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Evaluation of Bioagents Against *Rhizoctonia solani* Kuhn Incitant of Cowpea (*Vigna unuiculata* L. Walp.) Web Blight Disease

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ABSTRACT

Cowpea web blight caused by Rhizoctonia solani (Kuhn) is an emerging disease in cowpea growing areas of Rajasthan and causes considerable yield losses. Antagonistic potentiality of locally isolated eleven fungal and six bacterial antagonists were evaluated against R. solani. Four Trichoderma strains i.e. T. harzianum (Th-BKN), T. harzianum (Th-JJN), T. viride (Tv-BKN) and T. harzianum (JPR) and two bacterial antagonists i.e B. subtilis (Bs-BKN) and P. fluorescens (Pf-BKN) gave distinct antagonistic reactions, showing stunting of R. solani colony and a clear zone of inhibition between colonies of antagonist and the pathogen was developed. The mode of antagonism against R. solani was studied under both in vitro and in vivo conditions. The culture filtrate of the test bioagents checked the mycelial growth of R. solani. Maximum inhibition of the pathogen growth was recorded in media amended with culture filtrate of T. harzianum (Th-BKN), Volatile substances produced by T. harzianum (Th-BKN) and T. viride (Tv-BKN) checked more than 60 per cent mycelial growth. Rest of the three bioagents i.e. Trichoderma atroviride (Ta-7), B. subtilis (Bs-BKN) and P. fluorescens (Pf-BKN) also suppressed the mycelial growth of the pathogen.

Key words: *Web blight, Cowpea, Rhizoctonia solani, Bioagents, etc.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food legume which belongs to family Fabaceae. Cowpea originated in Africa and widely grown in tropical and subtropical regions of Africa, Asia, and Central and South America. Cowpea is also known as vegetable meat due to high amount of protein in the grain with better biological value on dry weight basis. In India the major cowpea growing states are Karnataka, Kerala, Madhya Pradesh, Rajasthan and Tamil Nadu.

The production has been low and static mainly because of its cultivation under rainfed areas, marginal and sub-marginal lands, low soil fertility, biotic and abiotic stresses. This global crop, encounters a number of operational constraints, including pests and diseases that limit its production and yield potentials from seedling to harvest[2]. Cowpea is attacked by at least 35 diseases. Diseases hampers crop establishment, impair forage quality and reduces green fodder and seed yield.

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Besides causing direct yield losses they also suppress nodulation and consequently negating the maximum nitrogen fixation. Soil borne plant pathogenic fungi cause heavy crop losses all over the world. Among the soil borne fungal diseases of cowpea, Web blight, caused by *Rhizoctonia solani* is an important disease. *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris* is a widespread and an ecologically diverse soil-borne fungus, causing different types of diseases in many plant species. It causes root rot, stem rot, fruit and seed decay, damping-off, foliar blight, stem canker and crown rot in various crops[6]. *R. solani* has prolonged saprophytic survival ability and a wide host range, the management of the disease is very difficult. Although the chemical fungicides have played an important role in increasing cowpea production and management of diseases like root rot and others, but their indiscriminate use for the control of diseases has led to several environmental problems, development of resistance, chemical residues and their adverse effect on beneficial microorganisms increasing interest of growers towards organic farming, more emphasis is being given on the management of soil borne disease using microbial antagonists *viz.*, *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., etc[12]. Biological control agents interact with phytopathogens directly or indirectly to reduce the population of pathogens or reduction in the ability of the pathogens to cause disease by implicated mechanisms *viz;* parasitism, antibiosis, competition for nutrients or space, production of enzymes and inactivation of pathogen enzymes, tolerance to stress through enhanced root and plant development induced systemic resistance and solubilization and sequestration of inorganic nutrients[8], but the information available on the antagonistic effect of rhizobacteria against *R. solani* is very scanty. In the present study, locally isolated bioagents from cowpea rhizosphere were isolated and evaluated against web blight causing pathogen (*Rhizoctonia solani*).

MATERIALS AND METHODS

Cowpea plants showing web blight symptoms were collected from cowpea field, Agriculture Research Station, Beechwal, Bikaner. The pathogenicity of the isolated pathogen (*R. solani*) was tested according to Koch's postulates.

Antagonistic efficacy of selected bioagents *in vitro.* Dual culture method for fungal antagonists

Dual culture method was followed in order to ascertain the antagonistic capacity of *Trichoderma* spp. and other fungal antagonists[3]. One mycelial disc (5 mm diameter) of each of the pathogen and antagonist was kept on the surface of potato dextrose agar medium in Petri dishes at 5 cm apart. The inoculated Petri dishes were incubated at 26 ^{0}C for 7 days. Three replications were kept for each fungal antagonist. In case of control, the Petri dishes were inoculated with mycelial discs of the test pathogen only. The mycelial growth of test pathogen was measured after seven days of inoculation. The inhibition of mycelial growth of the pathogen was calculated using the following formula:

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *R. solani* in control (mm)

T = Mycelial growth of *R. solani* in presence of antagonist (mm)

Paper disc method for bacterial antagonists

For bacterial antagonists paper disc method[11] was followed. Circular paper discs (5 mm dia.) of Whatman filter (No. 42) were cut and after dipping in suspension of bacterial antagonists placed 1 cm inward from the periphery of Petri dishes at four equidistance places, having in the centre the inoculum of pathogen (*R. solani*). The inoculated dishes were placed in an incubator at 26 ^{0}C for a week and observations were recorded. Three replications were kept for each treatment. In case of control, the Petri dishes were inoculated with mycelial discs of the test pathogen only. The mycelial growth of test pathogen was measured after seven days of inoculation. The inhibition of mycelial growth of the pathogen was calculated using the above mentioned formula.

Effect of volatile substances on pathogen

Experiments were conducted to study the effect of bioagents volatile substances on *R. solani* using paired plate technique[3]. For this purpose, mycelial discs (5 mm dia.) taken from the periphery of actively growing cultures of individual *T. harzianum* (Th-BKN), *T. viride* (Tv-BKN) and *T. atroviride* (Ta-7), was placed at the center of lower lid of respective Petri dishes containing Potato Dextrose Agar (PDA) medium. While on upper lid of the Petri dishes containing PDA medium, mycelial discs (5 mm diameter) taken from of actively growing culture of *R. solani* was placed at the center. The upper lid containing *R. solani* was inverted on to the lower lid having *Trichoderma* spp. and the Petri dishes were sealed using parafilm tape (HiMedia, Mumbai). In case of control, the lower lid of the Petri dishes contained only PDA without inoculation of *Trichoderma* cultures. For each bioagent, three replications were kept. The parafilm sealed Petri dishes containing the inoculated *Trichoderma* and *R. solani* were incubated at 26 ^{0}C. The mycelial growth of *R. solani* in inoculated upper lid of the Petri dishes was recorded after 7 days of incubation.

Two separate experiments were set to record the influence of volatile substances produced by the two bacterial antagonists *i.e. P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN). The method was essentially similar to that of fungal antagonists. However in these cases, the lower lid contained PAF media having streaked with *P. fluorescens* (Pf-BKN) while the upper lid had PDA inoculated with *R. solani*. For another bacterial bioagent the lower lid of the Petri dishes contained NA media having streaked with *B. subtilis* (Bs-BKN) and the upper lid contained the *R. solani* dishes. The Petri dishes were sealed and incubated at 26 ^{o}C. The mycelia growth of *R. solani* was recorded after 7 days of incubation.

Effect of culture filterate on pathogen

This experiment was conducted to test the effect of culture filtrates of the five test bioagents. The antagonists were grown in liquid media *i.e.* potato dextrose broth for *T. harzianum* (Th-BKN), *T. viride* (Tv-BKN) and *T. atroviride* (Ta-7), King's B broth and nutrient broth for *P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN), respectively. The antagonists were incubated at desired temperature in BOD incubator. In case of *Trichoderma* spp., the seven days old cultures were first filtered through double layered cheese cloth followed by filtering through Whatman No. 1 filter paper. The obtained culture filtrate was centrifuged at 10000 rpm at 4^{o}C for 15 minutes. The supernatant was then passed through bacterial proof filter and stored in refrigerator. The two bacterial antagonists *i.e. P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN) were raised in Kings' B broth and nutrient broth media, respectively, for 72 hours at 26 ^{o}C in BOD incubator. The broth media containing the bacterial growth was centrifuged at 10000 rpm for 15 minutes in 4 ^{o}C and the supernatant was passed through bacteria proof filter and stored in refrigerator for further studies. In order to study the inhibition potentiality, the respective supernatants were added to PDA at 1, 2.5 and 5 per cent concentrations at the time of pouring of the media in Petri dishes. Mycelial discs (5 mm dia.) taken from periphery of actively growing culture of *R. solani* was placed at the center of Petri dishes containing PDA medium previously amended with the respective supernatants. In case of control no supernatant was added to PDA. Three replications were kept for each type of supernatant/culture filtrate. The inoculated Petri dishes were incubated at 26^o C. Mycelial growth of *R. solani* was recorded after 7 days of incubation.

RESULTS AND DISCUSSION

In vitro evaluation of fungal and bacterial antagonists against *R. solani*

Efficacy of eleven fungal antagonists *viz.*, *T. harzianum* (Th-BKN), *T. harzianum* (Th-JJN), *T. harzianum* (Th-JPR) *T. viride* (Tv-BKN), *T. viride* (Tv-1), *T. atroviride* (Ta-7), *T. atroviride* (Ta-15), *T. longibrachiatum* (Tl-2), *Aspergillus niger* (Asp.-BKN), *Penicillium funiculosum* (Pf-BKN) and *Gliocladium virens*

(Gv -BKN) was tested against *R. solani* following dual inoculation technique in potato dextrose agar (PDA) medium. All the tested fungal antagonists significantly inhibited the mycelial growth of *R. solani*. The results given in Table -1 indicated that *T. harzianum* (Th-BKN) (89.77%) and *T. harzianum* (Th-JJN) (86.44%) and *T. viride* (Tv-BKN) (85.66%) were found highly inhibitory to *R. solani*. Another two fungal antagonists *i.e. T. harzianum* (Th-JPR) and *T. atroviride* (Ta-7), also effectively suppressed the growth of the test pathogen. *T. atroviride* (Ta-15) and T. *viride* (Tv–1) also significantly suppressed the growth of the test pathogen. Two antagonists *T. longibrachiatum* (Tl-2) and *Gliocladium virens* (Gv -BKN) were also observed more than 50 per cent inhibition. Further, the antagonists *Aspergillus niger* (Asp. BKN) and *Penicillium funiculosum* (Pf-BKN) were found least effective in suppressing the growth of the pathogen (Table -1). The similar observations in suppression of mycelial growth of *R. solani* by different microbial antagonists *viz; Trichoderma* spp., *P. fluorescens* and *B. subtilis* etc. have been reported by several workers on cowpea and other crop plants[9].

The antagonistic potential of six bacterial antagonists *viz., Pseudomonas fluorescens* (Pf-BKN), *P. fluorescens* (Pf-1), *P. fluorescens* (Pf- 2), *B. subtilis* (Bs-BKN), *B. subtilis* (Bs-1) and *B. subtilis* (Bs-2) was evaluated against *R. solani* following paper disc inoculation method. The mycelial growth of *R. solani* was suppressed by all the tested bacterial bioagents. The results revealed that the *B. subtilis* (Bs-BKN) was relatively more inhibitory to *R. solani* followed by *P. fluorescens* (Pf-BKN). The strains *B. subtilis* (Bs-1) and *P. fluorescens* (Pf-1) were relatively less effective for inhibition of mycelia growth of the pathogen. Among the tested strains *B. subtilis* (Bs-2) and *P. fluorescens* (Pf-2) were found least inhibitory to the test pathogen. Gupta *et al*.[7] tested efficacy of four bioagents, *T. viride, T. harzianum, T. virens* and *A. niger* under *in vitro* conditions against web blight (*R. solani*) of Frenchbean. Kumar *et al*.[10] reported plant growth promoting (PGP) and antagonistic activities of seven bacterial isolates, *Bacillus* Strain BPR7 has strongly antagonistic property resulting inhibited the growth of several phytopathogens (*in vitro*) such as *Rhizoctonia solani, Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani*, *Sclerotinia sclerotiorum*, and *Colletotricum* spp. Similarly findings also observed by Reetha *et al.,*[14] and Meena and Gangopadhyay[12].

Effect of volatile substances on growth inhibition of *R. solani*

The effect of volatile substances produced by the five test antagonists *viz., T. harzianum* (Th-BKN), *T. viride* (Tv-BKN), *T. atroviride* (Ta-7), *P. fluorescens* (Pf-BKN), and *B. subtilis* (Bs-BKN), on mycelial growth of *R. solani* was studied *in vitro*. The results revealed that all the five antagonists significantly checked the mycelial growth of the pathogen. The mycelial growth was least (30.67 mm) in presence of *T. harzianum* (Th-BKN) followed by *T. viride* (Tv-BKN) (32.56). Per cent growth inhibition by the two antagonists *T. harzianum* (Th-BKN) and *T. viride* (Tv-BKN) were 65.92 and 63.82 per cent, respectively. It was also observed that volatile substances produced by the fungal antagonist *T. atroviride* (Ta-7) and two bacterial antagonists *i.e. P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN) also inhibited the mycelial growth of *R. solani* to varying extent (Table -3). Similarly, the antagonistic activity of species of *Trichoderma* is based on production of antifungal metabolites, toxins, antibiotics, lytic enzymes, production of volatile substances mycoparasitism, and competition for nutrition and space reported by Alamri *et al*.[1].

Effect of culture filtrate on growth inhibition of *R. solani*

The effect of pure culture filtrate of five bioagents *viz., T. harzianum* (Th-BKN), *T. viride* (Tv-BKN), *T. atroviride* (Ta-7), *P. fluorescens* (Pf-BKN), and *B. subtilis* (Bs-BKN), on inhibition of mycelial growth of *R. solani* at three different concentrations *i.e*. 1, 2.5 and 5 per cent on PDA medium was studied. The results given in table-4 revealed that culture filtrate of the respective bioagents

checked the mycelial growth of *R. solani* to various extent. The maximum inhibition of the pathogen was recorded in media amended with culture filtrate of *T. harzianum* (Th-BKN). The inhibition of growth by *T. harzianum* (Th-BKN) was 23.17 per cent at 5 per cent concentration followed by *T. viride* (Tv-BKN) (20.38%) at 5 per cent concentration. The culture filtrate of *B. subtilis* (Bs-BKN), also checked the growth of the pathogen. The culture filtrates of *T. atroviride* (Ta-7) and *P. fluorescens* (Pf-BKN) were less inhibitory as compared to rest of the three antagonists. It was also recorded that the growth of the pathogen decreased with the increase in concentration of culture filtrate of all the five bioagents tested Table-5. Dev and Dawande[4] also found that the diseases caused by soil borne plant pathogen *R. solani* can be controlled by the antifungal activity of *Trichoderma* spp. and *P. fluorescens*. These two antifungal agents produces wide variety of enzymes such as beta 1, 4 glucanase, beta 1, 3 glucanase, chitinases etc. Similarly Ganesan and Sekar[5] also observed the suppressive effect of culture metabolites of five bacterial and two fungal antagonists against mycelial growth of *R. solani* causing web blight of groundnut. Mishra *et al.*[13] also reported the efficacy of culture filtrate of *T. viride* Tr 8 against *M. phaseolina* and other soil borne pathogens *in vitro*. However, decreased concentrations were less inhibitory to the growth of *R. solani.*

Table 1: Effect of fungal antagonists on mycelial growth of *Rhizoctonia solani*

S. No.	Antagonists	Mycelial growth (mm)	Inhibition of growth (%)
1.	*Trichoderma harzianum* (Th-BKN)	9.21 (17.67)*	89.77
2.	*T. harzianum* (Th-JJN)	12.25 (20.49)	86.44
3.	*T viride* (Tv-BKN)	12.96 (21.10)	85.66
4.	*T. harzianum* (Th-JPR)	13.30 (21.39)	85.22
5.	*T. viride* (Tv-1)	18.45 (25.44)	79.44
6.	*T. atroviride* (Ta-7)	14.00 (21.97)	84.44
7.	*T. atroviride* (Ta-15)	17.50 (24.39)	80.55
8.	*T. longibrachiatum* (Tl-2)	28.34 (32.16)	58.34
9.	*Aspergillus niger* (Asp.BKN)	41.67 (40.20)	38.72
10.	*Gliocladium virens* (Gv – BKN)	30.00 (33.21)	55.00
11.	*Penicillium funiculosum* (Pf-BKN)	44.00 (41.55)	35.29
12.	Control (without antagonist)	90.00 (71.57)	-
	S.Em.(±)	(0.08)	
	CD (P = 0.05)	(0.24)	-
	CV (%)	(3.05)	

* Figures in parentheses are angular transformed values

Table 2: Effect of bacterial antagonists on mycelial growth of *Rhizoctonia solani*

S. No.	Antagonists	Mycelial growth (mm)	Per cent Inhibition of growth
1.	*P. fluorescens* (Pf-BKN)	30.67 (33.63)*	58.55
2.	*P. fluorescens* (Pf-1)	42.67 (40.79)	42.34
3.	*P. fluorescens* (Pf-2)	50.67 (45.38)	31.53
4.	*B. subtilis* (Bs-BKN)	23.67 (29.11)	68.01
5.	*B. subtilis* (Bs-1)	33.67 (35.47)	54.5
6.	*B. subtilis* (Bs-2)	47.0 (43.28)	36.48
7.	Control (without antagonist)	74.0 (59.34)	-
	S.Em.(±)	(1.31)	
	CD (P=0.05%)	(3.82)	
	CV (%)	(3.18)	

* Figures in parentheses are angular transformed values

Table 3: Effect of volatile substances produced by bioagents on mycelial growth of Rhizoctonia solani

S. No.	Bioagents	Mycelial growth (mm)	Per cent growth Inhibition
1.	*Trichoderma harzianum* (Th-BKN)	30.67 (33.63)*	65.92
2.	*Trichoderma viride* (Tv-BKN)	32.56 (34.79)	63.82
3.	*Trichoderma atroviride* (Ta-7)	44.53 (41.86)	50.53
4.	*Pseudomonas fluorescens* (Pf BKN)	52.67 (46.53)	41.85
5.	*Bacillus subtilis* (Bs-BKN)	42.55 (40.72)	52.72
6.	Control	90.00 (71.57)	-
	S Em (±)	(2.03)	
	CD (P = 0.05)	(5.92)	
	CV (%)	(2.80)	

* Figures in parentheses are angular transformed values

Table 4: Effect of culture filtrate of different bioagents on per cent growth inhibition of *R. solani*

S. No.	Antagonist	Per cent growth inhibition in different concentration of culture filterate			Mean
		1 per cent	2.5 per cent	5 per cent	
1.	*Trichoderma harzianum* (Th-BKN)	22.05 (28.01)*	22.73 (28.47)	23.17 (28.77)	22.60 (28.39)
2.	*T.viride* (Tv-BKN)	18.38 (25.39)	19.27 (26.04)	20.38 (26.84)	19.30 (26.06)
3.	*T.atroviride* (Ta-7)	9.44 (17.89)	9.66 (18.11)	10.22 (18.64)	9.70 (18.15)
4.	*P. fluorescens* (Pf-BKN)	9.62 (18.07)	10.77 (19.16)	10.97 (19.34)	10.70 (19.09)
5.	*B. subtilis* (Bs-BKN)	10.55 (18.95)	14.3 (22.22)	14.70 (22.54)	12.80 (20.96)
	Mean	14.0 (21.97)	15.34 (23.06)	15.88 (23.48)	
Bioagent Culture filtrate Bioagent x Culture filtrate		S Em (±) 0.40 0.56 0.87	CD (P=0.05) 1.16 1.63 2.54		CV (%) 4.55

* Figures in parentheses are angular transformed values

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