Bioefficacy of *Trichoderma* isolates against pathogenic fungi inciting spinach (*Spinacea oleracea* l.)

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ABSTRACT

Biological control of plant pathogens using antagonistic fungi can be successfully utilized especially within the frame work of integrated disease management system. Efficacy of different isolates of *Trichoderma* species against two pathogenic fungi causing leaf spot (*Alternaria spinaciae*) and wilt (*Fusarium oxysporum* f. sp. *spinaciae*) on spinach (*Spinacea oleracea* L.) by dual culture technique under *in vitro* conditions. Generally *Trichoderma* used as a biological control for the inhibition of growth of the pathogens. Different isolates i.e. *Trichoderma viride* (12), *T. harzianum* (12), *T. koningii* (07), *T. pseudokoningii* (05) and *T. virens* (10) strains were isolated *and* used for antagonistic study. Results indicated that, among *T. viride* isolates, Tv_1 *and* Tv_5 showed maximum antagonism, but in case of Tv_7 and Tv_{10} showed minimum antagonism against *A. spinaciae*. In case of *F. oxysporum* f. sp. *spinaciae* showed significant inhibition by Tv_4 and reduced the percent inhibition by Tv_8 and Tv_6. In case of *T. harzianum* isolates, Th_3 and Th_{10} showed maximum inhibition on *A. spinaciae* while in *F. oxysporum* f. sp. *spinaciae*, Th_5 increased the inhibition and decreased in Th_3 and Th_7. *Trichoderma koningii* showed better results in Tk_7 isolate whereas decreased Tk_4 over *A. spinaciae*, but in *F. oxysporum* f. sp. *spinaciae* reduced rate was more in Tk_4 and Tk_2. Isolates of *T. pseudokononggii* found maximum inhibition in Tp_5 over *A. spinaciae* and significant inhibition in Tp_3 over *F. oxysporum* f. sp. *spinaciae*. *Trichoderma virens* isolates were found significant inhibition in Tvr_4 and Tvr_9 and 50% reduction over *A. spinaciae* and Tvr_4 increased the percent inhibition and decreased the Tvr_7 in case of *F. oxysporum* f. sp. *spinaciae*.

Key words: *Alternaria spinaciae,* dual culture, *Fusarium oxysporum spinaciae, Trichoderma*

INTRODUCTION

Spinach (*Spinacea oleracea* L.) is an important vegetable crop. Although many diseases have been reported as externally and internally seed borne (Correl and Monelok, 1994; Sumner, 1991; Walker, 1952; Bhale, 2002; Raid and Kucharek, 2003) which causes discolouration, spoilage of seeds, germination effects, rotting and wilting of seedlings, stem gall, blight, leaf spot, powdery mildew, downy mildew, rust and anthracnose. Out of which leaf spot (*Alternaria spinaciae* Allesch *and* Noack) and wilt (*Fusarium oxysporum* f. sp. *Spinaciae* (Sherb) Synder *and* Hansen) diseases to be severe. Since the diseases are soil and seed borne, is difficult to manage. Only practical solution seems to be use of resistant variety. However, resistant varieties for these diseases are not available. In this situation crop loss due to these diseases is substantial as it leads to capital losses up to 20 to 60%. Since, biocontrol agents for protection of seeds and control of seed borne diseases offers farmers an alternative source of chemical fungicides which is highly effective (Callan *et al*., 1997). Fungicides such as metalaxyl, are however expensive and growers tend to use lower than optimum dosages (Csoinos *and* Bertrand, 1994). Furthermore, the use of fungicides, besides being expensive and involving risks to the environment associated with the application of chemicals, is not totally effective and may lead to the appearance of new, resistant strains of pathogens (Bruin *and* Edgington, 1980). *Trichoderma* has been the focal point of a number of studies concerning their ability to control plant pathogens. Strains of the species *T. harzianum* has shown effectiveness when used in disease control caused by several fungi, including *Sclerotium rolfsii*, a widely distributed and highly destructive plant pathogen (Benhamou and Chet, 1996), and *Sclerotinia sclerotiorum* affecting runner beans (Inbar *et al.* 1996). In a similar fashion, strains of

T. harzianum, *T. koningii*, and *T. longibrachiatum* have been effective in *Armillaria* control in tea (Osando and Waudo, 1994), while *T. koningii* has been used for *Fusarium solani* corn rot control (McAllister *et al.* 1994).

It is therefore necessary to develop alternative ways of control. One such alternative is biological control, in which microorganisms are selected for their ability to antagonize pathogens. Therefore an investigation was made to evaluate the different isolates of *Trichoderma* species against the pathogenic fungi on spinach.

MATERIAL AND METHODS

Isolation of *Trichoderma* spp

Rhizosphere soils of irrigated and non irrigated plants were collected from different parts of Marathwada region of Maharashtra state. From the rhizosphere soil samples, desired strain of *Trichoderma* species were isolated by using potato dextrose agar (PDA) and *Trichoderma* selective medium (TSM) by dilution plate technique. The isolated strains were identified by reculturing on another petriplates containing sterilized TSM. The isolated strains were identified up to species level based on colony characters, growth of fungus, structure of mycelium, conidiophores and conidia. All *Trichoderma* spp. fungi were purified by hyphal tip technique and identified on the basis of cultural, microscopic and morphological characters (Kubicek and Harman, 2002). The isolated strains of *Trichoderma* species were maintained on PDA and TSM slants. This was maintained throughout the study by periodical transfers on (PDA) medium under aseptic conditions, to keep the culture fresh and viable.

Isolation of test pathogens

Test fungi *A. spinaciae* and *F. oxysporum* f. sp. *spinaciae* were isolated from the diseased part viz leaf spot and wilt of spinach (*Spinacea oleracea* L.) respectively Earlier, the pathogencity of test fungi were confirmed in inoculating spinach seedlings properly. The isolated test fungi were purified on aseptic conditions and maintained on PDA slants.

Dual culture experiment

Antagonistic efficacy of different isolates of *Trichoderma* species, *T. viride, T. harzianum, T. koningii, T. pseudokoningii* and *T. virens* were tested against the isolated pathogenic test fungi, dual culture experiment was described by Morton and Stroube, (1955). *Trichoderma* species and test fungi were inoculated at 6 cm apart. Three replications were maintained for each treatment and incubated at 27 ± 2° C for 7 days. Monoculture plates of both are served as control. After 7 days, radial growth of test fungi and *Trichoderma* isolates were measured. Colony diameter of test fungi in dual culture plate was observed *and* compared with control. Antagonistic effect of *Trichoderma* spp., as decrease of the mycelial growth of pathogenic fungi,was determined using the Vincent (1947) method.

RESULTS AND DISCUSSION

Isolation of *Trichoderma* species test pathogens

Five species of *Trichoderma*, T. *viride(12), T. harzianum*(12*), T. koningii*(07), *T.pseudokoningii* (05*)* and *T. virens*(10) were isolated from irrigated and non irrigated rhizosphere soil of Marathwada region. Perusal of results indicated that *A. spinaciae* and *F. oxysporum* f. sp. *spinaciae* were significantly influenced by various isolates of *Trichoderma* species under study at 7 days after inoculation (DAI).

Dual culture experiment

Table 1 indicated that *T. viride* isolates on growth of *A. spinaciae* Tv_1 and Tv_5 showed maximum antagonism as compared to others but Tv_7 and Tv_{10} decreased the antagonism. While in case of *F. oxysporum* f. sp. *spinaciae* showed greater inhibition by Tv_4 followed by Tv_3, but Tv_8 and Tv_6 reduced the percent inhibition. *Trichoderma harzianum* isolates on growth of *A. spinaciae* were found more percent inhibition in Th_3 and Th_{10}. In case of *F. oxysporum* f. sp. *spinaciae* increased the percent inhibition in Th_5 whereas decreased in Th_3 and Th_7 (Table 2).

Trichoderma koningii isolates on growth of *A. spinaciae* showed more antagonism in Tk_7 but decreased in Tk_4. But in case of *F. oxysporum* f. sp. *spinaciae* found more percent inhibition in Tk_3 (67.50%) as compared to other but reduced the percent inhibition Tk_4 and Tk_2 (Table 3).

Table 1. Influence of *Trichoderma viride* isolates on growth of *Alternaria spinaciae* and *Fusarium oxysporum* f. sp. *spinaciae*

Isolates	Locations	Radial growth of *A.spinaciae* (mm) (7DAI)	Inhibition %	Radial growth of *F.oxysporum* f. sp.*spinaciae.* (mm) (7DAI)	Inhibition %
Tv_1	Naldurg	14.00	84.44	40.22	55.50
Tv_2	Osmanabad	17.20	81.58 I	27.30	69.66
Tv_3	Latur	22.30	75.22	12.50	86.11
Tv_4	Nanded	35.10	61.00	09.00	90.00 I
Tv_5	Jalna	16.95	81.16 I	44.23	50.85
Tv_6	Aurangabad	27.00	70.00	55.15	38.72 D
Tv_7	Beed	55.15	38.72 D	27.00	70.00
Tv_8	Paranda	25.10	72.11	61.00	32.22 D
Tv_9	Ashti	18.95	78.94	47.60	47.12
Tv_{10}	Omerga	50.30	44.12 D	29.00	67.77
Tv_{11}	Ahmedpur	36.35	56.61	69.11	23.21 D
Tv_{12}	Hingoli	27.50	69.44	47.60	47.11
SE±			4.28		5.96
CD(P=0.05)			9.43		13.14

DAI- Days after inoculation, I - Increased percent inhibition, D- Decreased percent inhibition

Table 2. Influence of *Trichoderma harzianum* isolates on growth of *Alternaria spinaciae* and *Fusarium oxysporum* f. sp. *spinaciae*

Isolates	Locations	Radial growth of *A.spinaciae* (mm) (7DAI)	Inhibition %	Radial growth of *F.oxysporum* f. sp. *spinaciae.* (mm) (7DAI)	Inhibition %
Th_1	Tuljapur	20.12	77.70	30.12	66.10
Th_2	Kallam	34.12	62.08	37.10	58.77
Th_3	Ausa	45.00	50.00	57.00	36.67
Th_4	Nanded	12.00	86.66 I	61.37	31.81 D
Th_5	Badnapur	41.17	54.25	17.42	80.64 I
Th_6	Beed	22.39	75.12	35.00	61.11
Th_7	Paithan	24.11	73.21	50.23	44.18
Th_8	Paranda	27.67	69.25	29.14	67.62
Th_9	Patoda	35.00	61.11	47.17	47.58
Th_{10}	Nilanga	16.00	82.22 I	24.27	73.03
Th_{11}	Udgir	34.00	62.22	29.00	67.77
Th_{12}	Parbhani	40.22	55.31	55.52	38.3 D
SE±			3.38		4.59
CD(P=0.05)			7.44		10.11

DAI- Days after inoculation, I - Increased percent inhibition, D- Decreased percent inhibition

At 7 days after incubation (DAI), *T. pseudokoningii* isolates on growth of *A. spinaciae* was observed maximum in Tp_5 and in case of *F. oxysporum* f. sp. *spinaciae* was found maximum in Tp_3 (Table 4).Table 5 illustrated that *T. virens* isolates on growth of *A. spinaciae,* maximum percent inhibition showed Tvr_4 and Tvr_9 but 50% inhibition was found in case of Tvr_7. Maximum percent inhibition was observed in Tvr_4 and minimum in Tvr_7 in case of *F. oxysporum* f. sp. *spinaciae*.

Among all isolates of *T.viride*, isolate no 1 for *A. spinaciae* and isolate no 4 for *F. oxysporum f. sp. spinaciae* was found suitable for antagonism. In *T. harzianum*, isolate no 4 for *A. spinaciae* and isolate no 5 for *F. oxysporum f. sp. spinaciae* was found maximum percent inhibition. In case of *T. koningii*, isolate no 7 for *A. spinaciae* and isolate no 3 for *F. oxysporum f. sp. spinaciae* was found significant antagonism. In *T.pseudokoningii* isolate no 5 for *A. spinaciae* and isolate no 3 for *F. oxysporum f. sp. spinaciae* was observed bioefficacy. In *T.virens* isolates no 4 *and* 9 were suitable for antagonism but maximum percent inhibition was failed in *F. oxysporum f. sp. spinaciae*.

Table 3. Influence of *Trichoderma koningii* isolates on growth of *Alternaria spinaciae* and *Fusarium oxysporum* f. sp. *spinaciae*

Isolates	Locations	Radial growth of *A.spinaciae* (mm) (7DAI)	Inhibition %	Radial growth of *F.oxysporum* f. sp.*spinaciae.* (mm) (7DAI)	Inhibition %
Tk_1	Naldurg	26.00	71.10	35.00	61.10
Tk_2	Osmanabad	27.12	69.86	50.11	44.32 D
Tk_3	Latur	37.00	58.88	29.25	67.50
Tk_4	Aurangabad	51.50	42.77 D	57.44	36.17 D
Tk_5	Jalna	28.11	68.76	39.39	56.23
Tk_6	Parbhani	32.35	64.05	38.57	57.14
Tk_7	Nanded	12.33	86.30 I	31.11	65.43
SE±			5.01		11.87
CD(P=0.05)			4.29		10.18

DAI- Days after inoculation, I - Increased percent inhibition, D- Decreased percent inhibition

Table 4. Influence of *Trichoderma pseudokoningii* isolates on growth of *Alternaria spinaciae* and *Fusarium oxysporum* f. sp. *spinaciae*

Isolates	Locations	Radial growth of *A.spinaciae* (mm) (7DAI)	Inhibition %	Radial growth of *F.oxysporum* f. sp.*spinaciae.* (mm) (7DAI)	Inhibition %
Tp_1	Ausa	23.00	74.40	35.00	61.11
Tp_2	Tuljapur	29.25	67.50	37.25	58.61
Tp_3	Jalna	22.11	75.43	24.00	73.33
Tp_4	Sillod	39.15	56.50	47.00	47.77 D
Tp_5	Ardhapur	17.00	81.11 I	35.00	61.11
SE±			1.67		1. 67
CD(P=0.05)			4.65		4.65

DAI- Days after inoculation, I - Increased percent inhibition, D- Decreased percent inhibition

Table 5. Influence of *Trichoderma virens* isolates on growth of *Alternaria spinaciae* and *Fusarium oxysporum* f. sp. *spinaciae*

Isolates	Locations	Radial growth of *A.spinaciae* (mm) (7DAI)	Inhibition %	Radial growth of *F.oxysporum* f. sp.*spinaciae.* (mm) (7DAI)	Inhibition %
Tvr_1	Naldurg	24.00	74.00	27.00	70.00
Tvr_2	Murum	24.15	73.16	30.11	66.54
Tvr_3	Beed	29.11	67.65	34.39	61.78
Tvr_4	Badnapur	12.00	86.66 I	19.22	78.64
Tvr_5	Kannad	32.00	64.44	41.11	54.32
Tvr_6	Parbhani	21.37	76.25	33.13	63.18
Tvr_7	Nilanga	47.11	47.65 D	50.11	44.32 D
Tvr_8	Udgir	27.35	69.61	34.11	62.10
Tvr_9	Nanded	12.00	86.66 I	33.12	63.20
Tvr_{10}	Hingoli	31.34	65.17	28.05	68.83
SE±			3.60		2.91
CD(P=0.05)			8.13		6.59

DAI- Days after inoculation, I - Increased percent inhibition, D- Decreased percent inhibition

Several workers has been reported that the use of *Trichoderma* species against number of plant pathogenic fungi. *Trichoderma viride* and *T. koningii* were found effective against *Rhizoctonia solani* (Mukhopadhyay, 1987), *T. hamatum* against *R. solani* (Lewis *et al*., 1990). In *in vitro*,control of *R. solani*, *F. oxysporum* and *Macrophomina phaseolina* were achieved with *T. koningii*, *T. hamatum* and *T. harzianum* (Arora, 1990). *Trichoderma harzianum* and *T. viride* were antagonistic to *F. oxysporum* f. sp. *lycopersici* (Gaikwad *et al*., 1999) and to *R. solani* (Yadav and Tripathi, 1999). Recently, Waghmare and

Kurundkar (2011) reported efficacy of *Trichoderma* species against *F. oxysporum* f. sp. *carthami* causing wilt of safflower and found that isolates no 29 *and*33 were found minimum growth of the pathogen as compared to others. The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogens (Rajkonda *et al*., 2011).

According to Papavizas *and* Lumsden (1980) the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably: antibiosis, lysis, competition and mycoparasitism. However, Ayers *and* Adams (1981) indicated that interactions observed *in vitro* do not necessarily confirm their operation for the decrease in pathogen populations and reduction in diseases observed in natural conditions. Further investigations are needed in order to characterize the antagonist-host interactions observed during these studies.

The *in vitro* screening with our arbitrary system of bio-antagonists effective against soil borne pathogens is a simplistic approach to understand a small sector of biological system in diseases control. Therefore, it may be more prudent to search for biological antagonists against specific pathogen and evaluate blends of antagonists for wider applications (Baker and Cook, 1974). Our results show that although considerable success in biocontrol is achieved under laboratory conditions the outcome is not proportionate under field conditions. Hence, work is needed towards a better understanding and development of technologies that allow the biocontrol agent to spread and proliferate in soil. In addition as suggested by Papavizas (1985) research should be directed towards the improvement of strains of biological agents that are more capable of becoming established and surviving under adverse field conditions. Thus, it is obvious that biological control offers, durable environmentally safe and cost effective alternative to chemical for the efficient management of plant disease

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