on potato-dextrose-agar acidified (PDA). Antagonists isolation was made from a dilution of chicken-manure vermicompost of 1 x 10^{-2} in PDA medium culture more streptomycin and tetracycline. The antagonistic activity was tested by the dual culture confrontation methods. Two pathogens were obtained on tomato in the study area, *Fusarium* spp. and *Rhizoctonia* sp., presenting an incidence of 92% and 5%, respectively. Morphological characteristics were determined in cultivation of PDA. Molecular analysis identified *F. oxysporum*, *F. solani*, *F. subglutinans* and *Rhizoctonia* sp. Of 11 isolates of chicken manure vermicompost, only *Trichoderma* sp. and *Aspergillus* sp., had significant differences ($p \leq 0.05$) with respect to the control. Inhibition of *F. oxysporum* growth ranged from 45% to 48%, and 24% to 27%, in presence of *Trichoderma* sp. and *Aspergillus* sp., respectively; these antagonistic species inhibited growth of *Rhizoctonia* sp. by 38% and 25% , respectively.

Keywords: Biocontrol; Antagonists; *Solanum lycopersicum*; Wilt.

Resumen. Los objetivos de este trabajo fueron (1) aislar e identificar hongos patógenos de plantas de tomate con síntomas de marchitez, en plantaciones de seis localidades de la Ciénega de Chapala, Michoacán, México, y (2) determinar la capacidad antagónica de *Trichoderma* sp. y *Aspergillus* sp. asociados a vermicomposta de gallinaza. Los patógenos se aislaron a partir de un muestreo al azar en nueve plantaciones de las cuales se seleccionaron 45 plantas de tomate con síntomas de la enfermedad. Porciones de raíz y tallo fueron desinfectados y colocados en medio papa-dextrosa-agar acidificado (PDA). El aislamiento de antagonistas se hizo a partir de una dilución de vermicomposta de gallinaza de 1 x 10^{-2} en medio de cultivo PDA más tetraciclina y estreptomicina. El antagonismo fue evaluado mediante confrontación por cultivos duales. Dos fitopatógenos fueron obtenidos del área de estudio; *Fusarium* spp. y *Rhizoctonia* sp. con una incidencia de aislamientos de 92% y 5%, respectivamente. Las características morfológicas se determinaron en cultivo de PDA, y mediante un análisis molecular se identificó a *F. oxysporum*, *F. solani*, *F. subglutinas* y *Rhizoctonia* sp. De 11 cepas aisladas de la vermicomposta de gallinaza, solo *Trichoderma* sp. y *Aspergillus* sp., tuvieron diferencias significativas ($p \leq 0,05$) con respecto al testigo. La inhibición del crecimiento "*in vitro*" de *F. oxysporum* por *Trichoderma* sp. y *Aspergillus* sp. varió de 45% a 48%, y de 24% a 27%, respectivamente; estos antagonistas inhibieron el crecimiento de *Rhizoctonia* sp. en un 38% y 25%, respectivamente.

Palabras clave: Biocontrol; Antagonistas; *Solanum lycopersicum*; Marchitez.

[1] Instituto Politécnico Nacional, CIIDIR-U-MICHOACÁN, Justo Sierra No. 28, Col. Centro, C.P. 59510, Jiquilpan Michoacán, México.
[2] Facultad de Agrobiología, "Presidente Juárez" U.M.S.N.H. Uruapan, Michoacán.
[3] Colegio de Postgraduados. Producción de Semillas, Campus Montecillo, Texcoco, Edo. de México, C.P. 56230, México.
Address Correspondence to: Luis Fernando Ceja Torres, *e-mail:* lfceja@colpos.mx
Recibido / Received 13.XI.2012. Aceptado / Accepted 26.I.2013.

INTRODUCTION

A wide range of production problems occurs in commercial tomato (*Solanum lycopersicum*). Wilt diseases are often the most important cause of these problems, and fungi are the usual causal agents. *Fusarium* spp. and *Rhizoctonia* spp. are the major genera involved in tomato wilt (Lugo & Sanabria, 2001; Michael-Aceves et al., 2008) causing significant reductions in crop yield (Ascencio-Álvarez et al., 2004).

An increasingly favored alternative to chemical control is the use of biological control organisms. Antagonistic microorganisms can suppress growth of many plant pathogens and promote growth of a range of beneficial microorganisms. This might result in improved crop development associated with increased levels of protection against a range of pests and diseases (Ezziyyani et al., 2006).

In recent years, there has been renewed interest in the use of organic manures in crop production, and also in the use of biological control agents for managing plant pathogens. Examples are *Phytophthora nicotianae* var. *nicotianae* in cabbage (*Brassica oleracea* L.), *Fusarium oxysporum* f. sp. *lycopersici* in tomato, and *P. drechsleri*, *Rhizoctonia solani* and *F. oxysporum* in gerbera (*Gerbera jamesonii* H. Bolus) (Villa-Briones et al., 2008; Holguín-Castaño & Mora-Delgado, 2009). Biological control agents can reduce the negative impacts of a number of adverse environmental factors, and provide commercially significant levels of crop protection (Rodriguez & Montilla, 2002).

Vermicompost is widely used as an organic substrate to enhance growth and development of crop plants and raise soil fertility (Castillo et al., 2000; De La Cruz-Lázaro et al., 2010). These composts contribute to a useful range of plant nutrients, and contain a large number of microorganisms including bacteria, fungi and actinomycetes. These microbes break down organic residues into simpler substances during vermicomposting. The increased presence of these substances increases the range and numbers of soil microorganisms, including those potentially antagonistic to phytopathogens (Holguín-Castaño & Mora-Delgado, 2009).

Microbial antagonists most used for biological control are fungi of the genus *Trichoderma.* This genus can reduce growth of *P. infestans* isolated from tomato by 16-85%, and of *Alternaria solani* by 39-81% (Michael-Aceves et al., 2008). These authors observed a 48% inhibition of *F. oxysporum* f. sp. *lycopersici* (FOL) by *T. harzianum* on the same crop (Srivastava et al., 2010). This antagonistic species inhibited growth of *F. solani* isolated from passion fruit by 70% (Suárez-Meza et al., 2008).

The mechanisms through which antagonistic species suppress phytopathogenic soil microorganisms are mainly through the production of toxic metabolites (antibiotics); these metabolites inhibit the development of many phytopathogenic fungi (Villa-Briones et al., 2008). Other mechanisms involved in the beneficial effects might be: (1) the production of enzymes that destroy pathogen cell walls; (2) mycoparasitism, and (3) the induction in the crop plant of an increase in its systemic resistance to pathogens (Fernandez & Vega, 2001; Ezziyyani et al., 2006).

However, there are few studies on the microbiota found in vermicomposted chicken manure, and even fewer that deal with their effectiveness in controlling plant pathogens in tomato. The objectives of this study were to (1) isolate and identify pathogens of the below- and aboveground systems of tomato plants growing in the Cienega de Chapala, Michoacan, Mexico, and (2) evaluate the *in vitro* activities of *Trichoderma* spp. and *Aspergillus* spp. on two important species of tomato-wilt pathogens (i.e., *Fusarium* and *Rhizoctonia)*.

MATERIALS AND METHODS

Sampling. The research was conducted in 2011 at six locations in the Cienega de Chapala, Michoacán, where nine saladette tomato plantations were investigated. Forty five plants with symptoms of wilting or root and stem rot were collected randomly for analysis. Of these, 25 samples were of the variety "Toro", and 5 samples of the varieties "Palomo", "F1 Anibal", "Sun 7705", and "Juan Diego". Tomato plants showing the stated symptoms were placed in labeled plastic bags and taken to the laboratory of the Plant Pathology Unit, CIIDIR, of Michoacán for analysis. A total of 555 samples were collected. The number of samples of each tomato variety depended on the availability of diseased tissue (Table 1).

Table 1. Numbers of samples isolated from necrotic tissue obtained from the roots and stems of tomato plants (*Solanum lycopersicum*) showing symptoms of wilting in the Cienega de Chapala, Michoacán in 2011.

Tabla 1. Número de muestras aisladas de tejido necrótico mostrando síntomas de marchitez. Dicho tejido fue obtenido de las raíces y tallos de plantas de tomate (*Solanum lycopersicum*) en la Cienega de Chapala, Michoacán en 2011.

Locations	Tomato varieties	Tissue samples per variety	Tissue samples per location
Cojumatlán de Regules	Toro	90	185
	Palomo	95	
Jiquilpan	Anibal F1	25	60
	Sun 7705	35	
Nicolás Romero	Toro	80	80
El Platanal	Toro	85	85
Venustiano Carranza	Toro	45	85
	Juan Diego	40	
Villamar	Toro	60	60
Total		555	555

Isolation and identification of phytopathogens. Roots and stems showing necrotic symptoms were cut into sections about 1 cm long and surface-sterilized in 3% sodium hypochlorite solution for 2 min. Samples were thereafter rinsed three times with sterile distilled water, dried on sterile paper towels and placed in Petri dishes containing potato-dextrose agar (PDA, BIOXON®) medium. Samples were incubated for 5 days at 28 °C. When isolates grew, microscopic preparations were made using glass slides with lactophenol-cotton blue, and observed under a compound microscope (Zeiss® Ser. Nr. 993718). They were identified using the morphological taxonomic keys of Barnett & Hunter (1978) and of Nelson et al. (1983). Each isolate was purified by the hyphal-tip technique, and was also sent to the Colegio de Postgraduados for molecular identification.

Isolation of *Trichoderma* and *Aspergillus* spp. from chicken-manure vermicompost. The isolation of antagonists was made by mixing 10 g of chicken-manure vermicompost (VG) in 90 mL of sterile distilled water, from which serial dilutions were made to 10^{-2}. Aliquots (0.3 mL) of the 10^{-2} dilution were uniformly distributed with a glass rod on Petri dishes containing PDA + TS medium. Four replicates of each sample were plated and incubated at 28 °C for five days. The fungi that developed were subcultured to acidified PDA medium to obtain pure cultures. Colonies having obviously different cultural features were selected for morphological and molecular identification.

Genomic DNA extraction. Each isolate was grown on PDA medium at room temperature for 10 days. The mycelium was then scraped from the surface of the plate and crushed with a mortar in 1 mL of lysis solution (2% Triton-X 100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0) and 10 µL of protease K (10 mg/mL). Next, 600 µL of the sample were transferred to a 2 mL Eppendorf tube for DNA extraction following the protocol of Bainbridge et al. (1990) with slight modifications.

The DNA pellet from each tube was suspended in 50 µL of TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA). The quality of the DNA was confirmed by electrophoresis in a 1% agarose gel in 1x TAE buffer (Tris Acetate-EDTA) run at 87 V/cm for 1 h. The gel was stained with ethidium bromide (3 mg/L), and the bands were visualized using a Gel Doc 2000 UV transilluminator (Bio-Rad). The DNA concentration was quantified using a Perkin Elmer spectrophotometer (Lambda BIO10), and the samples were diluted to 20 ng/µL for PCR (Polimerase Chain Reaction).

PCR amplification of ribosomal RNA genes. The primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) were used to amplify the ITS region of the 18S rDNA gene (partial sequence); the 5.8S rDNA gene, internal transcribed spacers 1 and 2 (complete sequence); and the conserved domain in 28S rDNA gene (partial sequence). A fragment of 580 bp was expected.

A PCR master mix was prepared in a final volume of 25 µL containing 1x *Taq* DNA polymerase buffer, 2 mM $MgCl_2$, 0.8 mM deoxynucleoside triphosphates (0.2 mM of each), 100 ng DNA, 20 pmol of each primer, and two units of Go*Taq* DNA (Promega). PCR amplifications were performed with an initial denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min; followed by a single final extension cycle at 72 °C for 10 min.

All PCR reactions were carried out in a Peltier Thermal Cycler PTC-200 (Bio-Rad), and the PCR products were verified by loading 5 µL in a 1.2% agarose electrophoresis gel, which was stained as described above. The remaining amplified PCR products were purified using a purification kit (Promega) following the manufacturer's instructions. To ensure that there was no misreading, the PCR-products were sequenced in both directions using an Applied Biosystems model 3130 automated DNA sequencing system (Applied BioSystems).

Phylogenetic analysis of ITS. Sequences corresponding to both regions were assembled and edited using Bioedit software version 7.1.3 (Hall, 1999) and a consensus sequence of each isolate was created and submitted to BLASTN 2.2.19 (Zhang et al., 2000).

For evolutionary analyses, all consensus sequences were compiled into a single file (FASTA format) and aligned using the profile mode, Clustal W 1.81 (Thompson et al., 1994) included in the MEGA 5 software (Tamura et al., 2011).

Phylogenetic reconstructions were performed for the ITS dataset using the maximum parsimony method. This analysis was conducted using the Close Neighbour Interchange (CNI) search option (level = 1) with initial tree by random addition (10 reps), and gaps/missing data were considered as complete deletions.

To determine the confidence values for clades within the resulting tree, a bootstrap analysis was assessed with 1000 replicates (Felsenstein, 1985). The accession numbers of *F. oxysporum*, *F. subglutinans*, and *F. solani* deposited in the NCBI Gen Bank database were downloaded and included as reference species along with the sequences obtained in this study. *Phoma herbarum* (accession number EU082106) was designated as out group for construction of the evolutionary tree (Fig. 2).

Nucleotide sequences. The new sequences obtained in this study were deposited in the Gen Bank at the NCBI. For cases in which multiple isolates had an identical sequence, only one accession number was deposited, this representing the common sequence of those isolates for each region.

Microbial antagonism assay. To observe the effects between antagonistic fungi and the isolated pathogens, they were confronted by the dual cultures method (Larralde et al., 2008). One 5 mm-PDA disc of *Fusarium* spp. and/or of *Rhizoctonia* sp. were taken from a 10-day-old culture and placed at one extreme margin of a Petri dish containing acidified PDA medium. Two days later a 5 mm-PDA disc of *Trichoderma* sp. and / or of *Aspergillus* spp. from a 7-day-old culture was put at the other extreme. Petri dishes were then incubated for 8 days at 28 °C.

Visual and microscopic observations were carried out every 24 h to determine the action of *Aspergillus* sp. using the scale proposed by Aquino-Martínez et al. (2008), where the three possibilities were: a) invasion of the pathogen colony by the antagonist, b) cessation of the pathogen colony, and c) mutual antagonism.

The evaluation of *Trichoderma* sp. was based on the scale of Bell et al. (1982), where: 1 = *Trichoderma* completely covers the surface of the medium where the phytopathogen is growing, 2 = *Trichoderma* covers two thirds of the surface of the medium where the phytopathogen is growing, 3 = *Trichoderma* and the phytopathogen each colonize about half of the surface and none of them seems to dominate, 4 = the phytopathogen colonizes two-thirds of the surface and seems to resist the invasion of *Trichoderma,* and 5 = the phytopathogen occupies the whole plate area. This experiment involved four repeats per treatment and the percentage of inhibition was calculated using the formula described by Quiroz-Sarmiento et al. (2008):

Inhibition % = (D1-D2) / D1 x 100

where, D1 = radial growth of the pathogen without antagonist (control) and D2 = radial growth of the pathogen with antagonist.

Statistical analysis. Data were analyzed using ANOVA. When F tests were significant, differences between treatment means were compared using Tukey (p=0.05). Significance levels are reported as *=p<0.05, **=p<0.01, ***=p<0.001, and NS=not significant. The data presented are mean values ± SE (SAS, 2002).

RESULTS AND DISCUSSION

Fungi isolated from roots and stems of tomato plants with wilt symptoms. The localities of Jiquilpan, Cojumatlán of Regules and Nicolas Romero had the highest frequency of fungal isolates with 80%, 69% and 66% respectively. The variability in incidence between localities could be due to any or all of: the sample site, the climate, the crop management and the tomato variety (Quiroga-Madrigal et al., 2007).

In most locations, the incidence of fungi was higher in the root (50% to 74%) than in the stem (24% to 56%) of tomato plants (Fig. 1). This may be because fungi of the genus *Fusarium* mainly penetrate the root at transplanting or when secondary roots emerge. The process of stem colonization causes severe damage to the plant (Rodriguez & Montilla, 2002).

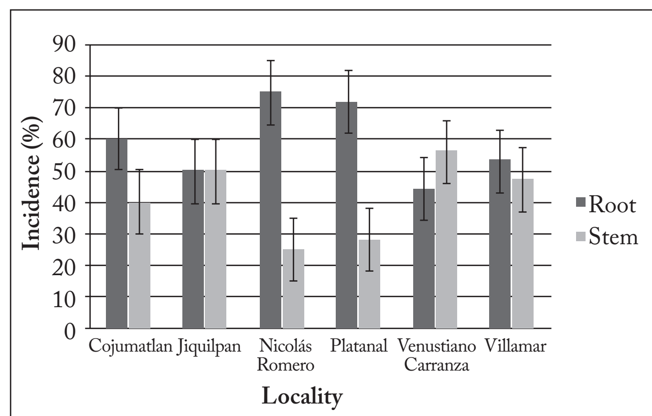

Fig. 1. Incidence of fungi in roots and stems of tomato plants (*Solanum lycopersicum*), in different localities.
Fig. 1. Incidencia de hongos en raíz y tallo de plantas de tomate (*Solanum lycopersicum*), en diferentes localidades.

A total of 28 strains of fungi were obtained from the tissue samples taken from the diseased tomato plants. From the morphological identification methods, 92% of these were *Fusarium* spp. and 5% were *Rhizoctonia* spp. Some strains (3%) could not be identified because sporulation did not occur. Similar results on the incidence of *F. oxysporum* (77% to 100%) were reported in fields of tomatoes in Sinaloa, México (Apodaca-Sánchez et al., 2002).

The isolates of *Fusarium* spp. developed colonies that were, variously: pale pink, purple, orange, red or white-grayish and cottony-looking. They presented microconidia, curved hyaline macroconidia and simple and branched conidiophores (Barnett & Hunter, 1978).

According to the morphological features for each species, *Fusarium oxysporum* microconidia presented ovoid, fusiform macroconidia with 3-5 septa, forming monophyalides, short conidiophores and chlamydospores developed alone or in pairs and usually thick-walled and globose (Nelson et al., 1983; Lugo & Sanabria, 2001).

Fusarium solani formed abundant unicellular microconidia and macroconidia that were cylindrical with rounded basal cells with monophyalides, long conidiophores and with single or paired chlamydospores. *Fusarium subglutinans* was characterized by the presence of poliphyalides with unicellular or septate oval microconidia and abundant macroconidia.

Morphological identification was confirmed at the molecular level by amplifying the Internal Transcribed Space (ITS) of rDNA in the Laboratory of Biotechnology and Seed Pathology at the Colegio de Postgraduados. Three clades were observed, grouped according to reference sequence. Clade I comprised isolates of the *F. oxysporum* species complex, clade

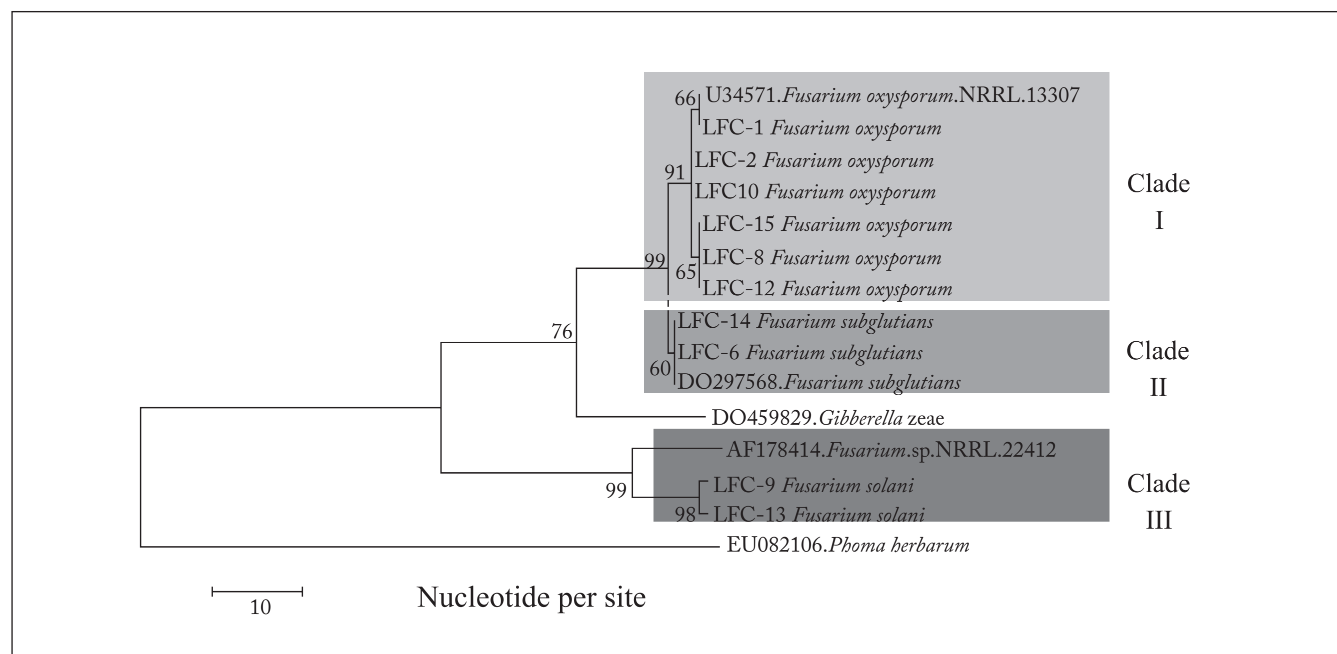

Fig. 2. Phylogenetic tree constructed with sequences corresponding to the Internal Transcribed Space (ITS) of rDNA of *Fusarium* species isolated from the roots and stems of tomato (*Solanum lycopersicum*) cv Saladette, growing in the Cienega de Chapala, Michoacán in 2011.

Fig. 2. Árbol filogenético construido con secuencias correspondientes al Espacio Transcrito Interno (ITS) del rDNA de especies de *Fusarium* aislados de las raíces y tallos de tomate (*Solanum lycopersicum*) tipo Saladette cultivado en la Ciénega de Chapala, Michoacán en 2011.

II isolates were of *F. subglutinans* and are considered within the *Giberella fujikuroi* species complex, and the species of clade III fell within the *F. solani* species complex due to the variability of the isolates within each species (Fig. 2).

The strain of *Rhizoctonia* sp. presented a septate mycelium with white pigmentation in young cultures and brown in mature cultures. Branch angle between the main hyphae was 90˚ (Barnett & Hunter, 1978; Agrios, 1988). The sequences of two isolates of *Rhizoctonia* were deposited in the NCBI Genbank**.**

Isolation and identification of antagonists. The total number of fungi ($6.5x10^4$ to $1.8x10^5$ conidia/g) quantified here in vermicomposted chicken manure was significantly higher than that reported (Nagavallemma et al., 2006) in vermicomposted cow manure ($8x10^4$ conidia/g). The difference in population density of these organisms depends on both the substrate (the sort of manure) and on the earthworm species (*Eisenia foetida*, *Eisenia eugeniae*, *Lampito mauritii* or *Perionyx excavatus*) used for vermicomposting (Parthasarathi et al., 2007). Each worm species has different nutritional requirements while the ingested microorganisms vary with the nature of the organic matter. Therefore, the microorganisms which develop depend on the nutritional makeup of the source (Guedez et al., 2009).

In all, eleven different fungi were obtained from the chicken-manure vermicompost suspensions and ten of these were identified as: *Trichoderma* sp. "VC 1", *Cladosporium* sp. "VC 3", *Curvularia* sp. "CV 4", *Fusarium* spp. "VC 5", "VC 6" and "VC 7", *Penicillium* sp. "VC 8", *Mucor* sp. "VC 10" and *Aspergillus* spp. "VC 9" and "VC 11". Only two of these showed antagonism against the phytopathogens examined here.

In a PDA medium, *Trichoderma* sp. "VC1" presented a white pigment in the early stages of growth and a green one in more mature colonies. It also developed branched conidiophores, simple and grouped phialides, and ovoid conidia cells presented in small terminal clusters. This fungus is very common as a saprophyte and is widely distributed in soils due to its high reproductive capacity and its ability to survive adverse conditions (Barnett & Hunter, 1978; Benitez et al., 2004). *Aspergillus* sp. "VC11" is characterized by developing a green pigmentation on PDA medium with extensive sporulation; morphologically simple, vertically-presented conidiophores, the conidia are formed of a single cell and the surrounding vesicle is globose or claviform (Barnett & Hunter, 1978). Saprophytic fungi such as *Aspergillus* spp. are usually supported by substrates exuded by the roots (Guedez et al., 2009).

Microbial antagonism tests. Of the eleven isolates obtained from chicken-manure vermicompost, only two showed antagonism, *Trichoderma* sp. "VC1" and *Aspergillus* sp. "VC11". Both differed significantly from the control (without antagonist fungus pathogen activity). Inhibition obtained with VC1

was between 45% (against *F. oxysporum*) and 48% (against *F. subglutinans*) (Fig. 3). Similar results obtained Michel-Aceves (2001), were *Trichoderma* spp. showed inhibition of 25% to 69% on *F. oxysporum,* and 30% to 73% on *F. subglutinans*. Also, *T. harzianum* showed a 48% inhibition of *F. oxysporum* f. sp. *lycopersici* (Srivastava et al., 2010). VC1 strain inhibited a 38% to *Rhizoctonia* sp. Based on the scale proposed by Bell et al. (1982), the fungus *Trichoderma* sp. developed a class 2 antagonism on *F. oxysporum*, *F. subglutinans* and *Rhizoctonia* sp., with overgrowth on two-thirds of the surface of the medium (Fig. 3).

Aquino-Martínez (2008) observed inhibition of mycelium growth in *F. oxysporum* f. sp. *dianthi* (FOD) by *Trichoderma lignorum* (Tl) attributing this to mycoparasitism. In general, it is well known that the antagonistic effect of *Trichoderma* spp. on pathogenic microorganisms is attributable to lytic enzymes produced by the fungus that degrade the cell walls of the host (mycoparasitism). Competition for nutrients also exerts a suppressing effect on phytopathogenic activity with these factors together contributing to biological control. *Trichoderma* spp. are also widely distributed in soils all around the world (Aquino-Martínez, 2008; Michael-Aceves et al., 2008).

Meanwhile, *Aspergillus* sp. "VC11" inhibited the growth of *F. oxysporum* by 27% and of *F. subglutinans* by 24%; for *Rhizoctonia* sp. the inhibition was 25% (Fig. 4). Responses of *Aspergillus* and *Trichoderma* as antagonists of *Fusarium* and *Rhizoctonia* were different than those reported in other studies. This is due to the selectivity that the species have, and the origin of evaluated strains (Quiroz-Sarmiento et al., 2008; Guédez et al., 2009).

Due to this antagonistic action, application of chicken-manure vermicompost has considerable potential as a management tool to control root diseases in vegetable crops such as tomatoes. Because it contains *Trichoderma* and *Aspergillus,*

Fig. 3. *Trichoderma* sp. "VC 1" (right) isolated from chicken-manure vermicompost inhibits the growth of (A) *F. oxysporum*, (B) *F. subglutinans* and (C) *Rhizoctonia* sp. (left).
Fig. 3. *Trichoderma* sp. "VC 1" (derecha) aislado de vermicomposta de gallinaza, inhibe el crecimiento de (A) *F. oxysporum,* (B) *F. subglutinans* y (C) *Rhizoctonia* sp. (izquierda).

Fig. 4. *Aspergillus* sp. "VC 11" (right) isolated from chicken-manure vermicompost inhibits the growth of (A) *F. oxysporum*, (B) *F. subglutinans* and (C) *Rhizoctonia* sp. (left).
Fig. 4. *Aspergillus* sp. "VC 11" (derecha) aislado de vermicomposta de gallinaza, inhibe el crecimiento de (A) *F. oxysporum*, (B) *F. subglutinans* y (C) *Rhizoctonia* sp. (izquierda).

and other potentially beneficial microorganisms, it has the potential to suppress the growth of agriculturally important phytopathogens. Moreover, such substrates can also promote growth of natural populations of microbial antagonists by improving soil conditions, thus reducing competition between similar soil microbiota, further enhancing the biological control (Rodríguez-Dimas et al., 2007).

CONCLUSIONS

The fungi *Fusarium* (incidence 92%) and *Rhizoctonia* (incidence 5%) were isolated and identified from tomato plants showing symptoms of wilting at Cienega de Chapala, Michoacán. Under *in vitro* conditions, *Trichoderma* sp. inhibited the development of *F. oxysporum* (by 45%), *F. subglutinans* (by 48%) and *Rhizoctonia* sp. (by 38%) with antagonism class 2. *Aspergillus* sp. also inhibited the same phytopathogens but less effectively (24% to 25%). Soil applications of chicken-manure vermicompost can significantly increase the soil microbe populations (6.5 x 10^4 to 1.8 x 10^5 conidia/g), thus diversifying the microbiota, and promoting the populations of these phytopathogenic antagonists.

REFERENCES

Agrios, G.N. (1988). Plant Pathology; 3rd ed. Academic, San Diego, CA, USA; 803 p.

Apodaca-Sánchez, M.A., E. Zavaleta-Mejia, R. García-Espinoza, S. Osada-Kawasoe & J.G. Valenzuela-Ureta (2002). Frecuencia de campos infestados con *Fusarium oxysporum* f. sp. *radicis-lycopersici* en Sinaloa, México, y su control. *Revista Mexicana de Fitopatología* 20: 1-7.

Aquino-Martínez, J.G. (2008). Biocontrol *in vitro* e *in vivo* de *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. y Delacr.) Snyder y Hans. con hongos antagonistas nativos de la zona florícola de Villa Guerrero, Estado de México. *Revista Mexicana de Fitopatología* 26: 127-137.

Ascencio-Álvarez, A., A. López-Benítez, F. Borrego-Escalante, S.A. Rodríguez-Herrera, A. Flores-Oliva, F. Jiménez-Díaz & A.J. Gámez-Vázquez (2008). Marchitez Vascular del Tomate: I. Presencia de razas de *Fusarium oxysporum* f. sp. lycopersisi (*Sacc.*) Snyder y Hansen en Culiacán, Sinaloa, México. *Revista Mexicana de Fitopatología* 26: 114-120.

Bainbridge, B.W., C.L. Spreadbury, F.G. Scalise & J. Cohen (1990). Improved methods for the preparation of high molecular weight DNA from large and small scale cultures of filamentous fungi. *Microbiology Letters* 66: 113-118.

Barnett, H.L. & B.B. Hunter (1978). Illustrated Genera of Imperfect Fungi. 4th. ed. MacMillan Publishing Co. New York, USA 217 p.

Benítez, T., A.M. Rincón, M.C. Limón & A.C. Codón (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* 7: 249-260.

Bell, D.K., H.D. Wells & C.R. Markham (1982). *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology* 72: 379-382.

Castillo, A.E., S.H. Quarín & M.C. Iglesias (2000). Caracterización química y física de compost de lombrices elaboradas a partir de residuos orgánicos puros y combinados. *Agricultura Técnica* (Chile) 60: 7-79.

De La Cruz-Lázaro, E., R. Osorio-Osorio, E. Martínez-Moreno, A.J. Lozano del Río, A. Gómez-Vázquez & R. Sánchez-Hernández (2010). Uso de compostas y vermicompostas para la producción de tomate orgánico en invernadero. *Interciencia* (Venezuela) 35: 363-368.

Ezziyyani, M., A.A. Sid, S.C. Pérez, M.E. Requena & M.E. Candela (2006). Control biológico por microorganismos antagonistas. *Revista Horticultura* 191: 8-15.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

Fernández, O. & L. Vega (2001). Microorganismos antagonistas para el control fitosanitario. *Manejo Integrado de Plagas* 62: 96-100.

Guédez, C., L.M. Cañizalez, C. Castillo & R. Olivar (2009). Micoflora asociada a dos sustratos orgánicos y su efecto en el control de *Rhizoctonia solani* Kühn. *Agronomía Colombiana* 27: 395-399.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* Ser. 41: 95-98.

Holguin-Castaño, V.A. & J. Mora-Delgado (2009). Dinámica microbiana en vermicompostas comerciales con y sin inoculación del hongo *Trichoderma* spp. *Revista Luna Azul* 29: 18-24.

Larralde, C.C., M.R. Santiago, R.A. Sifuentes, L.I. Rodríguez, P.M. Rodríguez, K. Shirai & Z.J. Narváez (2008). Biocontrol potential and polyphasic characterization of novel native *Trichoderma* strains against *Macrophomina phaseolina* isolated from sorghum and common bean. *Applied Microbiology and Biotechnology* 80: 167-177.

Lugo, Z.C. & N.H. Sanabria (2001). Características culturales y patogénicas en aislamientos de *Fusarium oxysporum* f. sp. *lycopersici* procedentes de plantaciones comerciales de tomate. *Agronomía Tropical* 51: 519-130.

Michel-Aceves, A.C., M.A. Otero-Sánchez, R.D. Martínez-Rojero, R. Ariza-Flores, A. Barrios-Ayala & A. Rebolledo-Martínez. (2008). Control biológico *in vitro* de enfermedades fungosas en tomate *Lycopersicum esculentum* Mill. Avances en Investigación Agropecuaria. Universidad de Colima, México 12: 55-68.

Nagavallemma, K.P., S.P. Wani, L. Stephane, V.V. Padmajan, C. Vineela, M. BabuRao & K.L. Sahrawat (2006). Vermicomposting: Recycling wastes into valuable organic fertilizer. India: International Crops Research Institute for the Semi-Arid Tropics. *SAT eJournal* 2: 16.

Nelson, P.E., T.A. Toussoun & W.F.O. Marasas (1983). *Fusarium* Species: An Ilustrated Manual for Identification. The Pennsylvania State University Press. University Park and London 193 p.

Parthasarathi, K., L.S. Ranganathan, V. Anandi & J. Zeyer (2007). Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. *Journal of Environmental Biology* 28: 87-97.

Quiroga-Madrigal, R., M. Rosales-Esquinca, P. Rincón-Espinosa & E. Hernández-Gómez. 2007. Enfermedades Causadas por Hongos y Nematodos en el Cultivo de Tomate (*Lycopersicon esculentum* Mill.) en el Municipio de Villaflores, Chiapas, México. *Revista Mexicana de Fitopatología* 25: 114-119.

Quiroz-Sarmiento, V.F., R. Ferrera-Cerrato, A. Alarcón & M.E. Lara-Hernández (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.

Rodríguez, D.A. & J.O. Montilla (2002). Disminución de la marchitez causada por *Fusarium* en tomate con extracto de *Citrus paradisi*. *Manejo Integrado de Plagas* 63: 46-50.

Rodríguez-Dimas, N., P. Cano-Ríos, E. Favela-Chávez, U. Figueroa-Viramontes, V. De Paul-Álvarez, A. Palomo-Gil, C. Márquez-Hernández & A. Moreno-Reséndez. (2007). Vermicomposta como alternativa orgánica en la producción de tomate en invernadero. *Revista Chapingo Serie horticultura* 13: 185-192.

Srivastava, R., A.K. Sharma & A. Khalid (2010). Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderm aharzianum* formulation against *Fusarium oxysporum* f. sp. l*ycopersici* for the management of tomato wilt. *Biological Control* 53: 24-31.

Statistical Analysis System SAS (2002). Statistical Analysis System for Windows Version 9. SAS Institute Inc., Carry, NC, USA.

Suárez-Meza, C.L., R.J. Fernández-Barbosa, N.O. Valero, R.M. Gámez-Carrillo & A.R. Páez-Redondo (2008). Antagonismo *in vitro* de *Trichoderma harzianum* Rifai sobre *Fusarium solani* (Mart.) Sacc., asociado a la marchitez en maracuyá. *Revista Colombiana de Biotecnología* 5: 35-43.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.

Thompson, J.D., D.G. Higgins & T.J. Gibson (1994). Clustal-W improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Villa-Briones, A., E. Zavaleta-Mejía, M. Vargas-Hernández, O. Gómez-Rodríguez & A. Ramírez-Alarcón (2008). Incorporación de vermicomposta para el manejo de *Nacobbus aberrans* en jitomate (*Lycopersicon esculentum* Mill.). *Revista Chapingo Serie horticultura* 14: 249-255.

White, T.J., T. Bruns, S. Lee & J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., D. H. Gelfand, J.J. Sninsky, & T.J. White (eds), pp. 315-322. PCR protocols: A guide to methods and amplifications. Academic Press, San Diego, California, USA.

Zhang, Z., S. Schwartz, L. Wagner & W. Miller (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203-14.