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Studies on Native Isolates of Fungal and Bacterial Bio-agents against Collar Rot of Chickpea

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A B S T R A C T

Sclerotium rolfsii Sacc, [teleomorph: *Athelia rolfsii* (Curzi)] causing collar rot of chickpea, is a devastating soil-borne fungus. It is reported almost all over the world with 10 to 30% yield loss is recorded annually according to severity of the disease. It is more serious at seedling stage causing plant mortality ranged from 54.7 to 95%. The present investigation was undertaken to find out bio-efficacy of twenty native isolates of *Trichoderma* spp. isolated from rhizospheric zone of eight different crops from different locations of Jabalpur by using serial dilution method against *Sclerotium rolfsii* and their effects on growth parameters of chickpea plant. *In vitro* screening of isolates (20) of *Trichoderma* spp. against *Sclerotium rolfsii* was done by dual culture technique with 87.41 and 39.78% of maximum and minimum per cent zone inhibition recorded with T- 20 and T- 7 isolate. On the basis of cultural and morphological characters best eight isolates; one from each host were selected for further studies in polyhouse. Tr- 7 was found to be most effective with minimum seedling mortality of 6.67%. Soil inoculation was found more effective as compared to seed treatment in controlling seed rotting as well as seedling mortality. In combination treatments some isolates of *Trichoderma* spp. were found to be synergistic (Tr- 2, Tr- 4, Tr-6, Tr- 7 and Tr- 8) whereas, some exhibiting antagonistic effect (Tr-1, Tr-3 and Tr- 5) with *Pseudomonas fluorescence.*

Introduction

Persoon (1794) was the first scientist to propose *Trichoderma*as a genus over two hundred years ago back. It was described as mealy powder enclosed by a hairy covering. Thakur and Norris (1928) from Madras isolated *Trichoderma* in India first time. It is a potent fungal bio-control agent against a wide range of plant pathogens. Especially, for the management of soil borne plant pathogens *Trichoderma* spp. was reported as important

biological control agents (Upadhyay and Mukhopadhyay, 1986, Kumar *et al.,* 2012andJabbar *et al.,* 2014). It was reported that *Trichoderma* spp. involves wide range of key characteristic mechanisms for disease control i.e, Mycoparasitism and hyphallysis, antibiosis, competition for nutrients and space and also promotion of plant growth (Rudresh *et al.,* 2005; Jash *et al.,* 2007 and Swathi *et al.,* 2015). Enhance plant growth response and productivity was induced with application of *Trichoderma* species and other root colonizing fungi reported in many crops such as beans, cucumber, pepper, Carnation, maize and wheat (Balasubramanian, 2003). Collar rotis a devastating soil-borne disease of chickpea caused by fungus *Sclerotium rolfsii* Sacc, [teleomorph: *Athelia rolfsii* (Curzi)], causing heavy economic losses (Kokub *et al.,* 2007). Wherever chickpea is grownall over the world it is almost reported everywhere and caused 10 to 30 per cent yield loss annually according to severity of the disease (Nene *et al.,* 1984).

Several control measures have been adopted for control of *S. rolfsii* by many scientists which includes biological control (Pandey and Chaube, 2004), cultural control (Blum and Rodriguez-kabana, 2006 and Pinheiro *et al.,* 2010), and chemical control (Paula *et al.,* 2011). Over conventional fungicides, biological control of plant pathogens has a number of advantages, as fungicides features only a temporary effect and require repeated applications during the growing period of crop while, the biological control agents are able to establish themselves, colonize and reproduce in the ecosystem (Melo and Faull, 2000).

Materials and Methods

Isolation Purification and identification of *Trichoderma* spp. and test pathogen

For isolation of different isolates of *Trichoderma* spp., soil samples were collected from different locations of Jabalpur. The soil samples were collected with the help of a soil augur up to a depth of 15-20 cm from rhizospheric zone individual plants, well dried and were stored in refrigerator 4±1^{o}C until further use. Soil sample of 5 g weight was weighed and placed in a beaker containing 95 ml of sterile distilled water. After shaking thoroughly, it was allowed to stand for a few minutes. Then serial dilution was made up to 10^{-4} dilution and 1 ml was drawn from each dilution and spread over the PDA plate and incubated at 28±1 ^{o}C. The plates were monitored regularly for the development of colonies. The *Trichoderma* isolates were subjected to sub-culturing on PDA medium for purification. Sub-culturing was done. These Petri plates were incubated at 28±1^{0}C for seven days with periodic observation for development of colonies of *Trichoderma* spp. The colonies of *Trichoderma* were identified by key based on branching of conidiophores, shape of phialides, emergence of phialides and spore characters (Gams and Bisset, 2002).Isolation of *Sclerotium rolfsii* was done from infected collar and upper root portion. Surface sterilized was done with 0.1% of $HgCl_2$ solution for 1 min, then aseptically transferred to Petri plates containing the sterilized PDA medium and incubated at 28±1 ^{o}C. By single sclerotia method under aseptic conditions pure culture was maintained (Pandey *et al.,* 2010).

Screening of antagonistic potential of *Trichoderma* spp. in vitro

Screening of twenty one isolates of *Trichoderma* spp. for their antagonistic potential *in vitro* against *S. rolfsii* was done by using dual culture method. Per cent inhibition of the pathogen over control was calculated by adopting the formula from Rudresh *et al.,* (2005).

I (%) = (C-T)/ C x 100

Where, I = Percent growth inhibition, C= Growth in control (monoculture), T= Growth in treatment (dual culture)

Evaluation of bio-efficacy of *Trichoderma* spp. and *Pseudomonas fluorescens* against *S. rolfsii in vivo*

Both the pathogen and antagonist were multiplied on sorghum grains. Sorghum grains were pre-soaked in 2 per cent sucrose solution overnight, drained and boiled in fresh water for 30 minutes and drained again. This was transferred into 1000 ml flasks @ 400 g and autoclaved at 15 lb psi (121.6 ^{0}C) for 20 minutes.

The flasks were allowed to cool at room temperature and inoculated with five mm discs of 3 to 4 days old culture of *S. rolfsii* grown on PDA. Seven discs per flask were added and flasks were incubated for three weeks at 28 ± 1 ^{0}C. Pot culture studies were conducted to evaluate efficacy of *Trichoderma in vivo*. By using two different application methodologies, i.e. seed treatment with 5 g/kg seed and soil application with 10 g/kg soil were used to assess the bio-control potential of *Trichoderma* isolates alone and in combination with *Pseudomonas fluorescens* in decreasing the collar rot of chickpea caused by *S. rolfsii* and also their effects on growth parameters of chickpea plant. Chickpea seed were sown @ 5 seed/pot and were watered time to time. Percent germination was recorded 10 days after sowing and final count after 30 days of sowing. Height (cm), dry weight and fresh weight (gm) were recorded to calculate vigour index mass, vigour index percentage and seedling mortality up to 30 days by using following formulae given by (Kharb*et al.*, 1994) ;

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of seedlings}} \times 100$$

Results and Discussion

Twenty isolates of *Trichoderma* spp. were collected from different locations of Jabalpur from rhizospheric zone of eight different crops *viz.*, Rice, Red gram, Okra, Soybean, Black gram, Green gram, Neem and Sesame (Table 1). These were designated with symbol T- 1, T- 2, T- 3 up to T- 20 according to the order they were collected.Online interactive keys of Samuels *et al.*, (2002) used for identification of the potential isolates of *Trichoderma* spp. based on the colony appearance and pigmentation, the presence, growth rate and branching patterns of conidiophores.

Radial growth rate of different twenty isolates of *Trichoderma* spp. was also recorded on PDA at different incubation periods at 28±1°C. *In vitro* screening of isolates of *Trichoderma* spp. against *Sclerotium rolfsii* done by Dual culture technique (Table 1). Maximum per cent zone inhibition of 87.41per cent recorded with T- 20 isolate, followed by 85, 77.3, 71.11, 69.11, 61.85 and 60.11 per cent with isolates T- 10, T- 1, T- 15, T- 2, T- 17 and T- 4 respectively against 0 per cent in control. Whereas, minimum per cent zone inhibition of 39.78 per cent recorded with T- 7 isolate, followed by 49.82 and 52.22 per cent in T- 13 and in T- 3 isolate respectively. Almost isolates were found to have significant variation among them, whereas differences among T- 5, T- 6, T- 8, T- 9, T- 12, T- 16, T- 18 and T- 19 isolates were found at par. T- 3, T- 2, T- 17, T- 14, T- 10, T- 20, T- 15 and T- 5were selected from each crop i.e., Red gram, Rice, Soybean, Sesame, Green gram, Black gram, Neem and Okra plant rhizosphere respectively and were re-designated as T- 1 as Tr- 1, T- 2 as Tr- 2, T- 17 as Tr- 3, T- 14 as Tr- 4, T- 10 as Tr- 5, T- 20 as Tr- 6, T- 15 as

Tr- 7 and T- 5 as Tr- 8 (Plate- 1) and were used for further studies.

Efficacy isolates of *Trichoderma* spp. against collar rot incidence of chickpea in Cvs JG 14 and JG 24

On seed treatment

It was observed that, on seed seed treatment significant increase in germination percentage up to 100 per cent was recorded with Tr- 2, Tr- 6 and Tr- 7 over control (66.67%) in variety JG 14. Whereas, effects of Tr- 1, Tr- 3, Tr- 5 and *Pseudomonas fluorescens* was found statistically at par (Table 2). Similarly, 100 per cent germination was also recorded with Tr- 7. Other treatments were also found significantly effective in enhancing germination percentages in JG 24 as compared to 60 per cent in pathogen treated control and 73.33 per cent in untreated control. Whereas, differences among treatments Tr- 1, Tr- 2, Tr- 3, Tr- 5 and Tr- 6 were found statistically at par.No seed rotting was recorded with Tr- 2, Tr- 6 and Tr- 7 treatments in variety JG 14 and with Tr- 7 treatment in variety JG 24. However seed rotting ranges from 0 to 33.33 and 0 to 40 per cent inJG 14 and JG 24 respectively with different treatments.

There was significant reduction in seedling mortality observed with different isolates of *Trichoderma* spp., out of which minimum seedling mortality of 6.67 per cent was recorded with Tr- 7 followed by Tr- 2 and Tr- 6, Tr- 1 and Tr- 3 and Tr- 5 exhibited 20, 21.67 and 30 per cent mortality respectively which differ significantly as compared to 77.22 per cent in pathogen treated control and 50 per cent in untreated control. Similarly, in variety JG 24 minimum seedling mortality of 6.67 per cent was found with Tr- 7, followed by Tr- 2 and Tr- 6, Tr- 1, Tr- 3 and Tr- 5 exhibited 20, 23.33, 21.67 and 28.33 per cent respectively. Other treatments also significantly reduce seedling mortality as compared to 77.78 per cent in pathogen treated control in variety JG 24. Whereas, differences among treatments Tr- 1, Tr- 2, Tr- 3 and Tr- 6 isolates were found statistically at par.

Soil inoculation

Data presented in the Table 3 revealed that, there was significant increase in germination percentage up to 100 per cent, recorded with Tr- 7 and Tr- 6 in variety JG 14. However, in other treatments 93.33 per cent germination recorded with Tr- 1, Tr- 2, Tr- 3, Tr- 4 and *Pseudomonas fluorescens,* and followed by 86.66 per cent with Tr- 5 against 66.67 per cent in pathogen treated control. Similarly, 100 per cent germination was recorded with Tr- 7 and Tr- 5 in variety JG 24. Other treatments were only found significantly effective in enhancing germination per cent in JG 24 as compared to 60 per cent in pathogen treated control and 73.33 per cent in untreated control. Whereas differences among treatments Tr- 1, Tr- 2, Tr- 3and Tr- 4 were found statistically at par.No seed rotting was recorded with Tr- 6 and Tr- 7 treatments in JG 14and with Tr- 7 treatment in variety JG 24. However seed rotting ranges from 0 per cent to 33.33 per cent in JG 14 and 0 to 40 per cent in JG 24 with different treatments.

Significant reduction in seedling mortality was observed with different isolates of *Trichoderma* spp., of which minimum seedling mortality of 6.67 per cent was recorded with Tr- 7 followed by 13.33per cent with Tr- 6 in variety JG 14 which differ significantly as compare to 77.22 per cent in pathogen treated control and 50 per cent in untreated control. Whereas, differences among treatments Tr- 1, Tr- 2, Tr- 3 and Tr- 5 were found at par. Similarly, in variety JG 24 minimum seedling mortality of 13.33 per cent was found with Tr- 7, followed by 20 and

21.67 per cent in isolates Tr- 2 and Tr- 6, Tr- 3 and Tr-5 respectively. Effect of Tr- 7 was found highly significant than other treatments whereas, differences among treatments Tr- 2, Tr- 3, Tr- 6 and *Pseudomonas fluorescens* were found statistically at par.

Efficacy isolates of *Trichoderma* spp. in combination with *Pseudomonas fluorescens* against collar rot incidence of chickpea in Cvs JG 14 and JG 24

Seed treatment

There was significant increase in germination percentage of 93.33% found with treatment Tr- 4 +*P. fluorescens* in variety JG 14 against 86.67 per cent when used alone with Tr- 4 isolate. In all most treatments i.e. Tr- 1 + *P. fluorescens*, Tr- 3 + *P. fluorescens* and Tr- 5 + *P. fluorescens* germination percentages decreased significantly. Whereas, in treatments Tr- 2 + *P. fluorescens* (100%), Tr- 6 + *P. fluorescens* (100%), Tr- 7 + *P. fluorescens* (100%) and Tr- 8 + *P. fluorescens* (86.67%) no change in germination percentage were recorded (Table 4). However, significant increased germination percentage was recorded in treatment Tr- 2 + *P. fluorescens* and Tr- 6 + *P. fluorescens* in variety JG 24 from 93.33 to 100 per cent. In treatments Tr- 1 + *P. fluorescens*, Tr- 3 + *P. fluorescens* and Tr- 5 + *P. fluorescens* germination percentages decreased significantly as compared to when applied alone. However, no change in germination percentage recorded with Tr- 4 + *P. fluorescens* (86.67%) and Tr- 7 + *P. fluorescens* (100%).

Table.1 Categorization of different isolates of *Trichoderma* spp. based on radial growth rate and per cent zone inhibition

Crop	Isolates	Radial Growth On 3rd Day (mm)	Per cent zone Inhibition of *S. rolfsii* (%)	Remark	Re-designation of selected Isolates
Red gram	T- 1	32.0	77.3	T- 1	Tr- 1
	T- 3	45.0	52.22		
	T- 6	34.0	57.85		
Rice	T- 2	32.3	69.11	T- 2	Tr- 2
	T- 4	31.0	60.11		
Soybean	T- 8	32.9	56.3	T- 17	Tr- 3
	T- 9	31.0	56		
	T- 17	34.0	61.85		
Sesame	T- 13	27.0	49.82	T- 14	Tr- 4
	T- 14	31.0	59.7		
Green gram	T- 10	32.5	85	T- 10	Tr- 5
	T- 12	44.5	57.11		
	T- 16	30.5	56.66		
Black gram	T- 18	33.5	55	T- 20	Tr- 6
	T- 20	32.5	87.41		
Neem	T- 11	32.5	59.47	T- 15	Tr- 7
	T- 15	41.0	71.11		
	T- 19	33.5	56.33		
Okra	T- 5	34.5	55.93	T- 5	Tr- 8
	T- 7	34.1	39.78		

Data presented in the table are average of three replications

Table.2 Efficacy of different isolates of *Trichoderma* spp. and *Pseudomonas fluorescens* as seed treatment against collar rot incidence of chickpea in Cvs JG 14 and JG 24

Verities	JG 14			JG 24		
Treatment	Germ. (%)	Percent Mortality		Germ. (%)	Percent Mortality	
		Seed rot	Seedling mortality		Seed rot	Seedling mortality
Tr- 1 isolate	93.33	6.67	21.67	93.33	6.67	23.33
Tr- 2 isolate	100	0	20	93.33	6.67	20
Tr- 3 isolate	93.33	6.67	21.67	93.33	6.67	21.67
Tr- 4 isolate	86.67	13.33	40.00	86.67	13.33	36.67
Tr- 5 isolate	93.33	6.67	30.00	93.33	6.67	28.33
Tr- 6 isolate	100	0	20.00	93.33	6.67	20
Tr- 7 isolate	100	0	6.67	100	0	6.67
Tr- 8 isolate	86.67	13.33	31.67	86.67	13.33	31.67
Pseudomonas fluorescens	93.33	6.67	28.33	86.67	13.33	23.33
Carbendazim + Thiram (1:1)	86.67	13.33	36.67	86.67	13.33	45
Inoculated soil +no treatment	66.67	33.33	72.22	60	40	77.78
Healthy soil + no treatment	80	20	50.00	73.33	26.67	52.78
SE m(±)	0.901	0.901	1.450	1.425	1.425	1.473
CD 5%	2.645	2.645	4.257	4.184	4.184	4.326

Data presented in the table are average of three replications

Table.3 Efficacy of different isolates of *Trichoderma* spp. and *Pseudomonas fluorescens* as soil inoculation against collar rot incidence of chickpea in Cvs JG 14 and JG 24

Verities	JG 14			JG 24		
Treatment	Germ. (%)	Percent Mortality		Germ. (%)	Percent Mortality	
		Seed rot	Seedling mortality		Seed rot	Seedling mortality
Tr- 1 isolate	93.33	6.67	21.67	93.33	6.67	28.33
Tr- 2 isolate	93.33	6.67	20.00	93.33	6.67	20
Tr- 3 isolate	93.33	6.67	26.67	93.33	6.67	21.67
Tr- 4 isolate	93.33	6.67	38.33	86.67	13.33	36.67
Tr- 5 isolate	86.67	13.33	21.67	93.33	6.67	28.33
Tr- 6 isolate	100	0.00	13.33	93.33	6.67	20
Tr- 7 isolate	100	0.00	6.67	100	0	13.33
Tr- 8 isolate	80.00	20.00	33.33	86.67	13.33	31.67
Pseudomonas fluorescens	93.33	6.67	28.33	86.67	13.33	23.33
Carbendazim + Thiram (1:1)	80.00	20.00	39.44	86.67	13.33	45
Inoculated soil +no treatment	66.67	33.33	72.22	60	40	77.78
Healthy soil + no treatment	80.00	20.00	50.00	73.33	26.67	52.78
SE m(±)	1.207	1.207	1.696	1.458	1.458	2.268
CD 5%	3.544	3.544	4.98	4.28	4.28	6.658

Data presented in the table are average of three replications

Table.4 Efficacy of different isolates of *Trichoderma* spp. in combination with *Pseudomonas fluorescens* as seed treatment on per cent germination and mortality of chickpea in Cvs JG 14 and JG 24

Verities	JG 14			JG 24		
Treatment	Germ. (%)	Percent Mortality		Germ. (%)	Percent Mortality	
		Seed rot	Seedling mortality		Seed rot	Seedling mortality
Tr- 1 isolate	93.33	6.67	21.67	93.33	6.67	23.33
Tr- 2 isolate	100	0	20	93.33	6.67	20
Tr- 3 isolate	93.33	6.67	21.67	93.33	6.67	21.67
Tr- 4 isolate	86.67	13.33	40	86.67	13.33	36.67
Tr- 5 isolate	93.33	6.67	30	93.33	6.67	28.33
Tr- 6 isolate	100	0	20	93.33	6.67	20
Tr- 7 isolate	100	0	6.67	100	0	6.67
Tr- 8 isolate	86.67	13.33	31.67	86.67	13.33	31.67
Tr- 1 isolate + *P. fluorescens*	86.67	13.33	40	86.67	13.33	36.67
Tr- 2 isolate + *P. fluorescens*	100	0	20	100	0	13.33
Tr- 3 isolate + *P. fluorescens*	80	20	50.56	80	20	58.83
Tr- 4 isolate + *P. fluorescens*	93.33	6.67	8.83	86.67	13.33	6.67
Tr- 5 isolate + *P. fluorescens*	86.67	13.33	36.67	86.67	13.33	38.33
Tr- 6 isolate + *P. fluorescens*	100	0	13.33	100	0	13.33
Tr- 7 isolate + *P. fluorescens*	100	0	6.67	100	0	6.67
Tr- 8 isolate + *P. fluorescens*	86.67	13.33	28.33	93.33	6.67	28.33
Pseudomonasfluorescens	93.33	6.67	28.33	86.67	13.33	23.33
Carbendazim + Thiram (1:1)	86.67	13.33	36.67	86.67	13.33	45
Inoculated soil +no treatment	66.67	33.33	72.22	60	40	77.78
Healthy soil + no treatment	80	20	50	73.33	26.67	52.78
SE m(±)	1.083	1.083	1.533	1.425	1.425	1.434
CD 5%	3.106	3.106	4.397	4.087	4.087	4.114

Data presented in the table are average of three replications

Table.5 Efficacy of different isolates of *Trichoderma* spp. in combination with *Pseudomonas fluorescens* as soil inoculation on per cent germination and mortality of chickpea in Cvs JG 14 and JG 24

Verities	JG 14			JG 24		
Treatment	Germ. (%)*	Percent Mortality		Germ. (%)	Percent Mortality	
		Seed rot	Seedling mortality		Seed rot	Seedling mortality
Tr- 1 isolate	93.33	6.67	21.67	93.33	6.67	28.33
Tr- 2 isolate	93.33	6.67	20	93.33	6.67	20
Tr- 3 isolate	93.33	6.67	26.67	93.33	6.67	21.67
Tr- 4 isolate	93.33	6.67	38.33	86.67	13.33	36.67
Tr- 5 isolate	86.67	13.33	21.67	93.33	6.67	28.33
Tr- 6 isolate	100	0	13.33	93.33	6.67	20
Tr- 7 isolate	100	0	6.67	100	0	13.33
Tr- 8 isolate	80.00	20.00	33.33	86.67	13.33	31.67
Tr- 1 isolate + *P. fluorescens*	86.67	13.33	53.33	80	20	33.33
Tr- 2 isolate + *P. fluorescens*	100	0	13.33	100	0	13.33
Tr- 3 isolate + *P. fluorescens*	86.67	13.33	30	86.67	13.33	30.00
Tr- 4 isolate + *P. fluorescens*	93.33	6.67	6.67	86.67	13.33	21.67
Tr- 5 isolate + *P. fluorescens*	73.33	26.67	28.89	80	20	33.33
Tr- 6 isolate + *P. fluorescens*	100	0	6.67	100	0	13.33
Tr- 7 isolate + *P. fluorescens*	100	0	6.67	100	0	6.67
Tr- 8 isolate + *P. fluorescens*	86.67	13.33	26.67	86.67	13.33	21.67
Pseudomonasfluorescens	93.33	6.67	28.33	86.67	13.33	23.33
Carbendazim + Thiram (1:1)	80	20	39.44	86.67	13.33	45
Inoculated soil +no treatment	66.67	33.33	72.22	60	40	77.78
Healthy soil + no treatment	80	20	50	73.33	26.67	52.78
SE m(±)	1.126	1.126	1.743	1.541	1.541	2.141
CD 5%	3.229	3.229	5.001	4.42	4.42	6.141

Data presented in the table are average of three replications

Plate.1 Pure culture of best eight isolates of *Trichoderma* spp. from each crop

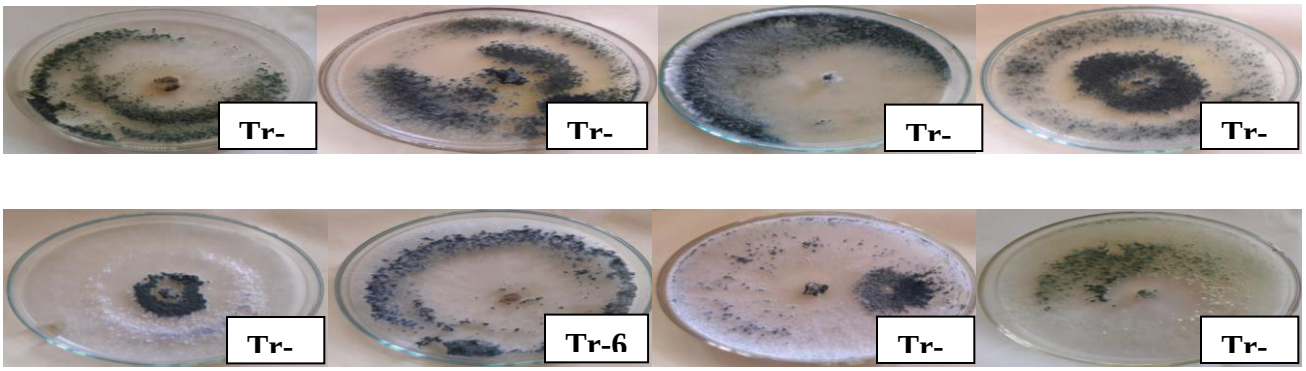


Plate.2 Bio-fficacy of different isolates of *Trichoderma* spp. and *Pseudomonas fluorescens*as seed treatment against collar rot incidence of chickpea in Cv JG 14

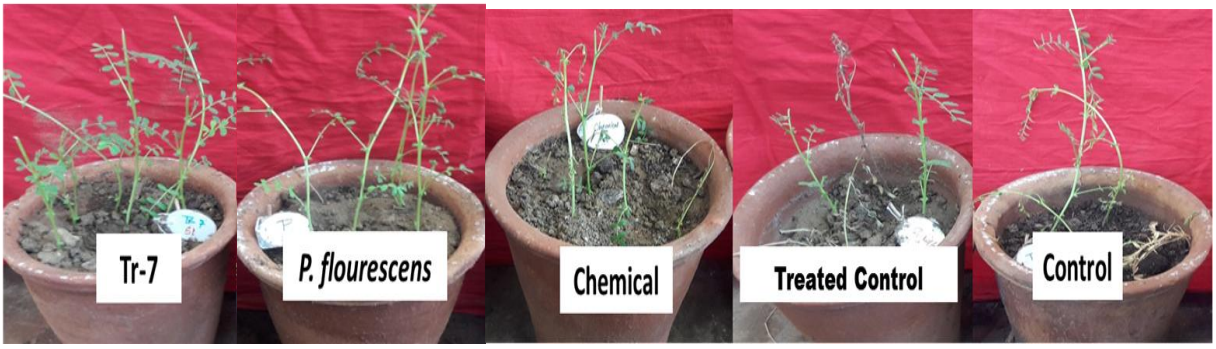
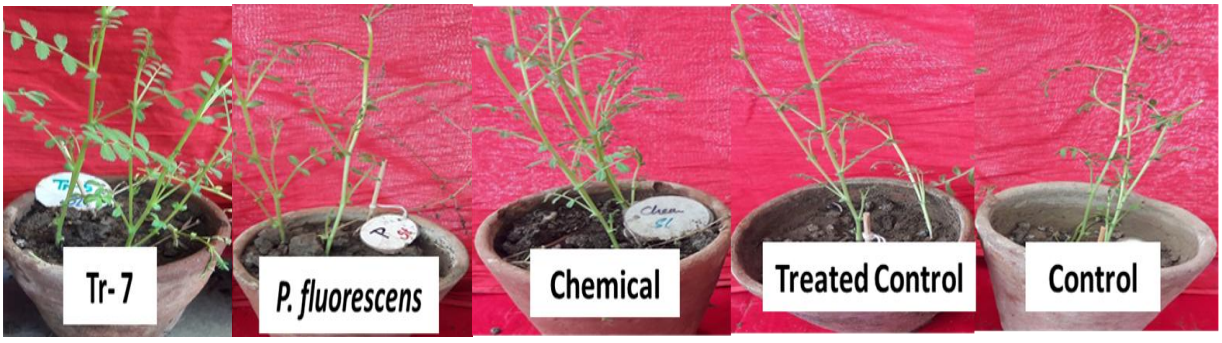


Plate.3 Bio-fficacy of different isolates of *Trichoderma* spp. and *Pseudomonas fluorescens* as soil inoculation against collar rot incidence of chickpea in Cv JG 14



There was significant decreased in seedling mortality was recorded in most treatments i.e. Tr- 4 + *P. fluorescens*, Tr- 6 + *P. fluorescens* and Tr- 8 + *P. fluorescens* with 8.83, 13.33 and 28.33 per cent respectively as compared to seedling mortality when applied alone in variety JG 14. However, increased in seedling mortality was recorded in treatments with Tr- 1 + *P. fluorescens*, Tr- 3 + *P. fluorescens* and Tr- 5 + *P. fluorescens* with 40, 50.56 and 36.67 per cent respectively as compared to seedling mortality when applied alone. Whereas, effects of Tr- 2 + *P. fluorescens*, Tr- 7 + *P. fluorescens* and Tr- 8 + *P. fluorescens*

were found statistically at par as compared to when applied alone. Similarly, decreased in seedling mortality was recorded in treatments Tr- 2 + *P. fluorescens*, Tr- 4 + *P. fluorescens*, Tr- 6+ *P. fluorescens*, and Tr- 8 + *P. fluorescens* with 13.33, 6.67, 13.33, and 28.33 per cent respectively as compared to seedling mortality when applied alone in variety JG 24.

However, significant increased in seedling mortality was recorded in treatments with Tr- 1 + *P. fluorescens* (36.67%), Tr- 3 + *P. fluorescens* (58.33%) and Tr- 5 + *P. fluorescens* (38.33%). Whereas, differences among the treatments Tr- 6+ *P. fluorescens*, and Tr- 8 + *P. fluorescens* were found statisticallyat par as compared to when applied alone.

Soil inoculation

There was significant increase in germination percentage was found in treatment Tr- 2 + *P. fluorescens* (100%) and Tr- 8 + *P. fluorescens* (86.67%) in variety JG 14 against 93.33% and 80% when used alone with Tr- 2 and Tr- 8 respectively (Table 5). In all most treatments i.e. Tr- 1 + *P. fluorescens*, Tr- 3 + *P. fluorescens* and Tr- 5 + *P. fluorescens* germination percentages decreased significantly. Whereas, in treatments Tr- 4 + *P. fluorescens* (93.33%), Tr- 6 + *P. fluorescens* (100%) and Tr- 7 + *P. fluorescens* (100%)effects was found at par. However, significant increased germination percentage was recorded in treatment Tr- 2 + *P. fluorescens* and Tr- 6 + *P. fluorescens* in variety JG 24 from 93.33 to 100 per cent. In treatments Tr- 1 + *P. fluorescens*, Tr- 3 + *P. fluorescens* and Tr- 5 + *P. fluorescens* germination percentages decreased significantly. However, no change in germination percentage recorded with Tr- 4 + *P. fluorescens* (86.67%), Tr- 7 + *P. fluorescens* (100%) and Tr- 8 + *P. fluorescens* (86.67%). Whereas, there was significant decreased in seedling mortality was recorded in most treatments i.e. Tr- 2 + *P. fluorescens*, Tr- 4 + *P. fluorescens*, Tr- 6 and Tr- 8 + *P. fluorescens* with 13.33, 6.67, 6.67 and 26.67 per cent respectively as compared to seedling mortality when applied alone in variety JG 14. However, significant increased in seedling mortality was recorded in treatments with Tr- 1 + *P. fluorescens* and Tr- 5 + *P. fluorescens* with 53 and 28.89 per cent respectively as compared to seedling mortality when applied alone whereas, differences among the treatments Tr- 3 + *P. fluorescens* and Tr- 7 + *P. fluorescens* were found statistically at par. Similarly, decreased in seedling mortality was recorded in treatments Tr- 2 + *P. fluorescens*, Tr- 4 + *P. fluorescens*, Tr- 6+ *P. fluorescens*, Tr- 7 + *P. fluorescens* and Tr- 8 + *P. fluorescens* with 13.33, 21.67, 13.33, 6.67 per cent and 21.67 per cent respectively as compared to seedling mortality when applied alone in variety JG 24. However, significant increased in seedling mortality was recorded in treatments with Tr- 3 + *P. fluorescens* (30%).Whereas, effect of treatments Tr- 1 + *P. fluorescens* (33.33%)and Tr- 5 + *P. fluorescens* (33.33%)were found statistically at par.

Efficacy isolates of *Trichoderma* spp. as seed and soil treatment against collar rot incidence of chickpea in Cvs JG 14 and JG 24

On seed treatment with different isolates of *Trichoderma* spp. significant reduction in seedling mortality was observed, out of which minimum seedling mortality of 6.67 per cent was recorded with Tr- 7 which differ significantly as compared to 77.22 per cent in pathogen treated control and 50 per cent in untreated control. Similarly, in variety JG 24 minimum seedling mortality of 6.67 per cent was found with Tr- 7, followed by 20 per cent in Tr- 6 and Tr- 2. Other treatments also

significantly reduced seedling mortality as compared to 77.78 per cent in pathogen treated control (Table 4). Similar report of efficacy of *Trichoderma* spp. in decreasing collar rot of chickpea through seed treatment method were also reported by Biswas and Sen (2000), Dutta and Das (2002), Patibanda *et al.,* (2002) and Jegathambigai *et al.,* (2010).

Minimum seedling mortality of of 6.67 per cent was recorded on soil treatment with isolate Tr- 7 followed by 13.33 and 20 per cent in Tr- 2 and Tr- 6 isolates respectively in variety JG 14 as compared to 77.22 per cent in pathogen treated control and 50 per cent in untreated control in variety JG 14. Similarly, in variety JG 24 exhibited minimum seedling mortality of 13.33 per cent with Tr- 7 followed by 20 per cent in both isolates Tr- 2 and Tr- 6 as compared to 77.78 per cent in pathogen treated control (Table 5). Srivastava *et al.,* (2010) and Montealegre *et al.,* (2010) had also reported the similar results.

Efficacy isolates of *Trichoderma* spp. in combination with *Pseudomonas fluorescens* as seed and soil treatment against collar rot incidence of chickpea in Cvs JG 14 and JG 24

There was significant decrease in seedling mortality was recorded on seed treatmentwith most treatments i.e. Tr- 4 + *P. fluorescens* (8.83%), Tr- 6 + *P. fluorescens* and Tr- 8 + *P. fluorescens*(28.3%) in variety JG 14 and Tr- 2 + *P. fluorescens* (13.33%), Tr- 4 + P. fluorescens (6.67%), Tr- 6+ *P. fluorescens* (13.33%), and Tr- 8 + *P. fluorescens* (28.33%) in variety JG 24. Similar finding of synergistic effect of *P. fluorescens* with *Trichoderma* spp. had also been reported by Duffy *et al.,* (1995) and Mishra *et al.,* (2011). However, increased seedling mortality was recorded in treatments with Tr- 1 + *P. fluorescens* (40%), Tr- 3 + *P. fluorescens* (50.56%) and Tr- 5 + *P. fluorescens* (36.67) in variety JG 14 and with Tr- 1 + *P. fluorescens* (36.67%), Tr- 3 + *P. fluorescens* (58.33%) and Tr- 5 + *P. fluorescens* (38.33%) in variety JG 24. Similar finding of antagonistic effect of *P. fluorescens* on *Trichoderma* spp. on seed treatment had also been reported by Hubbard *et al.,* (1883), Bin *et al.,* (1991) and Mishra *et al.,* (2011).

Similarly, on soil treatment both antagonistic and synergistic effect of *P. fluorescens* on *Trichoderma* spp. was found. Significant decrease in seedling mortality was recorded with Tr- 2 + *P. fluorescens* (13.33%), Tr- 4 + *P. fluorescens* (6.67%), Tr- 6+ *P. fluorescens* (6.67%) and Tr- 8 + *P. fluorescens* (26.67%) in JG 14 and in Tr- 2 + *P. fluorescens* (13.33%), Tr- 4 + *P. fluorescens* (21.67%), Tr- 6+ *P. fluorescens* (13.33%), Tr- 7 + *P. fluorescens* (6.67%) and Tr- 8 + *P. fluorescens* (21.67%) in JG 24. Present findings are similar to the results reported by Elad *et al.,* (2000) and Guetsky *et al.,* (2001). However, significant increase in seedling mortality was recorded in treatments with Tr- 1 + *P. fluorescens* and Tr- 5 + *P. fluorescens*. Present findings are similar to the results reported by reported by Bin *et al.,* (1991) and Mishra *et al.,* (2011) that *Pseudomonas fluorescens* exhibited the antagonistic effect on *Trichoderma* spp. against collar rot of chickpea.

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