RESEARCH ARTICLE

IN VITRO EVALUATION OF RHIZOSPHERE MICROBIAL ANTAGONISTIS AGAINST WILT PATHOGEN *FUSARIUM OXYSPORUM*IN CHILLI (*CAPSICUM ANNUUM* L.)

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Abstract: The indiscriminate use of highly hazardous chemical fungicides and pesticides in the process of disease management leading to serious threat to environment. Biological control represents a viable alternative to the use of chemical fungicides and it is considered to be a safe, effective and eco-friendly method for plant disease. The percent inhibition on the growth of the pathogen by the antagonist over control was calculated for every 24 hrs of inoculation up to seven days. The experimental data on 168 hr. of inoculation revealed that all the four rhizosphere antagonistic fungi inhibited the growth of the wilt pathogens at varying levels at a range of 16.19 to 84.14 per cent (Plate 1 and Table 1). Among which, RFA 2 showed the highest mycelial (84.14%) inhibition of pathogen followed by RFA 4 (75.84%) and RFA 1 (58.09%) where as among rhizospheric bacterial antagonists isolate RBA 1 which was identified as *B.subtilis* had shown highest inhibitory effect (64.14%) and out of two tested rhizospheric fluorescent pseudomonads RFP 1 has antagonistic effect against chilli wilt pathogen which inhibited 72.81 per cent of radial growth of the pathogen.

Keywords: Rhizospheric antagonists, Fusarium, Biocontrol,Chilli, Flourescent pseudomonads

INTRODUCTION

The narrow region of the soil which is very closer to plant roots that can be highly influenced by the root secretions of the plantscalled as rhizosphere, which always associated with microorganisms.Most of the beneficial microbes can reside in the rhizosphere which can inhibit the growth of the phyto pathogens. The beneficial rhizospheric microbes can be utilised as potential biocontrol agents as they can directly interfere by inhibiting the growth of the pathogen. The proper utilisation of rhizospheric fungal and bacterial antagonists can replace the chemical fungicides there by reducing the problem of environmental pollution and resurgence effect. The disease suppressive effect of rhizospheric microorganism may involve mechanism such as production of antibiotics (Hebber *et al*., 1992).

In this context,a roving survey was conducted in the major chilli growing areas of Andhra Pradesh to collect and isolate beneficial rhizospheric microbes.Twenty isolates were collected from the rhizosphere portion of healthy chilli plants. The antagonistic effect ofrhizosphere fungal and bacterial antagonists were tested towards the wilt pathogen of chilli by dual culture method. As an initial step, all the collected rhizosphere microbes were subjected to preliminary screening to know their antagonistic property. The rhizosphere antagonists which showed antagonistic activity in preliminary screening were further, tested individually to select the most efficient ones that showed better performance in dual culture against pathogen are the rhizospheric fungal antagonists RFA 1,RFA 2, RFA 3 and RFA 4 were identified as *T.asperellum*, *Penicillium* sp.,*Asperigillus flavus* and *T.harzianum* respectively. Whereas rhizospheric bacterial antagonists were identified as *Bacillus subtilis*, *B.pumilus* and *Pseudomonas fluorescence*.

MATERIALS AND METHODS

The antagonistic effect of rhizosphere fungal and bacterial antagonists was tested towards the wilt pathogen of chilli by dual culture method. As an initial step, all the collected rhizosphere microbes were subjected to preliminary screening to know their antagonistic property.

1. Preliminary screening of rhizosphere antagonists against the pathogen Fusarium wilt pathogen of chilli

Mycelial disc of the pathogen isolated from the chilli were taken from seven day old culture on PDA plate and incubated for 2 days. For screening of rhizosphere antagonistic bacteria, the isolates were inoculated one each on the four sides of the pathogen as a line of streak one cm away from the periphery of the petri dish. Similarly, for screening of rhizosphere antagonistic fungi, the mycelial discs of rhizosphere fungal isolates were placed one each on four sides of the pathogen at equidistance from the periphery of the petri dish one cm away from the edge of Petri dish. Plates with pathogen alone served as control. The inoculated plates were incubated at room temperature and observed for inhibition of the

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pathogen for five days or when there was full growth in the control.

The rhizosphere antagonists who showed antagonistic activity in preliminary screening were further, tested individually to select the most efficient ones.

1.1 *In vitro* evaluation of antagonistic rhizosphere fungi against chilli wilt pathogen

The fungal isolates were tested for their antagonistic potential against the *Fusarium* by dual culture method (Skidmore and Dickson, 1976). Mycelial disc of the pathogen from seven-day old culture grown on PDA was placed on one side of the plate and incubated at room temperature (26 ± 2°C) for two days. The mycelial disc (10 mm) of antagonistic fungi were placed on other side of the plate, four cm away from the pathogen and incubated. Three replications were maintained for each isolate. The pathogen grown as monoculture served as control. The plates were observed daily after 24 hrs. of inoculation of antagonists till the pathogen grew and covered the plate kept as control. The percent inhibition of the pathogen was calculated using the formula suggested by Vincent (1927).

$$PI = \frac{C - T}{C} \times 100$$

PI = Percent inhibition, C = Growth of the pathogen in control (mm), T = Growth of the pathogen in dual culture (mm).

Based on the percent inhibition of mycelia growth of the pathogen, the efficient antagonists were selected for further studies.

1.2 *In vitro* evaluation of antagonistic rhizosphere bacteria against chilli wilt pathogen

All the efficient rhizosphere bacterial antagonists were evaluated for their antagonistic effect by dual culture method (Utkhede and Rahe, 1983). Mycelial disc (10mm) taken from seven day old culture of the pathogen grown on PDA was placed at the centre of PDA mediated Petri dish and incubated for two days. The antagonistic bacteria were inoculated as a line of streak on both sides, one cm away from the edge of petri dish. For each isolate three replications were maintained. Plates with pathogen alone served as control. The plates were incubated at room temperature and observed daily,upto seven days of inoculation until the control exhibited full growth of the pathogen. The per cent inhibition was calculated by which the efficient ones can be selected.

RESULTS AND DISCUSSION

2. Antagonistic reaction of promising microbes against *fusarium* wilt in chilli

Ten bacteria, eight fungi and two fluorescent Pseudomonads were isolated from healthy rhizosphere portion of chilli by following standard serial dilution method as described in materials and methods (Plate 1 and Plate 2).

2.1 Rhizosphere fungal antagonists (RFA)

A total of eight fungal antagonists isolated from rhizosphere, were subjected to preliminary screening, out of which only 4 isolates have showed good inhibitory effect on radial growth of *Fusarium in vitro*. (table 1 and Plate 1). The efficient isolates obtained after preliminary screening were further evaluated for their antagonistic potential against the Fusarium wilt pathogen in terms of percent inhibition over the control.

The percent inhibition on the growth of the pathogen by the antagonist over control was calculated for every 24 hrs of inoculation up to seven days. (Table 1). The experimental data on 168 hr. of inoculation revealed that all the four rhizosphere antagonistic fungi inhibited the growth of the wilt pathogens at varying levels at a range of 16.19 to 84.14 per cent (Plate 1 and Table 1). Among which, RFA 2 showed the highest mycelial (84.14%) inhibition of pathogen followed by RFA 4 (75.84%) and RFA 1(58.09%), whereas the isolate RFA 3 showed the lowest inhibition of 16.19 per cent against Fusarium. The RFA 2 and RFA 4 had the fast mycelial growing capacity compared to the pathogen, they were overgrown and locked the growth of pathogen after 72 hrs. of inoculation µm (Plate 1). Hence the inhibitory effect of RFA2 was higher followed by RFA 4.

2.2 Rhizospheric bacterial antagonists (RBA)

Based on the performance of biocontrol activity in preliminary screening, out of ten bacteria isolated from rhizosphere, only four efficient bacteria were further evaluated for *in vitro* antagonistic activity. The results from the Table 1, Plate 2.) revealed that the isolate RBA 1 had shown highest inhibitory effect(64.14%) on *Fusarium* followed by RBA 3 (58.21%) and RBA 2 (23.44%) whereas the lowest inhibition of per cent was recorded by RBA 5 (18.84%) against *Fusarium oxysporum* at 168 hrs. of inoculation.

2.3 Rhizospheric fluorescent Pseudomonads (RFP)

Out of 2 fluorescent Pseudomonads isolates evaluated in the preliminary screening, only one *i.e.*, RFP 1 has antagonistic effect against chilli wilt pathogen which inhibited 72.81 per cent of radial growth of the pathogen.

The results are in agreement with Patel *et al.* (2017) who isolated a total of 35 isolates from the rhizospheric soil of tomato. Out of which, 11 isolates have shown antagonistic activity against *F. oxysporum* f. sp. *lycopersici*. Among those, two isolates showed maximum inhibition of fungal growth were MSS9 (83%) and MSS1(84.5%), respectively, against *F. oxysporum* f. sp. *lycopersici*. The similar results obtained by Hanan and Mohamed (2014) who observed the variability in degree of inhibition from one strain to another, *Trichoderma harzianum* was found highly antagonistic compared to *Trichoderma viride* isolates as it inhibited the

mycelial growth of *F. oxysporum* f. sp. *ciceris* and *F. solani* by 85.29% and 86.21% after 12 days of *invitro* incubation.Thesimilar results were obtained by Suraiya Yasmin *etal*. (2014), Sundaramoorthy *et al*. (2013), Ngueko *et al*., 2002, Goudar and Srikant kulkarni (1999) who reported that *T. harzianum* strain significantly reduced the mycelial growth of the *Fusarium oxysporum* upto 52 – 87%. Ramezani *et al*. (2010) and Basco *et al.* (2017) made extensive studies *in vitro* and concluded *T. harzianum* as the most promising antagonist for *F. oxysporum* f. sp. *lycopersici* by inhibiting the growth of *Fusarium* upto 95-100%. Basco *et al.* (2017) were evaluated for antagonistic activities against *F. oxysporum* f. sp. *lycopersici* after 4 days in dual culture assay. *B. subtilis* (83%) and *P. fluorescens* (88%) against the radial growth of *F. oxysporum* f. sp. *lycopersici*. Dilila Toua *et al*. (2013) have studied, the biocontrol capacity of *Pseudomonas fluorescens* against fusarium wilt of tomato and flax was evaluated both in vitro and *in vivo*. As compared to the untreated control, the mycelial growth decreased by 8.33 to 49.33%. Conidial germination and germ tube elongation weresignificantly inhibited and reduced, respectively, by 6.15 to 47.33% and by 1.63 to 45.45%. The Mycelial growth of *F. oxysporum* and *A. alternata* was strongly reduced in the presence of antagonistic fluorescent *Pseudomonas* sp. with the inhibition rate varying between 25 to 38%. This result was in agreement with Ommati and Zaker (2012), they found that *T. asperellum* had the significant inhibition of the growth of *F. oxysporum.* Dilila Toua *et al.* (2013) have studied, the biocontrol potential of *Pseudomonas fluorescens* against *Fusarium* wilt of tomato and flax both *in vitro* and in *vivo*. As compared to the untreated control, the mycelial growth decreased by 8.33 to 49.33%. Conidial germination and germ tube elongation was inhibited by 6.15 to 47.33% and 1.63 to 45.45% respectively.

Table 1.*Invitro* antagonistic activity of rhizosphere antagonists against *Fusarium oxysporum* at 168 hrs. of inoculation

S.No.	Rhizosphere antagonist	Radial growth of pathogen (mm)	PIOC (%)
1	RFA 1	37.00	58.09 (49.60)
2	RFA 2	14.00	84.14 (66.50)
3	RFA 3	74.00	16.19 (23.66)
4	RFA 4	21.33	75.84 (60.53)
5	RBA 1	31.66	64.14 (53.19)
6	RBA 2	67.66	23.44 (28.93)
7	RBA 3	36.33	58.21 (49.72)
8	RBA 4	71.66	18.84 (25.70)
9	RFP 1	24.00	72.81 (58.56)
10	CONTROL	88.33333	0 .00 (0)
	CD at 5%	0.479	
	SEM ±	0.161	

*Mean of three replications

**Figures in parenthesis are arc sin transformed values

Table 2. Percent inhibition of radial growth of pathogen by rhizosphere antagonists

S.no	Rhizosphere antagonists	Percent Inhibition Over Control (PIOC) %						
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.
1	RFA 1	7.53	37.34	48.83	50.32	56.6	59.05	58.09
2	RFA 2	56.62	63.84	72.45	74.33	77.16	80.63	84.14
3	RFA 3	1.86	4.8	5.5	7.54	13	13.78	16.19
4	RFA 4	37.71	48.19	56.69	59.81	65.25	70.49	75.84
5	RBA 1	16.98	26.5	40.9	47.16	48.35	56.21	64.14
6	RBA 2	3.73	7.23	23.62	28.3	23.98	17.01	23.44
7	RBA 3	13.19	24.07	40.16	42.83	46.72	50.67	58.21
8	RBA 4	3.73	7.23	7.56	10.69	10.83	13.33	18.84

9	RFP 1	35.84	46.99	52.09	58.49	60.84	66.8	72.81
10	Control	0	0	0	0	0	0	0

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