

***Trichoderma* sp Native from Chili Region of Poanas, Durango, Mexico Antagonist against Phytopathogen Fungi**

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Abstract: Problem statement: Presence of *Trichoderma* spp. in agricultural soils decrease incidence of diseases by phytopathogen fungi. Sanity diagnostic require to know if exist beneficial microorganism and what agricultural practices help to their propagation. **Approach:** Samples (30) were taken from soils and sick plants of ten sites in four localities of Valley of Poanas. *Phytophthora capsici* Leo, *Rhizoctonia solani* Kühn and *Trichoderma* sp were isolated in agar V8 and were identified by microscopy. **Results:** In the 30 samples analyzed the presence of *Phytophthora capsici* Leo and *Rhizoctonia solani* Kühn was determined. Two isolations of *Trichoderma* sp were obtained from soil, they had antagonist activity against to *P. capsici* and *R. solani* on agar-V8 medium and showed chitinase activity. Sugar production in chitinase (10 mg.mL^{-1}) by crude extract of *Trichoderma* growth in basal medium more chitin was determined. The average of sugar production from strains were 0.1175 and 0.1125 mg.mL^{-1} and standard deviations were 0.0567 and 0.0567 in four repetition. Interviews were applied to fifty farmers about cultivars and cultivation practices. At least seven types of chili were cultivated in the region of the Valley of Poanas, inorganic fertilization, irrigation systems by channel, gates and pumps were used. One hundred percent of farmers reported diseases of Damping off and Phytophthora root. Biocides were not used to control these diseases. **Conclusion:** The natural presence of *Trichoderma* spp was detected in Valley of Poanas, but some practices as inorganic fertilization and irrigation system can be contributing to propagation of phytopathogen fungi.

Key words: Damping off, Phytophthora root rot, chitinase activity, phytopathogen fungi, *Trichoderma* spp, *Capsicum annuum*, sick plants, antagonist microorganism, antagonist activity

INTRODUCTION

Mexico is the second producer of chili in the world and the state of Durango occupied the fifth place at national level. Cultivation of chilis (*Capsicum annuum*) in Valley of Poanas, Durango, Mexico is an important activity, generating an average of 158 wages per hectare. In the Valley 5, 000 ha are cultivated, contributing 75% of the production and surface dedicated to chili in Durango (SAGARPA, 2010). The Valley of Poanas is localized in the southeast of the state of Durango and is formed by mountain chain and plains belonging to Western Chain Mountain Mother ("Sierra Madre Occidental") at municipalities SÚchil, Vicente Guerrero, Nombre de Dios and Poanas, Dgo. The chili is the main vegetable cultivated in the region. This crop suffers different kinds of problem as hail,

floods and attack of phytopathogen. Damping off, stem and foliar blight, Phytophthora root rot, are pathologies caused by fungi as *Phytophthora capsici* Leo, *Fusarium* spp., *Rhizoctonia solani* Kühn., *Alternata* spp. and are factors limiting yield (Fayzalla *et al.*, 2009; Bnejdi *et al.*, 2010). One method to control the phytopathogen fungi is by antagonist microorganisms as *Trichoderma* spp. That is possible due to species of *Trichoderma* have lytic activity against to phytopathogen, compete for substrates, synthesize antibiotics and confer resistance to plants against phytopathogen fungi (Schuster and Schmoll, 2010), besides offer the advantages of: does not pollute, can be used with chemical fungicides and copper (Schuster and Schmoll, 2010). There are commercial products of *Trichoderma*, although species of this genus are distributed in agricultural soils. The objectives of this research were

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(1) know if there is the natural presence of *Trichoderma* spp. in crops affected by damping-off and Phytophthora root rot and (2) obtain native strains of *Trichoderma* spp. and identify them.

MATERIALS AND METHODS

Ten samples of soil, 10 of root and 10 of stem were taken from the region of the Valley of Poanas, Dgo during febrero to July of 2010. Ten sites were established in four localities of the Valley for sampling. In the Table 1 are described names of the cultivar, phase of the cultivar (seedbed, flowering and fructification), locality and coordinates. The places were selected randomly with a condition to have problem of damping-off and Phytophthora root rot.

Agar-V8 mediums were inoculated with samples of root, stem plant and soil. The petri dishes were incubated at 37°C during 24, 48 and 72 h. *Trichoderma* sp and phytopathogen fungi strains were isolated of the mediums and transferred to fresh medium. Mycelium of the isolates were visualized by microscopy and photographs were taken. Tests of parasitism were developed. Agar-V8 mediums were inoculated with *Phytophthora* and *Trichoderma* at the same time. Two types of assays were evaluated: (1) inoculum of each one in the extreme of the dish and (2) inoculum of each one in the middle of the dish in the same place. The inocula were obtained from one cm of the radio of center of the dish and their sizes were of 0.5 cm². The dishes were incubated at 37°C during 24, 48 and 72 h. Photographies were taken of the myceliums obtained. Tests of chitinase activity were developed according to (Gonzalez *et al.*, 2010); isolated strains of *Trichoderma* were cultivated in basal medium with chitin (5 mg. mL⁻¹) during 48 h. Crude extracts of 500 µL were transferred to new tube with 500 µL of chitin (10 mg. mL⁻¹) and were incubated at 37°C during 3 h. Production of sugars in crude extracts was quantified by dinitrosalicylic acid method. Interviews were done to 50 farmers about cultivar, kind of fertilization, irrigation system, pathologies observed and fungicides, insecticides applied.

RESULTS

R. solani and *P. capsici* were detected in 10 samples of each following: soil, roots and stems. Pathogens were identified by microscopy (those results are not show). Damping off was observed by the loss of plants and root rot in seedbed. Phytophthora root rot by wilt of plant and fungi in root. In the plots, ever there were plants healthy at sides of sick plants.

Trichoderma sp strains were isolated from two samples of soil from two different sites one of sampling in seedbed (site 3, Table 1) and other in plot at flowering (site 9). *Trichoderma* strains were identified by microscopy and by morphology in medium agar-V8 according description of (Infante *et al.*, 2009; Schuster and Schmoll, 2010). White mycelium appeared at 24 h of growth on agar v8 and after 48 h turned green. In microscopic views, it was possible watch: conidiophore were branched that finished in phialides where conidia were formed (Fig. 1).

Trichoderma sp inhibited the growth on agar v8 of *P. capsici* and *R. solani* as show in the Fig. 2. When the inocula were placed in middle of the dish *Trichoderma* sp covered the most part of the dish at 72 h and when the inocula of each one were placed in opposite side, at 72 h of growth *Trichoderma* began to invade the mycelium of *R. solani*. Parasitism of *Trichoderma* sp over *P. capsici* and *R. solani* was observed by microscopy (Fig. 3).

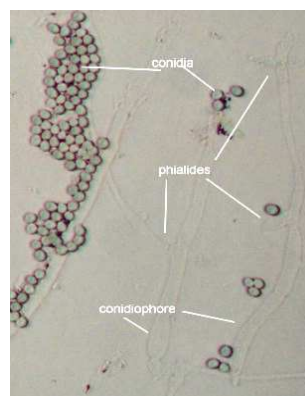


Fig. 1: Identification of *Trichoderma* sp by microscopic view (400x)

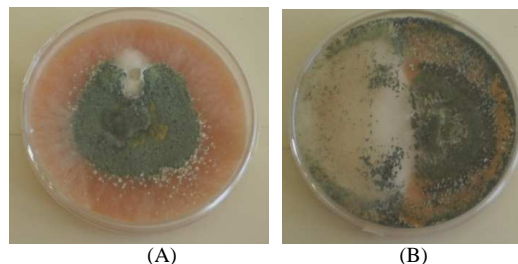


Fig. 2: Inhibition of propagation of *R. solani* by *Trichoderma* sp (A) *R. solani* and *Trichoderma* spp were inoculated in the center of the dish; (B) *R. solani* and *Trichoderma* sp were inoculated in opposite position in the dish

Table 1: Description of sites of sampling

Site	Type of chili	Phase of cultivar	Municipality of Durango, México	Coordinates
1	“Puya”	Seedbed	Sta. Cruz de Guadalupe	N 23°51.284´ W 104°10.624´ Altitude: 6079 ft
2	“Puya”	Seedbed	Potosi	N 23°56.832´ W 104°06.355´ Altitude: 6190 ft
3	Poblano “Puya” blond	Seedbed	Orizaba	N 23° 57.348´ W 104° 03.870´ Altitude: 6302 ft
4	“Puya” Poblano	Seedbed	Orizaba	N 23°57.411´ W 104°04.215´ Altitude: 6315 ft
5	“Puya”	Seedbed	Murguía	N 23°48.392´ W 104°05.656´ Altitude: 6207 ft
6	Poblano	Flowering	Orizaba	N 23°57.503´ W 104°04.010´ Altitude: 6320 ft
7	“Puya”	Flowering Fructification	Sta. Cruz de Gpe	N 23°51.014´ W 104°09.759´ Altitude: 6142 ft
8	“Puya”	Flowering Fructification	Sta. Cruz de Gpe	N 23°51.575´ W 104°09.723´ Altitude: 6135 ft
9	“Puya”	Flowering Fructification	Sta. Cruz de Gpe.	N 23°51.269´ O 104°09.812´ Altitude: 6112 ft
10	“chilaca” “Jalapeño” Poblano	Flowering Fructification	Potosi	N 23°56.871´ W 104°06.353´ Altitude: 6208 ft

Table 2: Production of sugar from chitin by crude extract of *Trichoderma* sp in equal volumes

Repetition	Production of sugars (mg.mL ⁻¹)	
	Strain 1 (site 3)	Strain 2 (site 9)
1	0.1000	0.1500
2	0.2000	0.0800
3	0.0700	0.1700
4	0.1000	0.0500
Average	0.1175	0.1125
Standard deviation	0.0567	0.0567

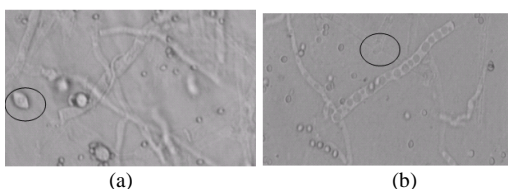


Fig. 3: Micoparasitism views. A: Hyphae coiled of *Trichoderma* sp on *P. capsici*. (Circle closed spore of *P. capsici*). B: Micoparasitism of *Trichoderma* sp on *R. solani* (Circle closed typical “T” form of *R. solani*)

Enzymatic activity was found, production of sugar was obtained from chitin (10 mg.mL⁻¹) with crude extract of *Trichoderma* sp. The quantities determined ranged from 0.05-0.2 mg.mL⁻¹ (Table 2).

The results of the interviews showed that types of (*Capsicum annum*) cultivated in The Valley of Poanas, Dgo. are: “puya or guajillo”, “jalapeno”, “serrano”, “habanero”, “güerito” “rat tail” “chilaca”, blond and “ancho or Poblano” (Fig. 4).

The farmers whom cultivate Poblano chili use creole or commercial seed. The name of commercial seed is Caballero chili. All others seeds of chilis used are creole. “Puya or guajillo” is the type of chili that 90% of farmers cultivate in major volume (more that 50% of crop) and this is commercialized dried. Fertilization is inorganic, the fertilizers used are urea, diammonium phosphate and potassium chloride. The organic fertilization is not used. Compost or liquid humus are applied occasionally when someone more recommends. Irrigation systems are by hose, multigates, channel and pump. The cultivar of chili is of irrigation although on july-august-september is rain time. Waterlogging are common in plots. The pathologies described in cultivar of chili by farmers and observed in field trips were: damping off, stem and foliar blight and *Phytophthora* root rot. Fifty of fifty farmers reported those diseases in cultivars of chili. The products used against pest and diseases are: Ridomil®, Captan®, Previcure®, Paration®, Tamaron®, MaxiGrow®, lime. Biocides are not used.

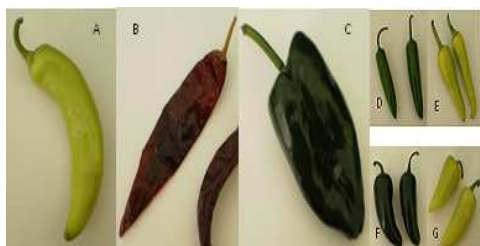


Fig. 4: Types of chilis (*Capsicum annuum*) cultivated in The Valley of Poanas, Dgo; (A) “chilaca”; (B) dried “puya”; (C) Poblano; (D) “serrano”; (E) “güerito” (F) “jalapeño”; (G) blond

DISCUSSION

The practices of farmers of cultivating different varieties, select seeds and rotation crops, permits to say that biodiversity of chili in the Valley of Poanas, Dgo. is preserved. The diseases of damping off and Phytophthora root rot are generalized in all chili cultivar of the region and are due to phytopathogen fungi as *R. solani* and *P. capsici* (Steiner *et al.*, 2008; Fayzalla *et al.*, 2009). In attention to observations that exist healthy plants at sides of affected plant, it's possible say there is natural resistance to phytopathogen. It's probably that natural presence of *Trichoderma* spp. in soil of plots is contributing to resistance like reported for (Ningthoujam *et al.*, 2009; Al-Taweil *et al.*, 2009); but some practices as entirely inorganic fertilization, irrigation systems used and waterlogging frequently are not recommend to bring down phytopathogen fungi and to increase the population of antagonist (Hayyan, 2009).

Trichoderma spp. are present in soils of Valley of Poanas, Dgo. The strains isolated have antagonist activity against *P. capsici* and *R. solani* as is mentioned for some species of *Trichoderma* (Argumedo *et al.*, 2009; Schuster and Schmoll, 2010). By microscopy we could see hyphae coiled of *Trichoderma* sp on *P. capsici* as described (Infante *et al.*, 2009). The isolation obtained have chitinase activity as described (Infante *et al.*, 2009; Gonzalez *et al.*, 2010). However, the problem of Damping off and Phytophthora root rot is generalized in plots. Addition of inorganic fertilizers, no disinfection of seed and use of insecticides can be affecting the presence of *Trichoderma* spp. in soils and favor the propagation of phytopathogen fungi (Al-Taweil *et al.*, 2009).

CONCLUSION

The natural presence of *Trichoderma* spp was detected in Valley of Poanas, but some practices as inorganic fertilization entirely and irrigation systems can be contributing to propagation of phytopathogen fungi. It's necessary to reconsider use of compost and liquid

humus, developing different irrigation systems as drip and implement disinfection of seed in order to stimulate the propagation of *Trichoderma* spp., decrease the level of phytopathogen fungi and improving the system production of chili in Poanas, Dgo.

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