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Induction of systemic resistance in tomato and cauliflower by *Trichoderma* spp. against stalk rot pathogen, *Sclerotinia sclerotiorum* (Lib) de Bary

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ABSTRACT: **In cauliflower and tomato *Trichoderma harzianum* and *T. viride* application at the sites spatially separated from the *S. sclerotiorum* inoculation resulted in a 30-70 per cent suppression of stalk rot symptoms, caused by a delay or suppression of spreading blighting/ blackening formation. Given the spatial separation of both microorganisms, this effect was attributed to the induction of systemic resistance by *Trichoderma* spp. The observations show the superiority of *T. harzianum* isolates over *T. viride* isolates in induction of systematic resistance (ISR). In tomato and cauliflower the effects of *Trichoderma* spp. were similar which confirmed that the induction of plant defense might also play role in biocontrol. Since, the suppression of the disease symptoms on leaves sprayed with *Trichoderma* spp. was on par with seed and seedling treated plants with *Trichoderma* spp., it indicates about the ISR-hypothesis. This study also shows that *Trichoderma harzianum* and *T. viride* may be effective in crop canopy.**

KEY WORDS: Cauliflower, induced systemic resistance, *Sclerotinia sclerotiorum*, *Trichoderma harzianum*, *T. viride*, tomato

INTRODUCTION

Stalk rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most serious problem in cauliflower and tomato crops and has been observed to reduce potential yield by 70-80 per cent in severely affected field. The fungus is soil borne in nature and its infection on stalks comes through the mid rib/petioles of the leaves touching the soil, which also helps in the multiplication of the secondary inoculums. Pathogenesis-related proteins have attracted considerable interest because their possible causal role in induced local and systemic resistance in the plants in response to pathogen (Stintizi *et al.*, 1993; Van Loon, 1997). Some studies have demonstrated that *Trichoderma* spp. can also affect the host plant physiology and induce plant defense reactions such as the hypersensitive response and production of phytoalexin (Meera *et al.*, 1994; Bigirimmana *et al.*, 1997; Chang *et al.*, 1997). This suggests an indirect biocontrol effect of *Trichoderma* through the enhancement of plant resistance and suppression of disease symptoms. Through the present study we aim to understand induced plant defense in tomato and cauliflower crops against *Sclerotinia sclerotiorum* by use of *Trichoderma harzianum* and *T. viride*.

MATERIALS AND METHODS

Planting materials

Tomato (*Lycopersicon esculentum* Mill) 'Pusa Sheetal' and cauliflower (*Brassica oberecea* var. *botrytis)* 'Pusa Him Jyoti' seeds were grown in the greenhouse at 20-25 °C in pots (diam 17cm) with potting compost soil. Plants were fed weekly with appropriate fertilizer and not treated with fungicides. Ten and 12 weeks old cauliflower and tomato plants, respectively were used for experiments.

Microorganisms and culture condition

On tomato and cauliflower plants were infected with *S. sclerotiorum* obtained from naturally infected plants. The fungus was grown and maintained on potato dextrose agar (PDA) in the growth room at a photon flux density of 220-μmol m^{-2} under a 10h photoperiod at 22 °C. Conidia from 7-10 day-old cultures were collected in water and cleared from mycelia debris by filtration through cheesecloth. Conidial concentration was determined with the help of haemocytometer and adjusted when necessary. The biocontrol agents *T. harzianum* (Th) and *T. viride* (Tv), that control stalk rot in greenhouse vegetable crops were multiplied and maintained on *Trichoderma* specific medium (Elad *et al.*, 1981).

Application of biocontrol agents

In tomato and cauliflower isolates of *T. harzianum* and *T. viride* were applied in a $4x10^6$ conidia ml^{-1} suspension. Seedlings were dipped for half an hour in conidial suspension. Tomato and cauliflower leaves were also sprayed with the conidial suspension before the challenge inoculation. While spraying the first pair of trifoliate of tomato and leaves of cauliflower, targeting of other plant parts was avoided to ensure *Trichoderma* would not be applied to the first trifoliate/leaves that was later challenged with *S. sclerotiorum.* Seeds were soaked for five minutes in a 10^8 ml^{-1} conidial suspension and subsequently planted in a soil amended with 10^8 ml^{-1} conidial suspension of *T. harzianum* and *T. viride.* Control seeds and seedlings were soaked in water and grown in untreated soil.

S. sclerotiorum inoculation and scoring of disease symptoms

Tomato and cauliflower plants were inoculated with *S. sclerotiorum* by modification in the technique of petal colonization method (Kapoor *et al.*, 1985), in which stalks of seedlings were impregnated with the matchstick covered with the pathogen. Leaves were also sprayed with a $5x10^5$ conidia ml^{-1} suspension and placement of sclerotia on leaf axil/base. Disease severity on whole plants was evaluated according to the percentage coverage of rot. The percentage of leaves that detached from the stem was also recorded. In this case, disease severity was evaluated by slight modification in the method described by Singh and Kalda (1995) and standardized for the study of systemic resistance in the 0-7 index, where no disease, 1 = blackening <5 mm on leaf, 2= blackening >5mm on leaf, 3 = blackening >5 and < 10 mm on leaf, 4 = blackening > 10 mm on leaf, 5 = blackening > 10 mm start of stem colonization, 6 = blackening > 10 mm severe stem colonization and 7 = plant completely dead. Subsequently, diameters of spreading blackening were recorded and sum per plant to make the total blackening diameter, which served as disease severity parameter. After challenge with *S. sclerotiorum*, plants were incubated at 20°C and 95 per cent relative humidity.

Spatial separation of *T. harzianum, T. viride* and *S. sclerotiorum*

In treatment where *T. harzianum* and *T. viride* were not applied to the leaf challenged with *S. sclerotiorum*, a possible *T. harzianum* and *T. viride* colonization of the challenged leaf was routinely checked on some plants. Leaves were macerated in sterile demineralized water and plated on half strength potato dextrose agar amended with

50mg^{l} rose bengal and 250mg^{l} chloramphenicol. Plates were incubated at 24 °C for two to four days and observed for *T. harzianum* and *T. viride* colonies.

Experimental design

Experiment with tomato and cauliflower consisted of 6 replicates per treatment and was laid out in a complete randomized design. For statistical analysis, arcsine transformed data were subjected to analysis of variance and treatments were prepared with Fisher's protected least significant difference (LSD) test. The total blackening diameter (TBD) was analyzed as a general liberalized model, in which experiments were pooled because interaction between treatment and experiment was not significant. Contrast analysis to determine the additive effect of leaf and seedling treatment was performed with the coefficients –1, 1, 1 and –1 for control, leaf only, seedling only, and leaf and seedling treatments, respectively. For all techniques the P= 0.05 threshold was used.

RESULTS AND DISCUSSION

Effect of *T. harzianum* and *T. viride* on Sclerotinia stalk rot of cauliflower and tomato

The seedling treatment with *Trichoderma harzianum* and *T. viride* before *Sclerotinia sclerotiorum* challenge significantly reduce stalk rot severity in cauliflower and tomato. (Table 1). For tomato *Sclerotina* stalk rot, coverage in the canopy did not give a complete picture of *S. sclerotiorum* development because leaves covered by the pathogen tend to detach from the stem. The cauliflower and tomato treated with *T. harzianum* and challenge inoculated with the pathogen did not show any stem rot infection at 10th and 30th day. However, the plant treated with *T. viride* showed some infection at 10th day but none on 30th days after pathogen challenge (Fig. 1).

Table 1. Influence of *T. harzianum* and *T. viride* application site on *S. sclerotiorum* infection

Treatment	Per cent disease severity			
	Tomato		Cauliflower	
	Th	Tv	Th	Tv
Leaf	38.13 (38.52)	39.23 (38.80)	39.35 (38.25)	40.97 (39.80)
Seedling + Seed	16.23 (23.73)	17.64 (24.80)	17.57 (24.73)	18.56 (25.50)
Seedling + Leaf	32.31 (34.63)	34.54 (35.98)	33.86 (35.55)	35.45 (36.58)
Seed + Leaf	14.12 (22.06)	15.42 (23.11)	16.56(23.97)	17.67 (24.80)
Seedling + Seed + Leaf	11.64 (19.91)	13.26 (31.35)	13.38 (21.42)	14.56 (22.40)
Untreated control	50.34 (45.17)	53.86 (47.18)	53.35 (46.91)	55.35 (48.07)
CD (P=0.05)				
Treatment	3.46		4.32	
Isolate	0.12		0.15	
Treatment x isolate	1.02		1.12	

Figures in parentheses are angular transformed values.

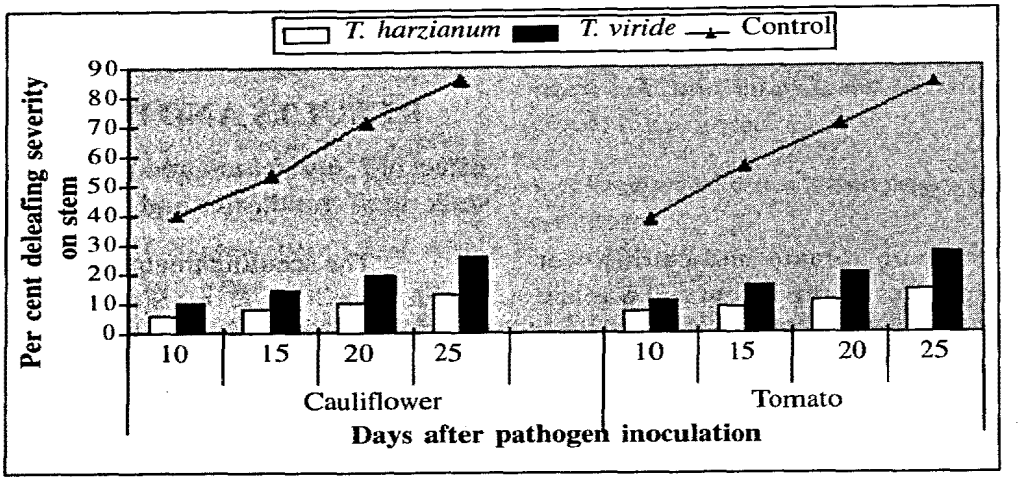

Figure 1. Effect of *T. harzianum* and *T. viride* seedling treatment on disease severity

Effect of *T. harzianum* and *T. viride* application way on stalk rot infection

The study revealed that *T. harzianum* and *T. viride* seedling treatment, seed treatment and leaf treatment significantly reduced the total blackening diameter (TBD). Combinations of seedling, seed and leaf treatment was even more effective than their alone treatment and reduce TBD, compared to control (Table 1). Contrast analysis showed that a model including seed + seedling + leaf treatment as additive factors could explain better *S. sclerotiorum* control.

Figure 2 clearly indicates that *T. harzianum* seedling treatment also reduce the deleafing of tomato and cauliflower plants followed by *T. viride*. As a result, the suppression of stalk rot coverage on the leaves should be combined with the suppression in deleafing to estimate the actual suppression in *S. sclerotiorum* development in tomato and cauliflower. The *S. sclerotiorum* spray inoculation also caused a low amount of stem infection when plants were pre-treated with *T. viride*. While non-biological treated control plants had coverage of 30.10 per cent and 35.12 per cent in cauliflower and tomato, respectively.

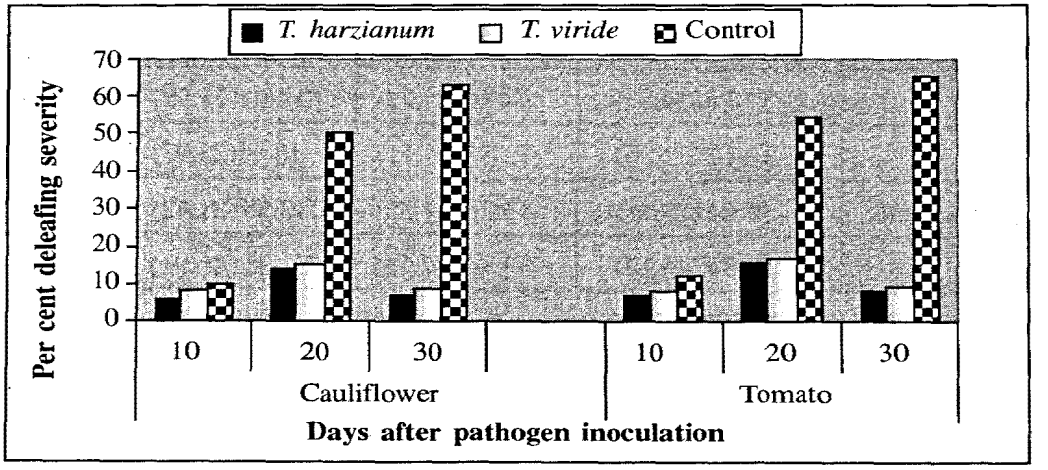

Figure 2. Effect of *T. harzianum* and *T. viride* seedling treatment on deleafing

Effects of *T. harzianum* and *T. viride* application site and time on *S. sclerotiorum* stalk rot infection

Leaf and seedling treatment resulted in a quite similar suppression in disease severity, when biocontrol agents were applied at the same time point before the pathogen challenge inoculation (Fig. 3 and 4). However, there was a little difference is disease severity between *T. harzianum* and *T. viride* treatments and between cauliflower and tomato plants. The result indicates that the site of biocontrol agents' application did not influence the developmental stage of the *S. sclerotiorum* infections. Though, the spreading of established *S. sclerotiorum* in *T. harzianum* leaf treated plants was 25-50 per cent slower than control and seedling-treated plants.

The effect of *Trichoderma* application at sites spatially separated from the *S. sclerotiorum* inoculation became particularly significant when, in control plants, established *S. sclerotiorum* infections developed into rapidly spreading blackening causing leaf spots coverage levels of more than 20 per cent. The *Trichoderma* spp. treated cauliflower and tomato plants showed less extensive blackening as compared to control, which indicates that *S. sclerotiorum* is restricted in the early stage of development. The restriction of *S. sclerotiorum* development was in a delay or suppression of spreading blackening formation depending on the host plant and *Trichoderma* application time. It was observed (Fig. 3 and 4) that time interval (1-3days) between *Trichoderma* and *S. sclerotiorum* inoculation reduce the disease severity. However, the time interval more than 7 days contrastingly responsible for increased disease severity.

Given the generally constant growth rate of spreading *S. Sclerotiorum* blackening, this indicates that blackening formation was delayed. Therefore, the TBD ultimately became the most constant indicator of disease severity because it represents both parameters when a constant growth rate is assumed for spreading blackening development.

Development of *S. sclerotiorum* infection was consistently reduced after a prior *T. harzianum* and *T. viride* treatment on plant parts spatially separated from the site of *S. sclerotiorum* inoculation. *T. harzianum* and *T. viride* seedling treatment reduced *S. sclerotiorum* stem and leaf infection in cauliflower and tomato. Though, the suppression in disease index was slightly greater in *T. harzianum* than that of *T. viride* treatment and in cauliflower than that of tomato. Because in the these experiments

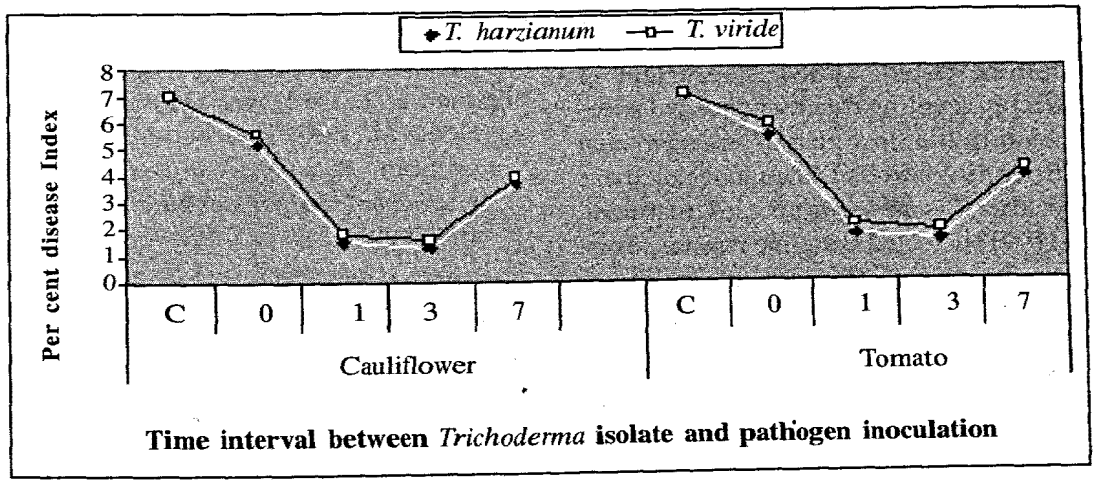

Figure 3. Influence of *T. harzianum* and *T. viride* application site and time on *S. sclerotiorum* infection

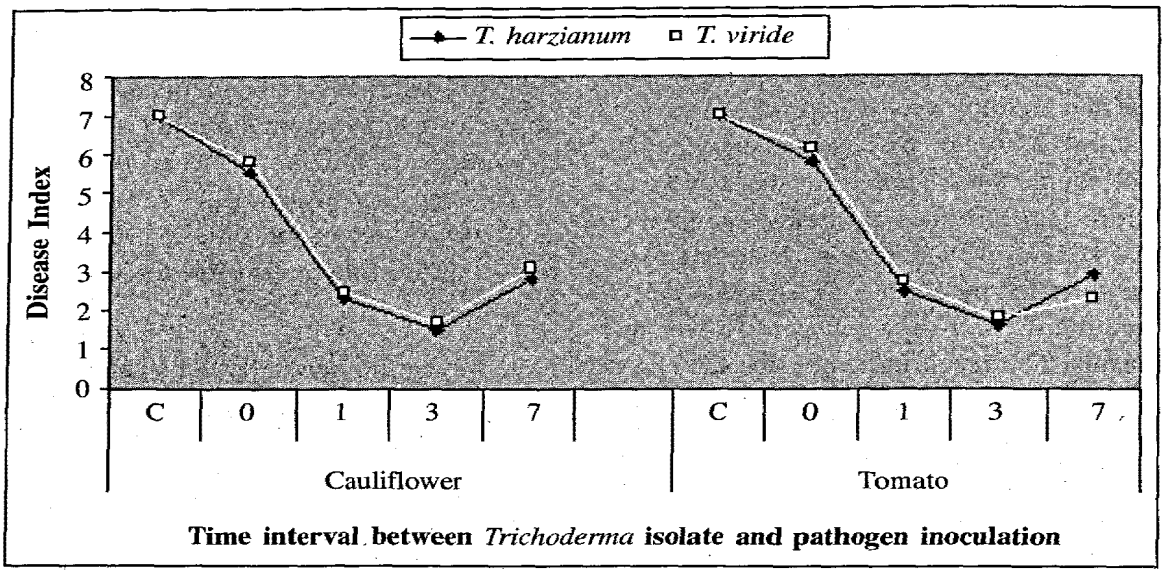

Figure 4. Influence of *T. harzianum* and *T. viride* application site and time on *S. sclerotiorum* infection

Trichoderma species could not directly interact with *S. sclerotiorum* or affect the environment for *S. sclerotiorum* development for reasons of spatial separation, induction of systemic resistance is by exclusion of alternatives, the most likely explanation of the *S. sclerotiorum* control.

Trichoderma spp. are known to work like ISR and SAR (Kloepper *et al.*, 1997; Ryals *et al.*, 1997) against several pathogens. *T. harzianum* seedling treatment reduced anthracnose symptoms of *Colletotrichum lindemuthianum* in bean (Bigirimmana *et al.*, 1997), gray mould of *Botrytis cinerea* in tomato, lettuces, pepper, bean and tobacco (Mayer *et al.*, 1965) and also reduced white mould in lettuce (Elad, 1988). The effect of *Trichoderma* spp. seedling treatment needed some time to develop in the host plant *S. sclerotiorum* was only efficiently controlled when inoculated one or more days after *Trichoderma* species treatment. Smith *et al.* (1991) detected systemic resistance one day after inoculation with *Pseudomonas syringae* pv. *syrirgae.*

The observation that *S. sclerotiorum* blackening spread was slow on *Trichoderma* spp. treated leaves than on leaves of control or *Trichoderma* spp. seedling treated plants, indicates that induced resistance is not the only mode of action involved. Therefore, further work is needed to evaluate the importance of induced resistance in *S. sclerotiorum* control on *Trichoderma* treated leaves.

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