The Pharma Innovation Journal 2022; 11(2): 1242-1245

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(2): 1242-1245

www.thepharmajournal.com
Received: 09-12-2021
Accepted: 24-01-2022

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Efficacy of bioagents and fungicide against root rot of chilli caused by *Rhizoctonia solani* Kuhn

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Abstract
Efficacy of antagonists and fungicide were evaluated under *in vitro* conditions for the mycelial growth inhibition. The antagonistic actions of three species of *Trichoderma* and two spp. of bacteria were evaluated against the test fungus by dual culture and paper disc technique, respectively. It was observed that all the biocontrol agents were significantly superior in inhibiting the mycelial growth of test fungus over control. Maximum growth inhibition was recorded in *T. harzianum* (64.81%) followed by *T. viride* and *T. atroviride*. Among the bacterial bioagent *Pseudomonas fluorescens* inhibit (41.48%), this was statistically at par with *Bacillus subtilis* (34.81%). The efficacy of Tebuconazole 2DS on mycelial growth of *R. solani* was recorded after 3, 5 and 7 days with the concentrations of 1.0, 10, 25, 50 and 100 ppm. The maximum inhibition of mycelial growth was observed in100 ppm where it was 16.66 mm, 17.33 mm after 3, 5 and 7 days respectively followed by 50 ppm where it was 34 mm, 35.70 mm and 36.33 mm after 3, 5 and 7 days, respectively. While 10 ppm and 1 ppm concentration was not much effective and it was descending order of merit. Thus 100 ppm and 50 ppm concentration effectively check the mycelial growth of *R. solani* in all the growth stage period *i.e.* 3, 5 and 7 days.

Keywords: Antagonists, biocontrol agents, fungicide, growth inhibition

Introduction
Chilli (*Capsicum annum* L.) is one of the most important commercial crop of India belongs to the Solanaceae family mainly cultivated for its green fruits as vegetable and dry chilli as spice purpose. It comprises numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, proteins, fibers and mineral elements. Capsicum fruits may serve as a source of natural bactericidal agents to be used in food and medicinal systems. Chilli is commercially important for two qualities, *i.e.*, its red colour is due to the pigment capsanthin and its biting pungency is due to capsaicin.

This crop is a native of tropical America and West Indies and was introduced in India from the Portuguese in the seventeenth century. Since then it has gained importance as an inevitable condiment and vegetable. It is cultivated about 831000 hectares area in India with annual production of 1872000 MT (Anonymous, 2016-2017) [4]. However, in Rajasthan the area under chilli is about 5139 hectares with annual production of 9449 MT (Anonymous, 2015-16) [3]. The major chilli growing states are Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu and Rajasthan. In Rajasthan Jodhpur, Ajmer, Bhilwara, Pali, Sikar, Alwar, Bharatpur and Swai madhopur are major chilli growing districts.

Among the fungal diseases, root rot complex of chilli is a serious infestation. In standing plants, yellowing and wilting are usually preceded by light to dark brown lesion on the stem adjacent to the ground followed by drooping and wilting of infected leaves and gradual wilting of infected leaves and gradual wilting of the whole plant. Mature plants dry suddenly. Seedlings affected by this infestation die soon after germination.

Ogoshi, (1996) [16] found that the Species of *Rhizoctonia* infect over 500 plants, mainly in the family's Compositae, Gramineae, Leguminosae, Solanaceae and Cruciferae. Malhotra *et al.*, (2011) [14] studied that *Rhizoctonia solani*, which causes damping-off disease of seedlings as well as root and stem rot in young transplants, is a major soil-borne pathogen of chilli (*Capsicum annum* L.). *R. solani* is a seed and soil borne pathogens. In the year 2001 root rot of chilli was first time reported from Rajasthan near Jaipur chilli growing areas, where the sever mortality of chilli plants during March-April was observed (Kalmesh and Gurjar, 2001) [11].

The chilli crop is suffered by various diseases caused by fungi, bacteria and viruses at different stages of crop.

Among the major diseases of chilli, root and stem rot caused by *R. solani* has become a serious problem in chilli growing areas.

As Chilli is a vegetable crop, the use of chemicals for disease control is not advisable in view of its residual problems. Biocontrol of plant pathogens using antagonistic fungi and bacteria therefore plays a significant role. However biocontrol agents can only play a partial role in integrated disease management of the disease, so in present study we have used fungicide and biocontrol agents for effective management of *Rhizoctonia solani* in *in vitro* condition.

Materials and Methods

The infected samples of *Rhizoctonia solani* Kuhn incitant of root rot were collected from diseased field of chilli from different districts of Rajasthan, *viz.* Sri Ganganager, Hanumangarh, Bikaner (Agricultural Research Station and farmer's field) and Jaipur. The samples collected from diseased plants were used for isolation. The roots were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were surface sterilized with 0.1 per cent sodium hypochloride solution for one minute, three washings with sterilized distilled water were given, placed on PDA slant under laminar flow and incubated at 28 ± 1^0C temperature for growth for seven days. To maintain the pure culture of *Rhizoctonia solani*, singal hyphal tip isolation technique was adopted.

Rhizoctonia solani was isolated from diseased chilli root tissues. After isolation, the fungus culture was purified by adopting single hyphal tip method. Observations on growth characteristics and sclerotial formation were recorded.

1) Testing of antagonists against *Rhizoctonia solani in vitro*

Microorganisms isolated during the course of studies were tested for their antagonistic activity against *Rhizoctonia solani* on PDA.

a) Dual culture method

The antagonistic potential of each antagonist *i.e Trichoderma harzianum*, *T. viride* and *T. atroviride*, were studied. A 5.0 mm diameter disc of test antagonist was placed individually at one end of the Petri dish containing PDA and just opposite to that a 5.0 mm diameter disc of the pathogen (*R. solani*) was placed. Three replications were maintained for each antagonist. In control, the pathogen alone was inoculated in petriplate. The petri dishes were incubated at 28 ± 1^0C for seven days in a BOD incubator and observations were recorded. The per cent inhibition of pathogen was calculated by using the following formula:

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

Where: C = Radial growth of in *R. solani* in control (mm), T= Radial growth of *R. solani* in presence of antagonist (mm)

b) Paper disc plate method

For antagonist potential of bacterium, *Bacillus* sp. and *Pseudomonas* sp. were evaluated by paper disc plate method (Loo *et al.*, 1945) [12]. Circular discs (5 mm dia.) of whatman filter (No. 42) were cut and after dipping in suspension of *Bacillus* sp., placed 1 cm inward from the periphery of Petri dishes at four equidistance places, having in the centre the inoculum of pathogen (*Rhizoctonia solani*).

Radial growth of *R. solani* was recorded and percent inhibition was calculated by using following (Dennis and web star, 1971) [7] formula:

$$\text{Per Cent Growth Inhibition} = \frac{C-T}{C} \times 100$$

Where: C = Radial growth of in *R. solani* control (mm), T= Radial growth of *R. solani* in presence of antagonist (mm)

2) Evaluation of fungicides against *R. solani* under *in vitro* condition

Five concentrations of Tebuconazole 2DS *viz*. (1, 10, 25, 50 and 100 ppm) were evaluated against the pathogen on PDA by using poisoned food technique (Dhingra and Sinclair 1985) [9]. Fungicidal suspensions of different concentrations were prepared by dissolving requisite quantities of each fungicide in PDA just prior to pouring. The fungicides were thoroughly mixed with the medium by shaking with hands after autoclaving. About 15 ml of sterilized medium was poured in each 9 cm sterilized petri dish. After solidification, the plates were inoculated by placing 5 mm discs of PDA cultures of *R. solani*. Three replications of each treatment along with control, were maintained in completely randomized design, incubated at 28 ± 1^0C for seven days in a BOD incubator and the observations were recorded. The radial growth of fungus in each treatment (per cent growth inhibition) was calculated by using above mentioned formula:

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

Where

PGI = Per cent growth inhibition, C = linear area of test fungus in control (mm),

T = linear area of test fungus in respective treatment (mm)

Result and Discussion

Efficacy of antagonists against *Rhizoctonia solani in vitro*

The antagonistic actions of three species of *Trichoderma* and two spp. of bacteria were evaluated against the test fungus by dual culture and paper disc technique, respectively. Based on observations of radial growth of antagonist and test fungus, per cent inhibition was calculated. The results are expressed in [table 1 and depicted in figure 1]. The results revealed that all the biocontrol agents were significantly superior in inhibiting the growth of test fungus over control. *Trichoderma* spp. Inhibited above 35 per cent growth of the test fungus. Maximum growth inhibition was recorded in *T. harzianum* (64.81%) followed by *T. viride* and *T. atroviride*. After that, in descending order of inhibition *i.e. Pseudomonas fluorescens* (41.48%), this was statistically at par with *Bacillus subtilis* (34.81%).

Table 1: Effect of antagonists on mycelial growth of *R. solani*

Antagonist	Per cent inhibition
Trichoderma harzianum	64.81 (54.00)*
T. viride	59.26 (50.31)
T. atroviride	36.30 (37.02)
Pseudomonas fluorescens	41.48 (37.91)
Bacillus subtilis	34.81 (36.14)
Control	(0.0)
S.Em (±)	0.73
CD (P=0.05)	2.29
CV (%)	3.55

*values in parenthesis are angular transformed values

Antibiotics secreted by antagonistic organisms can be used for inhibiting the development of pathogen in soil, thereby, reducing the intensity of soil borne diseases incited by plant pathogenic organisms, ultimately leading for biological control.

Antagonistic potential of fungal and bacterial bioagents was evaluated against *R. solani in vitro* employing dual and paper disc inoculation methods. The fungal antagonists *T. harzianum*, *T. viride* and *T. atroviride* and two bacterial antagonists *i.e. Pseudomonas fluorescens* and *B. subtilis* significantly inhibited the mycelial growth of *R. solani in vitro*.

Several authors reported that the inhibition of *R. solani* by *Trichoderma* species could probably be due to the secretion of extracellular cell degrading enzymes such as chitinase β-1, 3-glucanase, cellulose and lectin, which help mycoparasites in the colonization of their host (Agarwal, 2002; Shalini *et al*., 2007; Malhotra *et al.*, 2011; Shabir *et al*., 2012) [1, 21, 14, 20]. Mathur *et al*., (2002) studied that *T. viride* and *T. harzianum* showed maximum inhibition of mycelial growth and sclerotial production of *R. solani*., coiling of antagonists hyphae around the pathogen and finally lysis were also observed. Devi and Reddy, (2002) [8] also reported that *T. harzianum* is the most potential antagonist among the five isolates of *Trichoderma* spp., *P. fluorescens*, and *Bacillus* spp. against *R. solani* causing damping off in groundnut. The inhibition of pathogen may also be attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin. The similar observations on suppression of mycelial growth of *R. solani* pathogenic to chilli and certain other host plants by different microbial antagonist *viz*., *Trichoderma* spp., *P. fluorescens*, *B. subtilis* etc. have been reported by various workers. (Devi and Reddy, 2002; Malhotra *et al*., 2011; D'aes *et al*., 2011; Rehman *et al*., 2013; Babal *et al*., 2017) [8, 14, 6, 5].

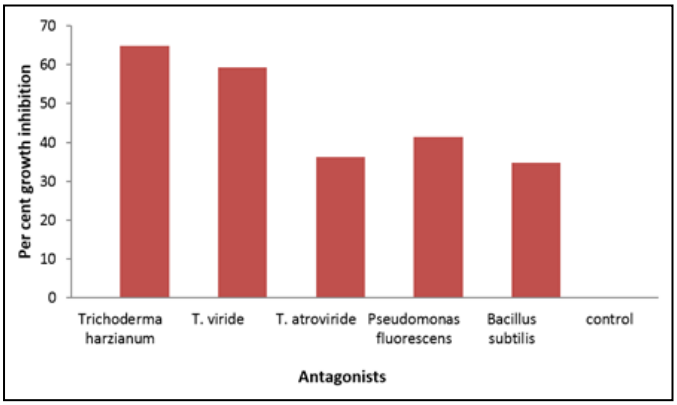

Fig 1: Effect of antagonist on mycelial growth of *R. solani*

Efficacy of fungicide against *Rhizoctonia solani in vitro*

In the present study, evaluated the efficacy of different concentrations *i.e.* 1.0, 10, 25, 50 and 100 ppm of Tebuconazole 2DS on mycelial growth of *R. solani* on PDA. The result presented in table 2 revealed that after 3 days, minimum mycelial growth of 16.16 mm was recorded by 100 ppm followed by 34 mm in 50 ppm fungicidal solution, while maximum growth of 59.66 mm was recorded in 1 ppm fungicidal solution. Similar trend of mycelial growth was observed after 5 and 7 days where minimum mycelial growth 17.33 mm and 17.66 mm was recorded after 5 and 7 days respectively in 100 ppm solution followed by 50 ppm where 35.70 mm and 36.33 mm radial growth was observed. Both the concentrations, nearly checked the radial growth after 5 and 7 days while in other concentrations, the growth was gradually increased after 5 and 7 days respectively. 1 ppm and 10 ppm concentrations became uneffective after 7 days where maximum 90 mm radial growth was observed and hence these concentrations were least effective in inhibiting the fungal growth at longer time. The results are expressed in table (2) and depicted in figure (2) indicates minimum mean radial growth of 17.21 was recorded in 100 ppm solution followed by 50 ppm where mean radial growth was 35.34 mm. it indicates that 100 ppm and 50 ppm concentrations of fungicide effectively checked the mycelial growth from 3 days and remain effective after 5 days and 7 days, respectively.

Table 2: Effect of Tebuconazole 2DS on mycelial growth of *R. solani*

Concentration of Fungicide (ppm)	Mycelial growth (mm)*			Mean
	After 3 days	After 5 days	After 7 days	
1	59.66	83	90.00	77.55
10	50.00	79.50	90.00	73.00
25	46.33	50.66	57.33	51.44
50	34.00	35.70	36.33	35.34
100	16.66	17.33	17.66	17.21
S.Em (±)	0.77	0.80	0.42	
CD (P = 0.05)	2.47	2.56	1.34	
CV (%)	3.24	2.61	1.25	

***** Mean of three replications

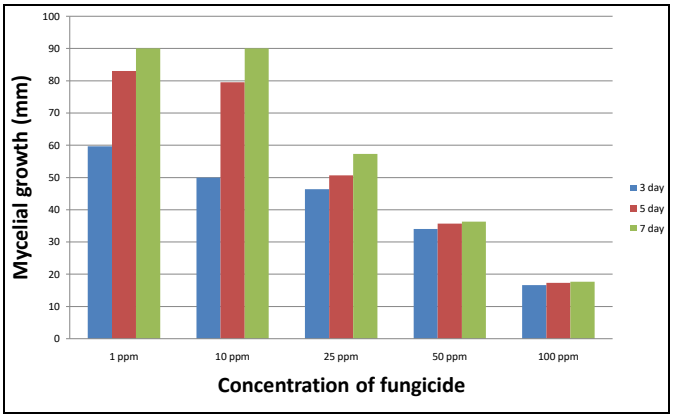

Fig 2: Effect of Tebuconazole 2DS on mycelial growth of R. solani

The concentration of fungicide inhibited the mycelial growth of pathogen to various extents. The inhibition of *R. solani* by Tebuconazole 2DS could probably be due to the chemical inducers which have direct antimicrobial effect. The concentration of 10 and 25 ppm also suppressed the growth of the pathogen but it was least effective as compared to 100 and

50 ppm. Conclusively, 100 ppm concentration was most inhibitory followed by 50 ppm to the pathogen. The similar observation on inhibition of mycelial growth of *R. solani* by different fungicide have been reported by various workers (Patel *et al.*, 2014; Rehman *et al.*, 2013) [17]. The percent growth inhibition of mycelia growth of *R. solani* was found effective in treatment of Tebuconazole 2DS which was in accordance to the result of Madhavi and Bhattiprolou, 2011; Ann and Mercer, 2017 [13, 2].

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