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In vitro biological control of *Fusarium oxysporum*-causing wilt in *Capsicum annuum*.

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Abstract

Five species of *Trichoderma* viz., *Trichoderma Viride, T. harzianum, T. Koningii, T. aureoviride* and *T. pseudokoningii* were evaluated for their *in vitro* antagonistic potential against *Fusarium oxysporum*, the cause of wilt disease in sweet peppers (*Capsicum annum*). Among the *Trichoderma* species *T. viride* showed the best performance *in vitro* biological control of *Fusarium oxysporum* followed by *T. harzianum, T. aureoviride, T. koningii* and *T. pseudokoningii*, respectively, resulting in 62, 36, 24, 18 and 6% reduction in colony growth of the test pathogenic fungus respectively.

Keywords: *Capsicum annuum*, Biological control, *Fusarium oxysporum*, Sweet pepper, *Trichoderma* spp.

Introduction

Sweet pepper (*Capsicum annuum* L.) belongs to the family solanaceae, which is an important group of vegetables cultivated extensively in Pakistan and also widely cultivated in almost every country of the world. Sweet pepper is a summer crop and its total area under cultivation in Pakistan is about 91800 hectare, with total annual production of 115,000 tonnes. It thrives best in warm climate, where frost is not a problem during growing seasons. In general, it requires temperature ranging 25-35 ˚C. (Govt. of Pak., 2001).

Fusarium oxysporum Schlecht is associated with wilt disease of sweet pepper (Mushtaq and Hashmi, 1997). *Fusarium* wilt is the most important disease caused by F. *oxysporum* in sweet pepper plants. *Fusarium* wilt first appears as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by F. *oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant. Browning of the vascular tissue is an strong evidence of *Fusarium* wilt in sweet pepper (Agrios, 1988).

Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant with its sporangial germ tube or mycelium by invading the plant's roots. The roots can be infected directly through the root tips, through wounds in the roots, or at the formation point of lateral roots. Once inside the plant, the mycelium grows through the root cortex intercellulary. When the mycelium reaches the xylem, it invades the vessels through the xylem's pits. (Agrios, 1988).

Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly. The resulting spores which on their turn act as new inoculum for further spread of the fungus (Agrios, 1988).

Currently effective means of controlling *F.oxysporum* include: disinfestation of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Jones *et al*., 1982; Agrios, 1988; Smith *et al*., 1988)

Use of environmentally friendly biological control agents can more affectively control the soilborne phytopathogens. (Nam *et al*., 1988; Park ,1989; Saleem *et al*., 2000).

From several studies, it has been confirmed that *Trichoderma spp.* have antagonistic and biologically control potential against a diversity of soil borne pathogens.(Grondona *et al*., 1997; Hanson and Howell, 2004; Bajwa *et al*., 2004).

Present study has been carried out to biologically control wilt disease in sweet pepper by using *Trichoderma spp*. as biocontrol agents.

Materials and Methods

Isolation of the Pathogen

Root samples of 10 sweet pepper plants infected with wilting were collected from vegetable garden, Quaid–e–Azam campus, University of the Punjab, Lahore, Pakistan during June 2005. The root samples were cut into small pieces up to 1.5 cm length and surface sterilized by 15 % H_2O_2 for 30-45 seconds. And then rinsed with distilled water for three times. These surface sterilized roots were placed onto 2% Malt Extract Agar (MEA) medium in Petri plates and incubated at 25°C After 6 days the fungal isolates appearing on the root pieces were identified and transferred to 2% MEA medium Petri plates for purification.

***In vitro* Biological Control**

Cultures of *F.oxysporum*, isolated from roots of diseased (wilted) sweet pepper plants, were maintained on 2% malt extract agar (MEA) medium . Cultures of various antagonistic fungi *Trichoderma Viride* Pers. ExGray, *T.harzianum* Rifai, *T.Koningii* Oudem, *T.aureoviride* Rifai, *T.pseudokoningii* Rifai were obtained from First Fungal Culture Bank of Pakistan , Department of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan and maintained on 2% MEA medium. The antagonistic effect of various test fungal species in inhibiting the growth of *Fusarium oxysporum* was studied by Well method. For this purpose 6mm (0.6cm) diameter plugs of *F. oxysporum* and various antagonists were taken with the help of sterilized cork borer and placed at the opposite sides of the Petri plates of 9cm diameter having 2% MEA medium. After inoculation plates were incubated at 25°C. Petriplates with only *F.oxysporum* served as control. Each treatment was replicated for three times. Data on mycelial growth in terms of colony diameter of the pathogenic fungus were taken after 5 days of inoculation. Data was analyzed by using X^2 test and level of significant was exact (P=0.05) followed by Duncun Multiple Range (DMR) test (Steel and Torrie, 1980) using computer software programme SPSS 10.

Results and Discussion

Effect of *Trichoderma* species on *in vitro* growth of *F. oxysporum* is shown in fig 1& 2. Data recorded after 5 days of inoculation of biological control plates showed much more influence of antagonistic *Trichoderma* species over the colony growth of *F.oxysporum*. Among the *Trichoderma* species, *T.viride* showed the best performance where the colony growth of the pathogenic fungus *F.oxysporum* was 1.9cm as compared to 5cm in control treatment (Fig. 1). *Trichoderma viride*, antagonistic fungus, suppressed the colony growth of pathogen by 62% (Fig. 2). *T.viride* is also known to biologically control chestnut blight caused by *Cryphonecteria parasitica* in chestnut (Arisan-Atac *et al*., 1995). *T.harzianum* followed *T.viride* in performance and decreased colony growth of *F.oxysporum* by 36%. Rest of the three species of *Trichoderma* viz. *T.aureoviride*, *T.koningii* and *T.pseudokoningii* reduced the colony growth of *F.oxysporum* 24, 18 and 6% respectively. Effect of all the tested *Trichoderma* species except *T.pseudokoningii* was statistically significant against the pathogenic fungus *F.oxysporum*. Inoculation of sweet pepper (*Capsicum annuum*) plants with the tomato wilt pathogen, *Fusarium oxysporum f.sp. lycopersici* (FOL), partially protected pepper plants from subsequent infection with *Phytophthora capsici*, with no damage to plant.(Diaz *et al* ., 2005). It has been observed that antagonistic fungi are specific in their antagonistic activity against specific fungi (Saleem *et al*, 2000). *Trichoderma* species are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity(Elad *et al*., 1982). Brasier (1975) reported that volatiles released by *Trichoderma* species reduced the growth of *Phytophthora spp*.

From the present study it is concluded that *Trichoderma* species have the potential to suppress the colony growth of *F.oxysporum* which is the pathogen of wilt in sweet peppers. Further studies are required to apply this technique of biocontrol in field of sweet pepper.

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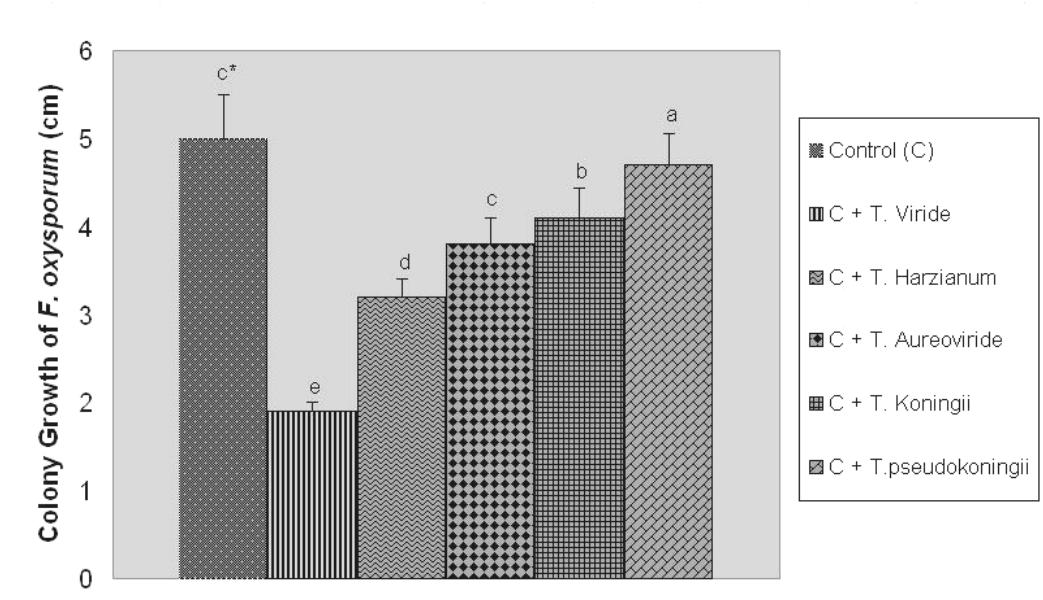

Fig. 1: Colony growth of *F. oxysporum* in control (c) and in the presence of various antagonistic *Trichoderma* species. Bars with different letters at their tops show significant difference (P=0.05) as determined by DMR test.

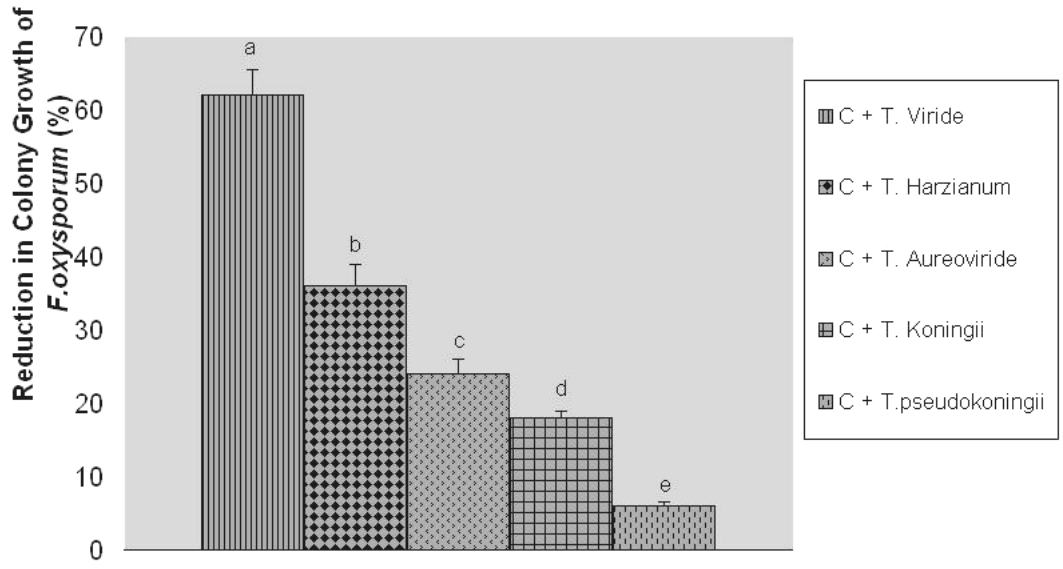

Fig. 2: Percentage reduction in Colony growth of *F.oxysporum* due to various antagonistic *Trichoderma* species. Bars with different letters at their tops show significant difference (P=0.05) as determined by DMR test.

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