CULTURAL CHARACTERISTICS AND BIOCONTROL POTENTIAL OF LOCALLY ISOLATED *TRICHODERMA HARZIANUM*

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ABSTRACT

Five isolates of *Trichoderma harzianum* (TH) T1, T2, T3, T4 and T5 were screened for their ability to grow on a range of pH and temperature. TH radial diameter was found maximum on pH 5 and minimum with pH 11. T4 (89.3 mm) produced maximum radial diameter at pH 5 and minimum was recorded with T5 (43.0 mm) at pH 11. Increase and decrease in the pH of the culture medium from optimum, drastically reduced the radial growth of the *TH* isolates. On CDA medium radial diameter of T4 (89 mm) was recorded maximum at 25°C. At 35°C all the isolates showed very slow growth and no growth in the radial diameter was recorded at 40°C. Growth of *Trichoderma* is directly dependent on the temperature of the environment. A broad range of temperature tolerance for growth and sporulation of *Trichoderma* is very interesting feature for suitability of the antagonists as biocontrol agents. *TH* isolates effectively inhibited the growth of pathogenic fungi in the dual culture. Against *Foc*, T3 showed maximum inhibition (64.3%) which was significantly ($P<0.01$) different from all the others. Minimum growth inhibition was registered by T5 (50.7%). *Trichoderma* isolate T3 was most promising against all the isolates of pathogen. TH isolate T3 was most promising in controlling the *Foc* and it also produced higher radial diameter at different pH and temperature as compared to others. Ability of isolate T3 to tolerate higher level of pH and temperature make it suitable for application as biocontrol agent against *Foc* under diverse agro-climatic conditions.

KEYWORDS: Bio-control, *Fusarium oxysporum* f. sp. *chrysanthemi, Trichoderma harzianum.*

INTRODUCTION

Chrysanthemum is one of the most leading commercial floriculture crop, grown for cut and loose flowers and also as pot plants throughout the globe. Commercial cultivation of *Chrysanthemum* is carried out in Karnataka, Tamil Nadu, Maharashtra, Rajasthan, Gujarat, Haryana, West Bengal, Delhi, U.P. etc. Demands of the *Chrysanthemum* flowers are increasing day by day both in domestic and international market (Bhattacharjee and De 2003). *Chrysanthemum* plant is infected by various fungal, bacterial and viral diseases of which wilt caused by *Fusarium oxysporum* f. sp. *chrysanthemi* (Foc) is one of the most wide spread and destructive disease. Selection and use of resistant cultivars of *Chrysanthemum* have a limited success in controlling *Foc.* Various chemicals and fungicides such as Benomyl, Ethazol + Thiophanate-Methyl, Bitertanol, Triadimefon, Thiabenzadole, Iprodione, Carboxin etc have been used to control the wilt pathogen of *Chrysanthemum* (Strider 1985). Benomyl was effective but at very higher doses (1.2 g.a.i./liter) as compared to above. The plants treated with higher dosages of chemical fungicides shows phyto-toxicity and reduction in the growth (Strider 1985). Use of the chemicals and fungicides for controlling the pathogens leads to severe environmental pollutions and also the population of beneficial microbes are reduced. *Trichoderma* spp. have a unique ability to control the plant pathogens which includes mechanism of mycoparasitism, antibiosis, competition, siderophore production, induction of systemic resistance, growth promotion etc (Chet 1987, Dennis and Webster 1971, Upadhyay and Mukhopadhyay 1986, Howell 2003). A critical review of the literature reveals that very little work has been done on the understanding of nature of this pathogen and its eco-friendly methods of control. The present study was undertaken to find out eco-friendly management of the wilt disease using efficient isolates of *Trichoderma harzianum.*

MATERIALS AND METHODS

Isolation of the antagonist:

Rhizospheric soil samples from gardens, nurseries and fields were collected in the fresh poly bags from Kanpur, Uttar Pradesh, India. Soil samples brought in to laboratory were dried under laminar air flow and isolation was carried out using serial dilution plate technique (Pramer and Schmidt 1956). Petri plates were incubated at 25 ± 2°C into a BOD incubator and observed periodically for the development of fungal colonies of antagonists from the soil samples. Purified cultures were maintained on PDA medium in refrigerator for further studies.

Cultural characteristics of the antagonists:

A detailed study on cultural characteristics of *Trichoderma* isolates was carried out by varying the temperature (15ºC, 20ºC 25ºC, 30ºC, 35ºC and 40ºC) and

pH (3, 5, 7, 9, 11) of the medium. Czapex,s Dox Agar (CDA) was prepared by adding Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5g, Potassium chloride 0.5g, Ferrous sulphate 0.01g and Sucrose 30g. For pH studies medium pH was adjusted with the help of Hydrochloric Acid (HCl) and Sodium Hydroxide (NaOH) solutions. The above prepared media were sterilized for 15 minutes at 121ºC temperature and 15 p.s.i. pressure. Five mm bits of the antagonistic fungi (7 days old culture) from the PDA Petri plates were cut with the help of a cork borer and inoculated over the CDA medium and data on radial growth were recorded periodically for 10 days.

Screening of antagonist against pathogen under *in vitro* conditions:

All the *Trichoderma harzianum* (TH) isolates were screened for their biocontrol potential against the *Fusarium oxysporum* f. sp. *chrysanthemi* (Foc) in *in-vitro* conditions using dual culture technique (Skidmore and Dickinson 1976). Potato Dextrose Agar (PDA) Petri plates containing five days old cultures of *T TH* and *Foc* were used for the dual culture studies. Five mm disc from cultures of TH and *Foc* were cut with the help of a sterilized cork borer and placed opposite to each other in 100 mm Petri plates containing PDA medium. Colony diameters of both antagonistic and pathogenic fungi were measured periodically up to 7 days. Controls of both the fungi were inoculated separately in the center of the Petri plates containing PDA medium. All the Petri plates of dual culture studies were incubated at 25±2°C and data on percent inhibition was recorded. All the combinations were in triplicates and the experiment was carried out twice.

The formula used for calculation of the percent inhibition in the dual culture Petri plates was:

$$\text{\% inhibition over control} = \frac{C - T}{C}$$

C = Radial diameter of fungus in the control Petri plates.

T = Radial diameter of fungus in the Dual Culture Petri plates.

RESULTS AND DISCUSSION

Radial diameter was found maximum on pH 5, followed by pH 7, pH 9, pH 3 and minimum radial diameter was recorded on the medium with pH 11. A variation in the rate of growth was recorded among isolates. Isolate T4 (89.3 mm) produced maximum radial diameter at pH 5 and minimum was recorded with T5 (43.0 mm) at pH 11. All the isolates showed growth on all the pH and medium. The results revealed a variation in radial diameter of TH isolates at different pH and medium. TH isolates grew on all the levels of pH and showed maximum growth at pH 5 with minimum at pH 11. Increase and decrease in the pH of the culture medium from optimum, drastically reduced the radial growth of the TH isolates. Ability of TH isolates to grow on different levels of pH (3 – 11) will make them suitable for its application as biocontrol agents in diverse soil type with varying pH. Kredics et al (2003) reported ability of *Trichoderma* isolates to grow at pH levels of 2.0 to 6.0. Similar were the findings of Jackson *et al* (1991).

Temperature effects on the growth of TH isolates was evaluated in order to determine the most suitable temperature for growth. On CDA medium radial diameter of T4 (89 mm) was recorded maximum at 25°C. At 35°C all the isolates showed very slow growth and no growth in the radial diameter was recorded at 40°C. At 15°C growth of the isolates were higher as compared to 35°C. Growth of *Trichoderma* is directly dependent on the temperature of the environment. Results showed that temperature of 25°C was most suitable for growth of all isolates. Growth of TH was reduced when the temperature was increased or decreased from the optimum (25°C). The ability of TH isolates to grow on varied temperatures will make them suitable to utilize as biocontrol agents in diverse agro-climatic conditions. At higher temperature growth of TH isolates was arrested. TH isolate T4 was fastest and T5 was slowest with respect to growth rate on the medium. Similar were the findings of Prasun and Raghu (1997). They reported maximum radial diameter of *Trichoderma* at 25°C and 30°C which reduced drastically with reduced or increase temperature. Temperature of the environment also influenced the biocontrol potential of *Trichoderma* isolates. Jayaswal *et al* (2003) reported temperature between 20°C - 37°C for good growth of of *Trichoderma* species. A broad range of temperature tolerance for growth and sporulation of *Trichoderma* is very interesting feature for suitability of the antagonists as biocontrol agents. The biocontrol formulations developed from the above isolate may be used in a wide range of geographical locations because of its versatility to grow on various pH and temperature.

TH isolates effectively inhibited the growth of pathogenic fungi in the dual culture. Against Foc, T3 showed maximum inhibition (64.3%) which was significantly ($P<0.01$) different from all the others. Minimum growth inhibition was registered by T5 (50.7%). There was no significant difference in the percent growth inhibition caused by T1, T2, T4 and T5.

Biocontrol potential of *Trichoderma harzianum* isolates against *Fusarium oxysporum* f. sp. *chrysanthemi*

Table 1. Effect of different pH and temperature on the growth of *Trichoderma*.

pH	Isolate	Radial Diameter (mm) on CDA	Temperature	Isolate	Radial Diameter (mm) on CDA
3	T 1	61.0 ± 0.00^{m}	15°C	T 1	71.6 ± 0.33^{j}
	T 2	63.0 ± 0.00^{l}		T 2	72.6 ± 0.33^{ij}
	T 3	59.6 ± 0.33^{n}		T 3	69.6 ± 0.33^{l}
	T 4	64.0 ± 0.00^{l}		T 4	73.0 ± 0.57^{i}
	T 5	58.6 ± 0.33^{n}		T 5	68.6 ± 0.88^{lm}
5	T 1	87.0 ± 0.00^{bc}	20°C	T 1	84.0 ± 0.57^{fg}
	T 2	87.6 ± 0.33^{b}		T 2	85.3 ± 0.33^{e}
	T 3	85.0 ± 0.57^{d}		T 3	83.0 ± 0.57^{g}
	T 4	89.3 ± 0.33^{a}		T 4	86.0 ± 0.00^{de}
	T 5	83.6 ± 0.33^{e}		T 5	81.6 ± 0.88^{h}
7	T 1	85.0 ± 0.00^{d}	25°C	T 1	87.3 ± 0.33^{bc}
	T 2	86.0 ± 0.00^{cd}		T 2	88.0 ± 0.57^{ab}
	T 3	83.6 ± 0.33^{e}		T 3	86.6 ± 0.33^{cd}
	T 4	87.0 ± 0.00^{bc}		T 4	89.0 ± 0.57^{a}
	T 5	81.3 ± 0.33^{f}		T 5	85.0 ± 0.57^{ef}
9	T 1	69.0 ± 0.57^{i}	30°C	T 1	85.0 ± 0.00^{ef}
	T 2	70.3 ± 0.33^{h}		T 2	85.6 ± 0.33^{de}
	T 3	67.3 ± 0.33^{j}		T 3	84.0 ± 0.00^{fg}
	T 4	71.6 ± 0.88^{g}		T 4	87.3 ± 0.33^{bc}
	T 5	68.3 ± 0.88^{ij}		T 5	83.0 ± 0.00^{g}
11	T 1	44.0 ± 0.00^{qr}	35°C	T 1	67.6 ± 0.33^{mn}
	T 2	45.6 ± 0.88^{p}		T 2	68.3 ± 0.33^{m}
	T 3	45.0 ± 0.57^{pq}		T 3	67.0 ± 0.00^{n}
	T 4	47.6 ± 0.33^{o}		T 4	68.6 ± 0.33^{lm}
	T 5	43.0 ± 0.57^{r}		T 5	65.0 ± 0.57^{o}

Values represent mean of 3 replicates with standard error. Values in each column followed by the same letter are significantly not different, according to Duncans test ($P < 0.01$).

Table 2. Percent inhibition of *Fusarium oxysporum* with *Trichoderma harzianum* isolates.

Sr No.	*Trichoderma* Isolates	Percent Inhibition of *Fusarium oxysporum*
1	T1	52.8±0.67^{bc}
2	T2	52.8±0.67^{bc}
3	T3	64.3±0.91^{a}
4	T4	55.6±1.83^{bc}
5	T5	50.7±0.72^{c}

Values represents means with standard error. Values in each column followed by same letters are significantly not different, according to Duncans test ($P < 0.01$)

(Foc) varied. Percent growth inhibition of 50.0% to 64.0% was caused by isolates of *Trichoderma* against wilt pathogens. *Trichoderma* isolate T3 was most promising against all the isolates of pathogen under dual culture studies. Similar were the findings of Orole and Adejumo (2009) who reported radial growth inhibition of *Fusarium* species from 25% to 75% by *Trichoderma*. Cundom *et al* (2003) reported variation in percent inhibition of *R. solani* from 38% to 59% with 9 isolates of *Trichoderma* species. There was a variation in percent inhibition by individual isolate of *Trichoderma* against Foc, used in dual culture studies. Different isolate have different potential to inhibit the growth of pathogen.

TH isolate T3 was most promising in controlling the Foc and it also produced higher radial diameter at different pH and temperature as compared to others. Ability of isolate T3 to tolerate higher level of pH and temperature make it suitable for application as biocontrol agent against Foc under diverse agro-climatic conditions.

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