Potential of Non-Pathogenic *Fusarium oxysporum* Isolates for Control of Fusarium Wilt of Tomato

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**ABSTRACT**
This study was done to evaluate the efficiency of non-pathogenic *Fusarium oxysporum* isolates (141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257) in controlling vascular wilt caused by *F. oxysporum* f. sp. *lycopersici*, race 2 (isolates C-21A, TO11, and TO245) in tomato (*Lycopersicon esculentum* cv. Viradoro seedlings). In order to determine the effect of non-pathogenic *F. oxysporum* isolates in tomato plants, the root system of 30-day-old seedlings was immersed in conidial suspensions (10^5 ml^-1) of each isolate and then transplanting them into substrates previously infested with isolates of *F. oxysporum* f. sp. *lycopersici*, race 2 (10^6 conidia ml^-1 of substrate). Evaluations were performed 35 days after transplanting, for severity in scale with 1=healthy plant to 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot and seedling height. The non-pathogenic *F. oxysporum* isolates were efficient in reducing the severity of the disease and maintaining normal plant development. These results provide evidence of the antagonistic activity of non-pathogenic *F. oxysporum* isolates in controlling vascular wilt caused by *F. oxysporum* f. sp. *lycopersici* race 2 in tomato.

**Additional keywords:** biological control, *Fusarium oxysporum* f. sp. *lycopersici* race 2, nonpathogenic *F. oxysporum*.

**RESUMO**

Potencial de isolados de *Fusarium oxysporum* não patogênico no controle da murcha de Fusarium do tomateiro

O trabalho avaliou a eficiência dos isolados (141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5 e 257) de *Fusarium oxysporum* não patogênico ao tomateiro (*Lycopersicon esculentum*) no controle da murcha vascular causada por *Fusarium oxysporum* f. sp. *lycopersici*, raça 2 em plântulas de tomateiro cv. Viradoro. Para verificar o efeito dos isolados de *F. oxysporum* não patogênicos, o sistema radicular de plântulas de tomateiro, com 30 dias de idade, foi imerso na suspensão de conídios (10^5 ml^-1) e as mudas transplantadas para substrato de cultivo. Após 35 dias do transplante foi verificado que esses isolados não foram patogênicos às plantas de tomateiro, nem afetaram o desenvolvimento das mudas. A eficiência dos isolados de *Fusarium oxysporum* não patogênicos no controle da murcha foi determinada imergindo-se as raízes de mudas de tomateiro em suspensão de conídios (10^5 conídios ml^-1) e transplantando-as em substratos previamente infestados com os isolados de *F. oxysporum* f. sp. *lycopersici*, raça 2 (10^6 conídios ml^-1 de substrato). Transcorridos 35 dias do transplante, foram realizadas as avaliações da severidade na escala de 1=planta saudável a 6=planta morta ou com vasos coloridos e folhas murchas até o ponteiro e altura das mudas. Os isolados de *F. oxysporum* não patogênicos foram eficientes em reduzir a severidade da doença e em manter normal o seu desenvolvimento. Esses resultados evidenciam a atividade antagônica dos isolados de *F. oxysporum* não patogênico no controle da murcha vascular do tomateiro, causada por *Fusarium oxysporum* f. sp. *lycopersici* raça 2.

**Palavras-chave adicionais:** controle biológico, *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* não patogênico.
reported in several countries. For this reason, alternative methods of controlling the disease have been studied, with emphasis on biological control. Soils naturally suppressive to Fusarium wilt (Garibaldi et al., 1990; Alabouvette, 1999) have been reported in different regions of the world. Although several antagonistic microorganisms have been evaluated to control Fusarium wilt, the most promising are non-pathogenic F. oxysporum isolates (Rouxel et al., 1979; Garibaldi et al., 1987; Minuto et al., 1995ab). Saprophytic species of Fusarium have been found to be effective in reducing F. oxysporum in cyclamen (Cyclamen persicum Mill.), gerbera (Gerbera jamesonii Hook.), basil (Ocimum basilicum L.), asparagus (Asparagus officinalis L.), eggplant (Solanum melongena L.), carnation (Dianthus caryophyllus L.), watermelon [Citrullus lanatus (Thumb.) Matsumi & Nakai], tomato, chick pea (Cicer arietinum L.) and cucumber (Cucumis sativus L.) (Mandeel & Baker, 1991; Postma & Rattink, 1992; Yamagushi et al., 1991; Hervás et al., 1995; Minuto et al., 1995ab; Larkin & Fravel, 1999; He et al., 2002; Reid et al., 2002).

The objective of this work was to evaluate the efficiency of non-pathogenic F. oxysporum isolates for biological control of tomato wilt caused by F. oxysporum f. sp. lycopersici race 2.

Tomato cv. Viradoro, resistant to race 1 but susceptible to race 2 of F. oxysporum f. sp. lycopersici, was used in all assays. The tomato seedlings were produced for transplanting on Multihort® planting substrate in a styrofoam tray (35 mm × 35 mm) in a greenhouse.

The F. oxysporum f. sp. lycopersici race 2 isolates were supplied by Dr. Sami J. Michereff, Universidade Federal Rural de Pernambuco (isolate C-21A) and by Dr. Angel Garibaldi, from Università degli Studi di Torino, Italy. The antagonistic isolates were introduced into Brazil through Laboratório de Quarentena Costa Lima (Brazil’s official quarantine facility), of Embrapa Meio Ambiente (MA Proceeding no. 21052.011767/99-04). The inocula of all isolates were produced in potato-dextrose broth in shake culture (150 rpm), for ten days, at 25±2 °C.

Effect of Fnp isolates on tomato

The root systems of tomato seedlings were washed in tap water, then immersed in a conidial suspension (10⁶ ml⁻¹) of respective non-pathogenic F. oxysporum isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, for five min. The seedlings were then transplanted to 500 ml pots containing the substrate. In addition to the non-pathogenic F. oxysporum isolates, the assay included a non-inoculated control and a control treated with the autoclaved PD culture medium. The plants were grown in the greenhouse and evaluations for disease severity and plant height were performed 35 days after transplanting, as previously described.

Effect of Fnp isolates on the control of Fusarium wilt in tomato

The tomato seedling root system was immersed in a conidial suspension (10⁴ ml⁻¹) of non-pathogenic F. oxysporum isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, for five min, after which the seedlings were transplanted to a substrate previously infested with a F. oxysporum f. sp. lycopersici isolates C-21A, TO11 and TO245 (10⁷ ml⁻¹ of substrates). The plants were grown in the greenhouse and evaluations for disease severity and plant height were performed 35 days after transplanting, as previously described.

Statistical analysis

A completely randomized experimental design with ten replicates was adopted for all assay. For the statistical analysis the data were transformed to sqrt (x + 0.5) and compared by the Tukey test at 5% probability, using the SAS System Software Package, version 8.

Race 2 of F. oxysporum f. sp. lycopersici isolates C-21A, TO11 and TO245 were found to be pathogenic to the cultivar Viradoro at all inoculum concentrations tested (Table 1), causing a drastic reduction of plant height. The isolate TO245 was the most virulent, causing the maximum diseases severity in plants grown in substrate infested with 10⁴ and 10⁵ conidia ml⁻¹ of substrate. These results agree with those
of Andrade & Micherref (2000), who demonstrated that tomato plants of different cultivars, inoculated with 10⁶ conidia ml⁻¹ of isolates C-1, C-7, C-21A, and F-23 of *F. oxysporum* f. sp. *lycopersici* race 2, showed a 50% disease incidence. He *et al.* (2002) also showed that 10⁶ CFU g⁻¹ soil of *F. oxysporum* f. sp. *asparagi* caused the death of asparagus plants.

Tomato seedlings whose root systems were immersed in the conidial suspension of non-pathogenic *F. oxysporum* isolates 141/3, 233, 233/1, 245, 245/1, 251, 251/2, 251/5, and 257, did not show symptoms of vascular diseases and developed normally. The non-pathogenic *F. oxysporum* isolates were obtained from carnation rhizospheres (Garibaldi *et al.*, 1985), so were not pathogenic to the tomato plants. This is important because the same non-pathogenic *F. oxysporum* isolates can be useful for other hosts, as demonstrated by Minuto *et al.* (1995ab) for cyclamen and basil and by Garibaldi *et al.* (1990) for melon (*Cucumis melo* L.) and radish (*Raphanus sativus* L.).

When the tomato seedling root systems were immersed in inocula of non-pathogenic *F. oxysporum* isolates and the plants were grown in substrate previously infested with race 2 of *F. oxysporum* f. sp. *lycopersici* isolates C-21A, TO11 and TO245, all non-pathogenic *F. oxysporum* isolates were efficient in controlling the disease; plants showed lower disease severity and greater height (Table 2), with no significant degree of difference between the non-pathogenic *F. oxysporum* isolates. These results agree with Garibaldi *et al.* (1987), Postma & Rattink (1992), and Minuto *et al.* (1995ab), who reported that non-pathogenic *Fusarium* spp. isolates, introduced by root immersion before transplanting, were efficient in colonizing the rhizosphere and in controlling Fusarium wilt. There are reports of non-pathogenic *F. oxysporum* that show they act by competing for infection sites and for nutrients, and by induction of resistance (Mandeel & Baker, 1991; Alabouvette & Couteaudier, 1992; Larkin & Fravel, 1999; Benhamou *et al.*, 2001). In order to control vascular wilt caused by *F. oxysporum* f. sp. *lycopersici*, with non-pathogenic *F. oxysporum* it is necessary to study the best method for applying the non-pathogenic *F. oxysporum*, i.e., by treating the root systems by deepening or by applying the non-pathogenic *F. oxysporum* in soil/substrate in which the tomato is grown.

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**TABLE 1** - Severity of Fusarium wilt and plant height (cm) of tomato (*Lycopersicon esculentum*) cv Viradoro grown in substrate infested with race 2 *Fusarium oxysporum* f. sp. *lycopersici* isolates

| Inoculum concentration (conidia ml⁻¹ of substrate) | Isolates of *F. oxysporum* f. sp. *lycopersici* |
|---------------------------------------------------|---------------------------------------------------|
| C-21A                                             | TO11                                              |
| **Severity***                                      | **Height**                                        |
| 0                                                 | 1.00c 51.36a                                     |
| 10⁵                                                | 3.16b 38.11b                                     |
| 10⁴                                                | 3.33b 35.71bc                                    |
| 10⁵                                                | 3.66bc 33.83dc                                   |
| 10⁶                                                | 4.16a 30.66c                                     |

*Severity – ratings: 1=healthy plant to 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot. Means followed by the same letter do not differ (Tukey p>0.05).*

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**TABLE 2** - Severity of Fusarium wilt and plant height (cm) of tomato (*Lycopersicon esculentum*) cv Viradoro treated with non-pathogenic *Fusarium oxysporum* isolates and grown in substrates infested (10⁵ conidia ml⁻¹ of substrate) with race 2 of *Fusarium oxysporum* f. sp. *lycopersici*

| Isolate of non pathogenic *Fusarium oxysporum* | Isolates of *F. oxysporum* f. sp. *lycopersici* |
|------------------------------------------------|--------------------------------------------------|
| C-21A                                           | TO11                                             |
| **Severity***                                    | **Height**                                       |
| Control                                         | 1.00b 52.33a                                     |
| Fol                                             | 3.66a 41.36c                                    |
| 233                                             | 2.33ab 49.88ab                                   |
| 233/1                                           | 2.16ab 50.18a                                   |
| 141/3                                           | 2.16ab 51.46a                                   |
| 251                                             | 2.33ab 52.61a                                   |
| 251/2                                           | 2.16ab 44.66b                                   |
| 245                                             | 2.16ab 47.11ab                                   |
| 245/1                                           | 2.00b 48.08ab                                   |
| 257                                             | 1.83b 49.25ab                                   |

*Severity – ratings: 1=healthy plant to 6=dead plant or plant showing vessel browning and wilted leaves up to the leader shoot. Means followed by the same letter do not differ (Tukey p>0.05).*
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