

## Control of *Fusarium* in chrysanthemum with sewage sludge, biofertilizer, hydrolyzed fish, chitosan, and *Trichoderma*

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**Abstract:** *Fusarium oxysporum* f.sp. *chrysanthemi* can cause severe losses in chrysanthemum (*Chrysanthemum morifolium*) in Brazil. This study was done to evaluate the efficacy of sewage sludge, biofertilizer, hydrolyzed fish, chitosan, and *Trichoderma harzianum* to control *Fusarium* in chrysanthemum in substrate composed of pine bark (pH 5.5; EC 0.6  $\mu$ S) obtained from pots of dead chrysanthemum plants. The infested substrate was or was not sterilized in water vapor (2 h; 100°C); with sewage sludge incorporated (0, 10, 20, and 30% v/v). Into these mixtures were added (or not) biofertilizer (14 ml/l), hydrolyzed fish (10 ml/l) and *Trichoderma* ( $10^8$  conidia/ml), transferred to pots (3 l) and planted with 'Yellow-Marino' chrysanthemum seedlings. For all treatments, half of the pots were sprayed with chitosan (200 mg/l) weekly. A multifactorial randomized experimental design with 24 replications, totaling 2560 pots, was adopted. The plants were grown in a commercial greenhouse and evaluations for disease severity (1=healthy plant; to 5=dead plant or wilted leaves) were performed at 8, 12, 14, 18 weeks after transplanting. In addition, plants were classified in commercial (class I, II, III) or not, after 18<sup>th</sup> week. The level of disease control was directly proportional to sewage sludge concentration incorporated in the substrate. Biofertilizer, hydrolyzed fish, chitosan and *Trichoderma* did not control the disease. In general, disease severities were higher in plants growing in sterile substrate when compared the plants growing in non-sterile substrate.

**Key words:** biological control, organic matter, sewage sludge, suppressive soil, *Trichoderma*

### Introduction

Chrysanthemum Fusarium wilt (*Chrysanthemum morifolium*) caused by *Fusarium oxysporum* f.sp. *chrysanthemi* is an important disease of this plant in Brazil, and the disease incidence has increased in recent years. In some growers, the incidence of Fusarium wilt has caused serious losses of around 80%. For the infection of Fusarium in chrysanthemum to take place, wounding is not necessary, but it contributes. Symptoms include yellowing, stunting and wilting of plants, lower leaves, vascular system tissues turning reddish brown and possible plant death. The control of Fusarium wilt in chrysanthemum is a problem because the most important cultivars are not resistant or tolerant and no chemical control is available. Then, the methods of controlling of Fusarium wilt should be preventive and should include the use of suppressive container media. Soil and container media amend with mature compost, fish emulsion, sewage sludge and other organic matters may suppress the soil-borne disease (Chef et al., 1983; Hoitink & Fahy, 1986; Ghini et al., 2002; Santos & Bettiol, 2003; Lazarovits et al., 2008). The use of organic matter originated from waste, besides improving the chemical, physical and biological soils characteristics, reduces the impact in the environment. This study was done to evaluate the efficacy of sewage sludge, biofertilizer, fish hydrolyzed, chitosan, and *Trichoderma harzianum* to control *Fusarium* in chrysanthemum in substrate composed of pine bark obtained from pots of dead chrysanthemum plants.

## Material and methods

The studies were conducted in the greenhouse of a grower with serious problems of *Fusarium* wilt on chrysanthemum. The container media, composed of pine bark (pH 5.5; EC 0.6  $\mu$ S), utilized in the two experiments was obtained from pots containing *chrysanthemum* with high severity of *Fusarium* wilt. The natural infested substrate was or was not sterilized in steam (2 h; 100°C) and sewage sludge was incorporated at these containers media at concentration of 0, 10, 20, and 30% (v/v). Into these mixtures, after 15 days of incubation, in the first experiment biofertilizer (B) (14 ml/l), and *Trichoderma* (T) ( $10^8$  conidia/ml) were added (or not), transferred to pots (3 l) and planted with 'Yellow-Marino' chrysanthemum health seedlings. In the second experiment, hydrolyzed fish (H) (10 ml/l) was used instead of biofertilizer. For all treatments, half of the pots were sprayed with chitosan (C) (200 mg/l) weekly, during 10 weeks. A multifactorial randomized experimental design with 24 replications, totaling 2560 pots, with one plant per pot, was adopted. The plants were grown in a commercial greenhouse (temperature is about 45°C afternoon) and the fertility levels were adjusted according to grower program. The evaluations for disease severity (1=healthy plant; 2= plant with main vascular system tissues totally reddish brown; 3= plant with main vascular system tissues totally reddish brown and at least one secondary reddish brown haste; 4= plant with wilt; 5=dead plant or wilted leaves) were performed at 8, 12, 14, and 18 weeks after transplanting. In addition, plants were classified in commercial (class I, II, III) or not, after the 18<sup>th</sup> week. We therefore calculated the area under the disease severity curve (AUDSC). After severity evaluations, sections from stems of wilted chrysanthemum plants were placed in Komada's medium to ensure correct diagnosis.

## Results and discussion

*Trichoderma* did not suppress Chrysanthemum *Fusarium* wilt, independently on the other treatments. In the experiments, there was a quadratic response with an inflection point at 10 and 20% for area under the disease severity curve for sewage sludge, independently on the other treatments (Fig. 1AB). Apparently, suppression of the *Fusarium* wilt in the sewage sludge is biological in origin, because in general, disease severities were higher in plants growing in sterile substrate when compared to the plants growing in non-sterile substrate. The effect of sewage sludge to reduce the incidence or severity of soil-borne plant disease was observed for *Sclerotium rolfsii* in bean (Santos & Bettiol, 2003); *F. oxysporum* f. sp. *lycopersici* race 1 in tomato (Cotxarrera et al., 2002); *Rhizoctonia solani* in radish, *S. rolfsii* in bean and *Rashtonia solanacearum* in tomato (Ghini et al., 2007). Possibly, the effect of sewage sludge on the suppressiveness induction on soil/substrate is specific to each pathosystem since Ghini et al. (2007) observed the absence of effects of sewage sludge in *F. oxysporum* f. sp. *lycopersici* and Bettiol (2003) observed that increasing concentrations of the same types of sludge resulted in increased incidence of corn stalk rot caused by *Fusarium*.

Biofertilizer, fish hydrolyzed, and chitosan did not suppress *Fusarium* wilt (Fig. 1AB). In the diseased plants, *Fusarium* was isolated from stems in Komada's medium, to stress the diagnosis. In all sewage sludge concentrations, the plants were classified in commercial class I and II.

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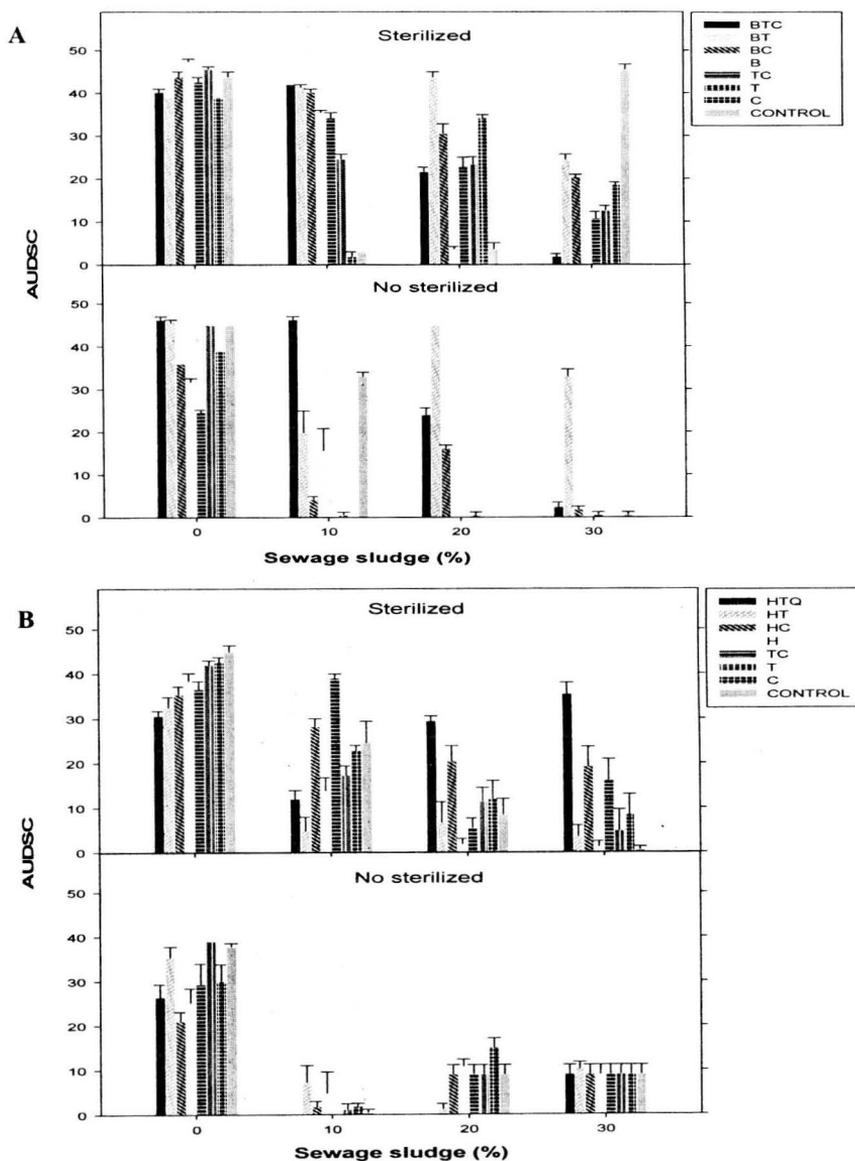


Figure 1. Effect of sewage sludge, biofertilizer (B), fish hydrolyzed (H), chitosan (C), and *Trichoderma harzianum* (T) to control *Fusarium* in chrysanthemum in substrate composed of pine bark naturally infested. \*Biofertilizer (A) and fish hydrolyzed (B).

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