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Biological management of cowpea web blight incited by *Rhizoctonia solani*

PRIYANKA[1]*, S L GODARA[2], SURESH YADAV[3], A C MATHUR[1] and ANAND KUMAR MEENA[1]

S K Rajasthan Agricultural University, Bikaner, Rajasthan, India

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ABSTRACT

Among the diseases of cowpea, web blight caused by *Rhizoctonia solani* Kuhn is the most serious soil borne disease which causes severe yield loss. A field trial was conducted during *kharif* 2016 to manage web blight disease of cowpea at ARS field of SKRAU, Bikaner. Combination of *T. harzianum* plus *B. subtilis* used as seed treatment (5 + 5 g/kg) and soil application (5+5 kg/ha) proved to be most effective against web blight incited by *R. solani* in field conditions and provided maximum disease control (79.5%). Seed emergence, plant survival, fresh and dry weight, root and shoot lengths and grain yield was higher and per cent cowpea plant mortality was lower in treatment *T. harzianum* plus *B. subtilis* used in combination as seed treatment (5 + 5 g/kg) and soil application (5 + 5 kg/ha). The bioagents used in the present investigation could be therefore, used as a part of integrated disease management (IDM) strategy for web blight of cowpea in areas where the disease is reported to cause substantial yield losses.

Keywords: Bioagents, Biological management, Cowpea, *Rhizoctonia solani*, Web blight

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important food legume, which is a major and cheap source of quality protein, green leaves and pods are also consumed as vegetable and the dried grain is used in many food preparations. Protein content of cowpea leaves range from 27–43% and protein concentration of the dry grain ranges from 21–33%. (Ddamulira *et al*. 2015, Abudulai *et al*. 2016). Cowpea encounters a number of operational constraints, including insect pests and several diseases that limit its production and yield potentials from seedling to harvest (Asiwe 2006) and about 35% grain yield loss (Amadioha 2003). Among the various diseases, web blight caused by a soil borne pathogen. *Rhizoctonia solani* Kuhn results in yield loss of 30% worldwide (Kankam *et al.* 2018). Cowpea is especially susceptible to this disease at seedling and vegetative stage when planted in moist soils coupled with high temperature and humid conditions (Thies *et al*. 2006). This Disease hampers proper crop development, impairs forage quality and reduces green fodder and seed yield. Besides causing direct yield losses pathogen also suppress nodulation and subsequently negating the maximum nitrogen fixation. *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 plant disease scenario has become more independent of chemicals because of their indiscriminate use 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 (Baysal-Gurel *et al.* 2019).The knowledge about this disease and its management through fungal and bacterial bio control agents is very meagre. Therefore, an experiment was conducted to assess the effects of biocontrol agents in field conditions as seed treatment or soil application in different combinations to develop an eco-friendly management strategy for menacing disease.

MATERIALS AND METHODS

Isolation identification and purification: Cowpea plants showing web blight symptoms were collected from different cowpea growing areas of Rajasthan, i.e. Bikaner, Jaipur, Nagour, Sikar, Jhunjhunu, Churu, and Sriganganagar (2016). For isolation of *Rhizoctonia solani* small pieces of infected stem tissues were washed and surface sterilized with 0.1% mercuric chloride for a minute and then washed three times with distilled water. After drying on blotter paper stem tissues were cut into small pieces and transferred aseptically on Potato Dextrose Agar (PDA). Plates were incubated in BOD at 28± 2^0C for six to eight days. The mycelia growth was observed and examined to confirm the association of *R. solani* with cowpea web blight. For identification of the pathogen cultural and morphological characters were

Present address: [1]RARI, Durgapura, SKNAU, Jobner; [2]SKRAU, Bikaner; [3]ICAR-IARI, New Delhi. *Corresponding author e-mail: pkpoonia93@gmail.com.

compared with the standard descriptions of Holliday (1980).

Pathogenicity: Pathogenicity of the purified cultures of *R. solani* was tested by growing cowpea plants in pots containing pathogen infested soil. The prepared sterilized media were inoculated with respective *R. solani* cultures and incubated at 26^0C for 10 days. The sand maize media inoculum (sand: maize meal: distilled water-980:20:250 ml) of respective *R. solani* was added to soil at 20 g/kg soil and mixed thoroughly. The inoculated soils were transferred to clean earthen pots (20 cm diameter). Ten healthy seeds of cowpea (RC-101) were sown in each pot. The pots were watered regularly to maintain suitable moisture regime. The web blight symptoms developed in seedlings were recorded periodically. Re-isolation of the pathogen was made from infested seedlings.

Mass culture of bioagents: Four bioagents were used in the study of which two were fungal, viz. *T. harzianum*, *T. viride* and two were bacterial bioagents, viz. *P. fluorescens* and *B.subtilis.* Bioagents were used on the basis of their antagonistic potentiality. Talc based formulation was prepared by mixing mass of conidia in order to keep the conidial population of 2×10^6 conidia/g in the case of fungal bioagents and the formulations of bacterial bioagents were prepared by mixing of culture broth containing a minimum population of 9×10^{-8} cfu/ml with sterile talc powder. This formulation was used @ 10 g/kg seed for seed treatment. Soil application of bioagents were used at 10 kg/ha alone and in combination with, farmyard manure (FYM) @ 10 tonnes/ha before 15 days of sowing. The field experiment was conducted at ARS field of SKRAU, Bikaner during *kharif* 2016. Total thirteen treatments including control were tested using randomized block design having plot size 2.7 × 3 m^2 (Table 1) along with 3 replications. Observations on disease incidence, seedling mortality, plant growth

Table 1 Effect of bioagents combinations seedling emergence and mortality of cowpea plants at different interval under field condition

Treatment	Seedling emergence (%)	Plant mortality % (DAS=Days after Sowing)			Plant survival (%)
		30DAS	45DAS	60DAS	
Trichoderma harzianum ST 5g/ kg	84.7 (66.97)	5.1 (13.05)	10.0 (18.43)	13.9 (21.9)	86.1
T. viride ST 5 g/kg	84.5 (66.82)	5.1 (13.05)	12.5 (20.70)	15.1 (22.8)	84.9
Pseudomonas fluorescens ST 5g/ kg	84.3 (66.66)	5.1 (13.05)	16.2 (23.73)	19.9 (26.4)	80.1
Bacillus subtilis ST 5 g/ kg	84.0 (66.42)	5.1 (13.05)	19.1 (25.91)	24.7 (29.8)	75.3
T. harzianum ST 5 g/kg + SA 5 kg/ ha	84.9 (67.13)	5.1 (13.05)	9.9 (18.34)	11.1 (20.2)	88.9
T. viride ST 5 g/kg + SA 5 kg/ha	85.8 (67.86)	5.1 (13.05)	10.0 (18.43)	13.2 (22.1)	86.8
P. fluorescens ST 5 g/kg + SA 5 kg/ ha	85.9 (67.94)	5.1 (13.05)	9.9 (18.34)	14.0 (20.7)	84.0
B. subtilis ST 5 g/kg + SA 5 kg/ ha	85.0 (67.21)	5.1 (13.05)	9.9 (18.34)	12.6 (21.1)	87.4
T. harzianum + *P. fluorescens* ST (5+5 g/kg) + SA (5+5 kg/ ha)	86.2 (68.19)	4.1 (11.68)	7.3 (15.68)	10.9 (19.2)	89.1
T. viride + *P. fluorescens* ST (5+5 g/kg) + SA (5+5 kg/ha)	86.5 (68.44)	3.8 (11.24)	7.2 (15.56)	10.1 (18.5)	89.8
T. harzianum + *B. subtilis* ST (5+5 g/kg) + SA (5+5 kg/ha)	87.3 (69.12)	2.0 (8.13)	3.8 (11.24)	6.5 (14.7)	93.5
T. viride + *B. subtilis* ST (5+5 g/ kg1) + SA (5+5 kg/ha)	85.9 (67.94)	4.9 (12.79)	8.2 (16.64)	12.0 19.4)	88.0
Control (without bioagents)	80.0 (63.43)	11.2 (19.55)	26.1 (30.72)	44.6 (41.9)	-
SEm (±)	2.36	3.30	4.50	3.30	
CD (P= 0.05%)	6.89	9.63	13.14	9.63	
CV (%)	9.20	8.80	10.50	9.90	

* Figures in parentheses are angular transformed values, *ST= Seed Treatment, *SA= Soil Application

parameters, yield and biomass were recorded.

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

RESULTS AND DISCUSSION

The present study revealed the antagonistic effect of various bioagents against *R. solani*. All the bioagents have reduced the disease severity and have shown significantly beneficial effect over the seedling mortality, plant growth parameters, yield and biomass. The detailed morphological characters of the isolated cultures were examined and compared with the standard description (Holliday 1980) and identified the pathogen as *R. solani*. Pathogenicity test following Koch's postulates proved it to be the causal organism of cowpea web blight. The characteristic symptoms were oval or spindle shaped brown black lesions at soil level near collar region and girdling the basal portion of the stem were developed (Vavilapalli *et al*. 2014). The treatment effect under field condition was found significant with respect to seed germination, percent seedling mortality, root and shoot length and yield in cowpea. Seedling emergence due to inoculation of the pathogen before sowing in the field was reduced marginally. Inoculation with *R. solani* grown in sand-maize-meal medium in field after emergence of seedling caused significant seedling mortality in all cases.

Effect of bioagents on seed germination and seedling mortality: Seedling emergence in the field was reduced marginally, but the treatment effect under field condition was found significant with respect to seed germination, percent mortality and survivability of plants (Table 1). Seed germination of cowpea under field condition was recorded in the range of 80.0–87.30%. Maximum seed germination 87.3% was recorded with combination of *T. harzianum* plus *B. subtilis* used as seed treatment and soil application followed by *T. viride*, along with *P. fluorescens* 86.5%. In case of control minimum seed germination was recorded 80.0%. The per cent mortality of cowpea plants due to web blight reached up to 44.6% at 60 DAS in untreated control. Situation improved significantly in all the treatments and after 60 DAS per cent mortality of cowpea reduced significantly. The results indicated that all talc-based formulations of bioagents exhibited sharp reduction in seedling mortality of cowpea. Minimum seedling mortality 6.5% in dual inoculation system was obtained when *T. harzianum* was applied with *B. subtilis* as seed treatment as well as soil application followed by *T. viride* along with *P. fluorescens* 10.1%. Combined treatment of *T. harzianum* and P. *fluorescens* also reduced the seedling mortality which was 10.9%. *T. harzianum* used as seed treatment and soil application also effectively control the seedling mortality 11.1% followed by *T. viride* along with *B. subtilis* 12.0%. *B. subtilis*, *T. viride* and P. *fluorescens* used as seed treatment with soil application also reduced seedling mortality, i.e. 12.6, 13.2 and 14.0%, respectively. The mortality percentage of cowpea plants due to *R. solani* infection, increased with days after sowing which was very drastic in control. It was also observed that *T. viride* plus *B. subtilis* provided satisfactory disease control 40.40%. Pan and Das (2011) also found significant effects of treatments with respect to seed germination, percent mortality and plant growth. Similar results were also concluded by Reddy *et al*. (2013).

Effect of bioagents on fresh and dry weight: Fresh and dry weight of cowpea plants significantly increased due to bioagent treatments used alone or in combinations. Both fresh and dry weight was highest in combination of *T. harzianum* along with *B. subtilis* followed by *T. viride* with *P. fluorescens* and *T. harzianum* with *P. fluorescens*. The results also revealed that fresh and dry weight was higher in seed treatment plus soil application of *T. harzianum* followed by combined treatment of *T. viride* plus *B. subtilis*. Fresh and dry weight was also higher in seed treatment plus soil application of *B. subtilis* and *T. viride*. Fresh and dry weight also high when *T. harzianum* used as seed treatment followed by *P. fluorescens* as seed treatment plus soil application. Fresh and dry weight was high when *T. viride*, *B. subtilis*, *P. fluorescens* applied only as seed treatment. Conclusively, the bioagents in combination increase fresh weight and dry weight more effectively as compared to sole application of bioagents.

Effect of bioagents on incidence of web blight: The results (Table 2) indicated that web blight incidence was significantly reduced in different bioagent treatments. *T. harzianum* plus *P. fluorescens* (14.10%) suppress the disease to maximum extent when used in combination as seed treatment and soil application followed by *T. viride* plus *P. fluorescens* (25.20%), *T. harzianum* plus *P. fluorescens*, (27.90%) and *T. harzianum* minimize the incidence to 35.60% when used as seed treatment and soil application as compared to other treatments. Combined treatment with *T. viride* + *B. subtilis* was also minimizing the web blight incidence to 41.20%. Seed treatment and soil application of *B. subtilis, T. viride* and *P. fluorescens* also minimized the web blight incidence which was 42.30, 45.70 and 54.20%, respectively. Only seed treatment with *T. harzianum* also reduced the disease incidence (47.10%). Three other bioagents *T. viride*, *B. subtilis*, *P. fluorescens* also proved to be effective when used only as seed treatment and the disease incidence was 56.60, 58.40 and 60.20%, respectively.

Disease control efficacy of four bioagents were found maximum in *T. harzianum* + *B. subtilis* (79.50%) followed by *T. viride* + *P. fluorescens* (63.60%) and *T. harzianum* in combination with *P. fluorescens* (59.70%), while in case of sole treatment of bioagents as seed treatment plus soil application, i.e. *B. subtilis, T. viride, P. fluorescens* control the web blight disease which was 38.90, 33.90 and 21.70 per cent, respectively. The results revealed that disease incidence was also reduced when bioagents used only as seed treatment but the per cent disease control was lesser than combinations. Per cent disease control with *T. viride, B. subtilis, P. fluorescens* was 18.20, 15.70 and 13.00%, respectively. A perusal of data clearly revealed that disease control was higher when the bioagents were used in combinations as seed plus soil application as compared to

Table 2 Effect of bioagents combination on growth parameters, disease incidence and yield of cowpea plants under field conditions

Treatment	Length (cm)		Whole plant (gm)		Disease incidence (%)	Disease control (%)	Yield (kg/ha)
	Root	Shoot	Fresh weight	Dry weight			
T. harzianum (Th-BKN) ST 5 g/kg	17.01	39.86	255.19	115.21	47.10 (43.34)	32.0 (34.45)	415.50
T. viride (Tv-BKN) ST 5 g/ kg	15.91	38.26	253.67	111.26	56.60 (48.79)	18.20 (25.25)	405.50
P. fluorescens (Pf-BKN) ST 5 g/kg	12.55	33.29	196.11	98.45	60.20 (50.89)	13.0 (21.13)	384.60
Bacillus subtilis (Bs-BKN) ST 5 g/kg	14.28	34.21	200.67	105.6	58.40 (49.84)	15.70 (23.34)	395.30
T. harzianum (Th-BKN) ST 5 g/kg + SA 5 kg/ha	19.30	46.56	340.19	132.11	35.60 (36.63)	48.6 (44.20)	467.50
T. viride (Tv-BKN) ST 5 g/ kg + 5 kg/ha	17.90	45.85	317.01	128.21	45.70 (42.53)	33.90 (35.61)	430.90
P. fluorescens (Pf-BKN) ST 5 g/kg + 5 kg/ha	16.95	38.66	295.91	112.67	54.20 (47.41)	21.7 (27.76)	412.00
Bacillus subtilis (Bs-BKN) ST 5 g/kg + SA 5 kg/ha	18.25	45.50	305.6	130.95	42.30 (40.57)	38.90 (38.59)	435.31
T. harzianum (Th-BKN) + *P. fluorescens* (Pf-BKN) ST (5+5 g/kg) + SA (5+5 kg/ ha)	23.10	52.10	358.89	155.98	27.90 (31.88)	59.7 (50.59)	628.50
T. viride + *P. fluorescens* ST (5+5 g/kg) + SA (5+5 kg/ ha)	24.70	54.53	364.99	156.37	25.20 (30.13)	63.60 (52.89)	628.90
T. harzianum + *Bacillus subtilis* ST (5 +5 g/kg) + SA (5+5 kg/ha)	26.67	56.40	370.21	164.29	14.1 (22.06)	79.5 (63.08)	647.30
T. viride+ *Bacillus subtilis* ST (5+5 g/kg) + SA (5+5 kg/ ha)	19.00	47.90	338.32	131.89	41.20 (39.9)	40.40 (39.47)	445.10
Control (without bioagents)	10.40	28.24	159.93	56.51	69.30 (56.3)	--	356.60
SEm (±)	1.10	1.90	3.10	(2.30)		4.60	
CD (P=0.05%)	3.21	5.54	9.05	(6.71)		13.43	
CV (%)	7.40	8.40	8.40	(9.80)		10.11	

* Figures in parentheses are angular transformed values

sole applications. In case of both seed treatment and soil application, the antagonist treatment *T. harzianum*, (48.6%) provided highest disease control followed by *B. subtilis*, (38.90%), *T. viride*, (33.90%) and *P. fluorescens*, (21.70%). Among the treatments, when *T. harzianum* used alone as seed treatment quite effectively protected the cowpea plants against web blight and provided (32.0%) disease control followed by *T. viride*, (18.20%), *B. subtilis*, (15.70%) and *P. fluorescens*, (13.00%).

Yield: The results given in the Table 2 showed that the grain yield of cowpea was significantly increased when bioagents were used alone or in combination as seed treatment and soil application**.** The grain yield was highest (647.3 kg/ha) in *T. harzianum* along with *B. subtilis* used in combination as seed treatment (5 + 5 g/kg) plus soil application (5+5 kg/ha) followed by *T. viride* along with *P. fluorescens* (628.90) and *T. harzianum* plus *P. fluorescens* (628.50 g/kg) applied as seed treatment (5 + 5 g/kg) plus soil application (5+5 kg/ha). The grain yield was also quite higher in *T. harzianum* (467.50 kg/ha) seed treatment (5 g/kg) plus soil application (5 kg/ha) followed by *T. viride* plus *B. subtilis* (445.10 kg/ha) used in combination as seed treatment (5 + 5 g/kg) plus soil application (5+5 g/kg). Higher grain yield was also recorded in *B. subtilis* (435.32 kg/ha) seed treatment (5 g/kg) with soil application (5 kg/ ha) closely followed by *T. viride* (430.91 kg/ha) used as seed treatment (5 g/kg) and soil application (5 kg/ha). *T. harzianum* (415.5 kg/ha) used as seed treatment (5 g/ kg) also produced higher yield followed by *P. fluorescens* (412.00 kg/ha) used as seed treatment (5 g/kg) and soil application (5 kg/ha). Again, the grain yield was positively influenced by three bioagents, i.e. *T. viride, B. subtilis* and *P. fluorescens* used as seed treatment (5 g/kg) which was 405.50, 395.30 and 384.66 kg/ha respectively. Conclusively, the bioagents in combination enhanced the yield more effectively as compared to sole application of bioagents.

The reduction in soil borne plant diseases and subsequent enhancement in the yield of different crops after treatment with formulations of *T. harzianum* have been reported by several workers (Harman *et al.* 2004 and Jaiman *et al.* 2009). In conclusion, the bioagents in combination effectively control the disease incidence, increase grain yield, growth parameter and biomass more effectively as compared to sole application of bioagent.

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