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Biological Control of *Fusarium oxysporum* f.sp. *lycopersici* Isolated from Algerian Tomato by *Pseudomonas fluorescens*, *Bacillus cereus*, *Serratia marcescens* and *Trichoderma harzianum*

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Abstract: Evaluation of the antagonistic activity of three bacterial and a fungi with direct confrontation method and the filtrates culture against the growth of *Fusarium oxysporum* f.sp. lycopersici showed the inhibition of the mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* with *Bacillus cereus* energized the low activity and it was more significant with *Serratia marcescens* and *Trichoderma harzianum* for the 2nd day but with *Pseudomonas fluorescens*, it was for the 5th day. The filtrates of culture of these antagonists showed that only *Serratia marcescens* and *Trichoderma* sp. have a rate of inhibition which varies between (40-95%) and of (20-30%) with *Pseudomonas fluorescens*.

Key words: Fusarium, biological control, pseudomonas, *Bacillus serratia*, trichoderma, antagonisms inhibition

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and widely cultivated vegetable crops. Fusarium is one of the most important genera of plant pathogenic fungi with a record of devastating infections in various economically important plants (Messian *et al*., 1991; Armstrong and Armstrong, 1981). The tomato grower is faced with the danger of losses due to *Fusarium oxysporum* f.sp. *lycopersici* (Fol) W.C. Snyder and H.N. Hans the causal agent of vascular wilt. The vascular wilt fungus *Fusarium oxysporum* is a soil borne facultative parasite. The fungus enters the host roots directly through penetration hyphae and colonizes the cortex by intracellular and intercellular growth (Fuchs *et al*., 1997; Di Pietro *et al*., 2001). As in other Fusaria, its identification has generally been based on morphological criteria such as the shape of micro and macroconidia, structure of microconidiophores and formation and disposition of chlamydospores (Henni *et al*., 1994; Di Pietro *et al*., 2003). The biocontrol of the phytopathogens depends primarily on the choices the antagonistic and costs also with the knowledge of the biological characteristics of the fungi or bacterial materials used. It must also take account of the influence which the medium (ground) exerts on the various stocks. One can also expander of beneficial fungi of the Trichoderma kind for a fight against various pathogenic. Colonizing micro-organisms of the anthers able to use the tartaric acid made it possible to reduce the rate of fusariose of ear (Khan *et al*., 2001). The objective of this research is to study *in vitro* the inhibiting effects of antagonistic agents with *Fusarium oxysporum* f.sp. *lycopersici* and to also know some their mechanisms of action. This *in vitro* study will give only one vision partial of these mechanisms of inhibition. From other that it is quite clear, the inhibiting actions *in vitro* are obtained under controlled conditions. These conditions can be different from those which exist in nature. However, this *in vitro* study is very significant because it will enable us to select the antagonists initially and to consider in the second time their use with the fields.

MATERIALS AND METHODS

***Fusarium oxysporum* isolates:** Diseased tomato plants were collected from west Algeria. Ten *F. oxysporum* isolates were obtained from xylem tissues showing typical. Pieces of vascular tissue were excised and placed aseptically on PDA medium. Plates were incubated at 22°C under 12 h photoperiodic. The identification of Fusarium isolates was made on basis of their morphological characters (Henni *et al*., 1994). From each field one *F. oxysporum* monoconidial isolate was obtained and maintained on PDA media at 22°C.

Microorganisms used for antagonism tests: *Pseudomonas fluorescens*, *Bacillus cereus* and *Serratia marcescens* bacteria cultures and *Trichoderma harzianum*

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were obtained from the microbial collection of the laboratory applied microbiology of Faculty of Science, University of Oran.

***In vitro* plate assay for evaluation of antagonistic activity:** In order to test bacterial and fungal activity against *Fusarium oxysporum* f.sp. *lycopersici* isolate, agar plugs containing both categories of fungal isolates were placed on the PDA plates about 5 cm apart from each other and assessed for about 10 days whether any inhibition zone was appeared or not. Three replicate plates were used for each fungal isolate (Abeysinghe, 2006).

Antagonism of bacteria: Discs (5 mm) of 7 days old mycelium of *Fusarium oxysporum* f.sp. *lycopersici* were placed in the centre of plats with PDA. Each colony of *Pseudomonas fluorescens, Bacillus cereus and Serratia marcescens* were placed equidistant sites 1 cm from plate periphery. After 7 days of inoculation at 25°C. The percentage of antagonistic colonies of bacteria inhibiting growth of *Fusarium oxysporum* f.sp. *lycopersici* mycelium in the whole population was assessed (Szczech, 1999; Agarry *et al*., 2005; El-Hamshary and Khattab, 2008).

Antagonism of fungi: Two agar discs (5 cm), one with the mycelium of *Fusarium oxysporum* f.sp. *lycopersici* and another with mycelium of *Trichoderma harzianum* were placed 2 cm apart in the centre of PDA plate. Three replicates with each fungus were prepared. The plates were incubated for 10 days at 25°C. Measurement of the percentage inhibition and intercolony distance were taken daily for 7 days (Szczech, 1999; Hibar *et al*., 2005; Agarry and Osho, 2005; Srinon *et al*., 2006; Nikam *et al*., 2007).

Method of the filtrates of culture: Agar discs (5 mm in diameter) of *Trichoderma harzianum* inoculated in 250 mL erlenmeyer flasks containing 50 mL of PD broth. Suspensions of *Pseudomonas fluorescens* and *Serratia marcescens* were inoculated individually with GN broth. For each experiment, flasks in triplicate were incubated at 30°C for 7 days for fungal and 48 h for bacteria. The filtrate culture of fungi and bacteria were removed by centrifuging the cultures at 9000 rpm for 30 min. The supernatant was used immediately (Agarry *et al*., 2005).

About 1 mL of filtrate culture of the fungi and bacterial was added in PDA, then disc of *Fusarium oxysporum* f.sp. *lycopersici* was placed in center of a petri plate containing PDA and filtrate culture. Plates were cultured for 7 days at 28°C and fungal growth was measured and compared to control growth where the filtrate culture was replaced with sterile distilled water.

RESULTS AND DISCUSSION

The resultants of the direct confrontation between *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Serratia marescens* with *Fusarium oxysporum* f.sp. *lycopersici* (Fig. 1) shows that the mycelia growth is inhibited after 3 days incubation with *Trichoderma harzianum* and for to 2 days contact with the bacterial colonies and in the 5th day growth of *Fusarium oxysporum* f.sp. *lycopersici* is inhibited completely showed that *Pseudomonas aeruginosa* and *Serratia marcescens* are antagonistic potentials agents Kumar *et al*. (2002) confirm that *Pseudomonas fluorescens* has a strong antifungal activity against *Fusarium oxysporum*, mainly by the production of the antifungal metabolites.

In the other hand with Bacillus *cereus*, one notes that the mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* is only very slightly affected. However, Gomez (1981) obtained with *Bacillus cereus* str.C-3 of good result with respect to *Fusarium*. On the other, Podilli and Dube (1985), one found that *Bacillus subtilus* does not inhibit F.o.l, *Fusarium oxysporum* f.sp. *vasinfectum* and *Verticillium dahliae* with multiplied by 10

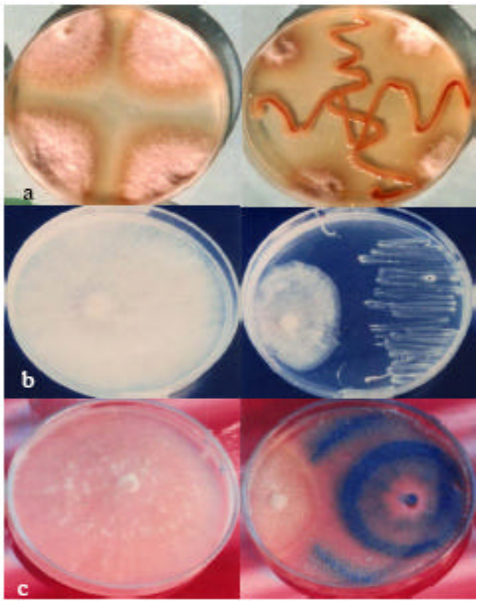

Fig. 1: Direct confrontation between *Fusarium oxysporum* f.sp. *lycopersici*; a) *Serratia marescens*, b) *Pseudomonas fluorescens* and c) *Trichoderma harzianum*

concentrations (Basha and Ulaganathan, 2002) isolated *Bacillus* BC121 of the rhizosphere of the sorghum which showed a high antagonistic activity against *Curvularia lunata* and the study of the antifungal activity of *Bacillus coagulans* against three pathogenic species of *Fusariurn* (Czaczyk *et al*., 2002).Abdel-Salam *et al*. (2007) test the capacity of seven antagonistic potential strains, four *B. subtilis*, *P. aeruginosa, P. fluorescens* and *E. cloacae* to inhibit the plant fungal pathogen *F. oxysporum*, these researchers demonstrated the highest antagonistic was *P. aeruginosa* followed by *E. cloacae* and *P. fluorescens*, these resultants concord and confirmed *Pseudomonas fluorescens* strong antifungal activity against *Rhizoctonia bataticola* and *Fusarium oxysporum*, mainly through the production of antifungal metabolites (Kumar *et al*., 2002). The filtrate culture of *Pseudomonas fluorescens* does not given any inhibiting effects on the parasite just after 5 days of incubation and the percentage of inhibition was 20-30% but the inhibition mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* showed clearly with only 1 mL filtrate culture of *Trichoderma harzianum* and *Serratia marescens* (Fig. 2) when the percentage of inhibition energized between 40-95% (Fig. 3). According to studies of Rosenzweig and Stotzky (1980) on

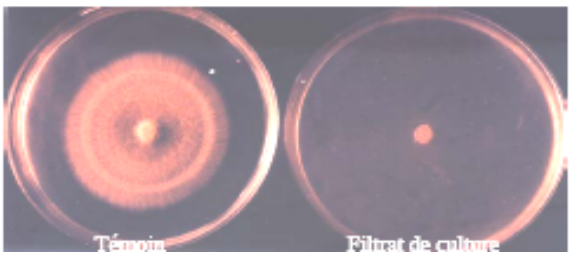

Fig. 2: Anatafonist activity of the filtrate culture of *Trichoderma harzianum* diffuse in PDA between *Fusarium oxysporum* f.sp. *lycopersici*

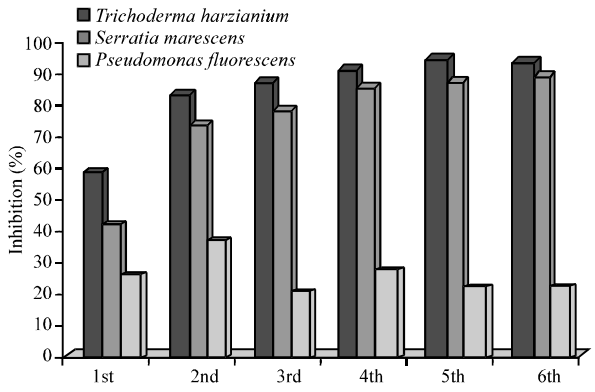

Fig. 3: Anatafonist activity of the filtrate culture of *Trichoderma harzianum* diffuse in PDA between *Fusarium oxysporum* f.sp. *lycopersici*

Aspergillus niger, the antagonistic action of *Serratia marescens* is primarily due to secretion in the medium of its pink pigment the prodigiosine.

Among 62 isolates tested against *Rhizoctonia solani*, 226 isolates had an ability to overgrow *R. solani* completely (Sawangsri *et al*., 2007), the tests of direct confrontation, on PDA medium between *Fusarium oxysporum* f.sp. *radicislycopersici* and *Trichoderma harzianum* revealed that the latest has inhibited mycelial growth of the pathogen by >65% compared to the control after 4 days (Hibar *et al*., 2005), the same when tested produced a metabolite on PDA medium and the filtrates cultures of *Trichoderma harzianum* that inhibited growth of plant pathogenic fungi *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum* and *F. moniliforme* (Kucuk and Kivan, 2003, 2004).

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