INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-1, Jan-Mar-2014

ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 13th Dec-2013 *Revised: 18th Dec -2013* *Accepted: 15th Jan-2014*

Research article

EFFICACY OF RHIZOSPHERIC AND ROOT ENDOPHYTIC BACTERIA AGAINST *RHIZOCTONIA BATATICOLA* AND COMPATIBILITY STUDIES WITH FUNGICIDES

G Amrutha Veena[1], N P Eswara Reddy[2], M Harshitha[3] and C Prathyusha[4]

Department of Plant Pathology, S V Agricultural College, Tirupati-517 502., A.P., India.

ABSTRACT: A total of 40 antagonistic bacteria were isolated, out of which 20 bacteria were obtained from rhizosphere soil and 20 bacteria from root as root endophytes in chickpea against dry root rot. The bacterial isolates from rhizosphere soil were designated as CRB-1 to CRB-20 followed by root endophytic bacteria as CREB-1 to CREB-20. Among the 20 rhizospheric bacterial antagonists tested, the isolate CRB-13 showed the maximum inhibition (86.66%) and among the 20 root endophytic bacterial antagonists tested, the isolate CREB-13 showed the maximum inhibition (95.55%) of growth of *Rhizoctonia bataticola.* In compatibility studies using spectrophotometric method, the isolate CREB-16 was more compatible with validamycin (84.13%) followed by copper oxychloride (78.27%).

Key words: Rhizospheric, Endophytic bacteria, *Rhizoctonia bataticola*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important food legume crop. In India, it is grown over an area of 8.74 m ha with an annual production of 7.35 million tonnes and productivity of 841 kg ha^{-1} [3]. In Andhra Pradesh, it is grown in an area of 5.84 lakh ha with an annual production and productivity of 7.19 lakh tonnes and 1233 kg ha^{-1} respectively [2]. Dry root rot by *Rhizoctonia bataticola* (Taub.) Butler cause considerable yield losses in chickpea which may be as high as 50 to 71 per cent [1. Effective and practical chemical control is not feasible for soil borne pathogens. Biological control appears to be the only solution for long-term sustainability and effective management of soil borne diseases. An integrated approach by including the fungicide tolerant native bacterial antagonists isolated from rhizosphere soil and root endophytes appears to be possible solution for effective management of dry root rot.

MATERIALS AND METHODS

Isolation of Pathogen

A virulent isolate of *Rhizoctonia bataticola* was isolated from infected chickpea plants showing symptoms like withering and drying, by using tissue segment method (Rangaswamy and Mahadevan, 1999). The pathogen culture was purified by single hyphal tip method and maintained on PDA by periodical transfer throughout the present investigation.

Isolation of native antagonistic bacteria from rhizosphere

Antagonistic bacteria were isolated by following serial dilution technique (Johnson and Curl, 1977). Composite soil sample was collected from rhizosphere of healthy plants. The soil was dried under shade and then used for serial dilution. To get 10^{-1} dilution, ten gram of this soil was dissolved in 90 ml of sterile distilled water. From this one ml of soil suspension was taken and added to nine ml of sterile distilled water to get 10^{-2} dilution. This was repeated until a final dilution of 10^{-6} for bacteria. Antagonistic bacteria were isolated on nutrient agar medium by using a dilution of 10^{-6}. One ml of final dilution of soil suspension was poured into sterilized Petri plates, and then the melted and cooled media was poured. Plates were rotated gently on the laminar air flow bench to get uniform distribution of soil suspension in the medium. Then the plates were incubated at 28 ± 2^0C and observed at frequent intervals for the development of colonies. One day old colonies of bacteria were picked up and purified by streak plate method.

Isolation of native antagonistic bacteria from root endophytes

For isolation of endophytes, five g of root was surface sterilized for 5 min with 70 per cent ethanol and homogenized in 20 ml of sterilized phosphate buffer (0.2M Na_2HPO_4 + 0.2M NaH_2PO_4) pH 7.0 using mortar and pestle. Appropriate dilutions (10^{-6} for bacteria) of these suspensions were plated on NA for the isolation of bacteria. The plates were incubated at 28 ± 2°C [8]. One day old colonies of bacteria were picked up and purified by streak plate method.

Identification of potential biocontrol agents

The efficacy of antagonistic bacteria from rhizosphere and root endophytes was determined by dual culture technique [6] under *in vitro* conditions.

Dual culture technique

To test the efficacy of antagonistic bacterium a 4 cm line was streaked at one side of the plate. On the opposite side to the antagonist, mycelial disc measuring six mm diameter from four day old culture of test pathogen was placed on sterile Petri plate containing PDA medium. The petri plates with pathogen inoculated at one end alone, served as control. The petri plates were then incubated at 28 ± 2°C. Three replications were maintained in each treatment. Growth of antagonists, pathogen and zone of inhibition were measured after recording full growth of the pathogen in control plate. Per cent inhibition of mycelial growth of test pathogen was calculated by the formula:

$$I = \frac{C - T}{C} X\ 100$$

where,
I = Per cent inhibition in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment.

Identification of compatibility of potential biocontrol agents with fungicides *in vitro*

Potential biocontrol agents were tested for their compatibility with the fungicides *viz.*, copper oxychloride (0.25%), captan (0.25%), hexaconazole (0.2%), tebuconazole (0.1%) and validamycin (0.1%) as these are commonly used against dry root rot in chickpea. The compatibility of antagonistic bacterial isolates with the fungicides were tested by spectrophotometic method [8] under *in vitro* conditions.

Spectrophotometric method

Five hundred microlitres of antagonistic bacterial cultures grown in Nutrient Broth (NB) for 16 h at 28 ± 2°C and 150 rpm were added to 50 ml of NB in 250 ml flasks containing different fungicides. Inoculated flasks were incubated at 28 ± 2°C and at 150 rpm on orbital shaker. Bacterial growth was determined in systronic spectrophotometer at 600 nm after 24 hours of incubation. Each treatment was replicated thrice.The flasks containing nutrient broth without fungicides was kept as control [8].

RESULTS AND DISCUSSION

Identification of pathogen

The fungus produced radial hyaline colonies, which later become carbonaceous brown to black. Mycelium was septate and dark brown in colour. Typical right angled branching of mycelium was observed. Sclerotia were black, varied from spherical to irregular in shape and measured 80 to 85µm in diameter. Pycnidial production was not observed in culture plates. The colony characters and morphological characters of mycelium and sclerotia were in agreement with the descriptions of Sajeena [13]. Thus, the fungus under present investigation was identified as *Rhizoctonia bataticola* (Taub.) Butler.

Isolation and identification of native antagonistic bacteria from rhizosphere soil and root endophytic region of chickpea against *R. bataticola*

A total of 40 bacteria were isolated out of which 20 bacteria were obtained from rhizosphere soil and 20 bacteria from root as root endophytes. The bacterial isolates from rhizosphere soil were designated as CRB-1 to CRB-20 followed by root endophytic bacteria as CREB-1 to CREB-20. Parmer and Dadarwal [9] isolated *Pseudomonas* and *Bacillus* sp. from rhizosphere and rhizoplane of healthy chickpea plants. Rangeshwaran and Prasad [11] isolated three hundred antagonistic bacteria against root rot and wilt from chickpea rhizosphere. Rangeshwaran [12] isolated five endophytic and two rhizospheric bacteria from healthy chickpea plants and identified them based on morphological, physiological and biochemical tests.

During the present investigation also several biocontrol agents were isolated from both rhizosphere and endophytic zone to evaluate their efficacy against *Rhizoctonia bataticola*.

In vitro evaluation of efficacy of antagonistic bacteria against _R. bataticola_ in dual culture technique.

Efficacy of antagonistic bacteria against *Rhizoctonia bataticola* was evaluated by using dual culture technique. The data pertaining to per cent inhibition of mycelial growth of *R. bataticola* due to antagonistic bacteria are presented in Table 1 and 2. Among the 20 rhizospheric bacterial antagonists tested, the isolate CRB-13 showed the maximum inhibition (86.66%) of growth of *R. bataticola* followed by CRB-18 (83.33%) and CRB-2 (81.48%). However, both were found to be on par with each other in inhibiting the pathogen. The isolate CRB-6 recorded least (27.77%) per cent inhibition. Among the 20 root endophytic bacterial antagonists tested, the isolate CREB-13 showed the maximum inhibition (95.55%) of growth of *R. bataticola* that was on par with CREB-6 (94.44%) and CREB-16 (93.33%) The isolate CREB-12 recorded least (44.44%) per cent inhibition. These results were in agreement with Khan and Gangopadhyay [7] who reported that the maximum reduction of mycelial growth of *R. bataticola*, incitant of dry root rot of chickpea in dual culture technique by *Pseudomonas fluorescens* strains PFBC-25 and 26. Vinod Kumar [15] tested the efficacy of *Pseudomonas fluorescens* isolates against *M. phaseolina* incitant of charcoal rot of chickpea under *in vitro* and reported that the isolate Pf 4-99 was found to be effective in inhibiting the mycelial growth of pathogen. Uppala [14] isolated 46 bacterial endophytes from amaranth and screened against *Rhizoctonia solani* under *in vitro* conditions using dual culture method. Among the endophytes screened, only six bacteria showed antagonistic activity against *R. solani*.

Table 1 _In vitro_ evaluation of efficacy of chickpea rhizosphere bacterial isolates against _Rhizoctonia bataticola_ in dual culture technique

S. No	Bacterial antagonists	Linear growth of *R. bataticola* (Cm)*	Per cent inhibition of mycelial growth of *Rhizoctonia bataticola*
1	CRB-1	2.3	74.44(59.63)
2	CRB-2	1.6	81.48(64.52)
3	CRB-3	3.9	56.66(48.83)
4	CRB-4	4.5	50.00(44.99)
5	CRB-5	3.0	66.66(54.74)
6	CRB-6	6.5	27.77(31.79)
7	CRB-7	5.2	42.22(40.51)
8	CRB-8	4.0	55.55(48.20)
9	CRB-9	5.5	38.88(38.56)
10	CRB-10	4.5	50.00(44.99)
11	CRB-11	2.8	68.88(56.10)
12	CRB-12	3.5	61.11(52.43)
13	CRB-13	**1.2**	**86.66(68.73)**
14	CRB-14	2.2	75.55(60.40)
15	CRB-15	4.2	53.33(46.90)
16	CRB-16	4.4	51.11(45.62)
17	CRB-17	4.0	55.55(48.18)
18	CRB-18	1.5	83.33(65.91)
19	CRB-19	4.8	46.66(43.07)
20	CRB-20	5.7	36.66(37.24)
21	Control	9.0	-
	S. Em ±		1.05
	C D (0.05)		3.02

* Mean of three replications

Figures in parenthesis are angular transformed values

Table 2 *In vitro* evaluation of efficacy of chickpea root endophytic bacterial isolates against *Rhizoctonia bataticola* in dual culture technique

S. No	Bacterial antagonists	Linear growth of *R. bataticola* (Cm)*	Per cent inhibition of mycelial growth of *Rhizoctonia bataticola*
1	CREB-1	2.8	68.88(56.13)
2	CREB-2	3.0	66.66(54.74)
3	CREB-3	3.5	61.11(51.41)
4	CREB-4	3.6	60.00(50.77)
5	CREB-5	4.8	46.66(43.07)
6	CREB-6	0.5	94.44(76.50)
7	CREB-7	4.3	52.22(46.27)
8	CREB-8	4.6	48.88(44.35)
9	CREB-9	1.9	78.88(62.67)
10	CREB-10	3.2	64.44(53.44)
11	CREB-11	4.7	47.77(43.71)
12	CREB-12	5.0	44.44(41.75)
13	CREB-13	**0.4**	**95.55(77.88)**
14	CREB-14	4.8	46.66(43.08)
15	CREB-15	3.3	63.33(52.76)
16	CREB-16	0.6	93.33(75.06)
17	CREB-17	1.4	84.44(66.82)
18	CREB-18	1.2	86.66(68.66)
19	CREB-19	2.0	77.77(61.90)
20	CREB-20	2.6	71.11(57.49)
21	Control	9.0	-
	S. Em ±		1.40
	CD (0.05)		3.94

* Mean of three replications

Figures in parenthesis are angular transformed values

In vitro evaluation of the compatibility of potential bacterial antagonists with different fungicides.

The highly potential antagonistic bacteria CREB-13, CREB-6, CREB-16 from root endophytic isolates and CRB-13 from rhizosphere isolates were selected for fungicidal compatibility studies since they have shown maximum inhibition of *Rhizoctonia bataticola* growth in dual culture studies when compared to all other antagonists. Spectrophotometric method was used to evaluate the compatibility of CREB-13, CREB-6, CREB-16 and CRB-13 bacterial isolates with different fungicides and the results are presented in Table 3. Higher OD values at 600 nm indicate high compatibility of the antagonist with that specific fungicides. It is evident from the data (Table 3) that the isolate CREB-13 was more compatible with validamycin (58.08%) followed by copper oxychloride (53.70%) and least compatible with hexaconazole (28.30%) and tebuconazole (35.04%). The isolate CREB-6 was more compatible with validamycin (56.19%) followed by copper oxychloride (51.33%), while it has shown least compatibility with hexaconazole (28.12%). The isolate CREB-16 was more compatible with validamycin (84.13%) followed by copper oxychloride (78.27%). However, both were found to be on par with each other. It has shown least compatibility with tebuconazole (25.51%). In the case of CRB-13, more compatibility was recorded with validamycin (66.12%) that was on par with copper oxychloride (59.03%) and least compatible with captan (20.96%). The present results indicated that the isolate CREB-16 was found to be most compatible antagonistic bacteria as it has shown highest per cent compatibility when compared to CRB-13, CREB-13 and CREB-6 isolates. The fungicide validamycin showed highest compatibility with all the four isolates followed by copper oxychloride and less compatibility was recorded in case of captan, tebuconazole and hexaconazole. Dean [4] carried out *in vitro* compatibility of the antagonistic bacterium *Bacillus subtilis* BSF4 strain with some fungicides and the results showed that it was compatible with azoxystrobin, penconazole, triadimenol and sulphur fungicides.

Khan and Gangopadhyay [7] reported that carbendazim and carboxin were least toxic to *Pseudomonas fluorescens* with the fungicides and revealed that carboxin and carbendazim were least toxic to *Pseudomonas fluorescens strain* PFBC-25 whereas captan was most inhibitory.

Table 3 *In vitro* evaluation of the compatibility of the potential bacterial isolates CRB-13, CREB-6, CREB-13 and CREB-16 with different fungicides by spectrophotometry

S. No.	Fungicides	CRB-13*	% Compatibility	CREB-6*	% Compatibility	CREB-13*	% Compatibility	CREB-16*	% Compatibility
1	Copper oxychloride (0.25%)	0.732	59.03 (50.24)	0.845	51.33 (45.76)	0.797	53.70 (47.18)	1.135	78.27 (63.25)
2	Captan (0.25%)	0.260	20.96 (27.19)	0.620	37.66 (37.84)	0.695	46.83 (43.17)	0.440	30.34 (33.37)
3	Hexaconazole (0.2%)	0.400	32.25 (34.49)	0.463	28.12 (32.00)	0.420	28.30 (32.09)	0.716	49.37 (44.64)
4	Tebuconazole (0.1%)	0.596	48.06 (43.81)	0.700	42.52 (40.68)	0.520	35.04 (36.28)	0.370	25.51 (30.15)
5	Validamycin (0.1%)	0.820	66.12 (54.74)	0.925	56.19 (48.59)	0.862	58.08 (49.65)	1.220	84.13 (68.25)
6	Control	1.240		1.646		1.484		1.450	
	S. Em ±		3.04		1.48		1.44		3.98
	CD (0.05)		9.93		4.84		4.72		12.99

* Mean of three replications
Figures in parenthesis are angular transformed values

REFERENCES

[1] Ahmed Q and Mohammad A. 1986. Losses in yield due to *Rhizoctonia* root rot of chickpea in Bihar. Indian Phytopathology. 39: 590-592.

[2] Annual Report, 2010-2011. Department of Agriculture and Cooperation Ministry of Agriculture, Government of India.

[3] Anonymous, 2009-10. Agricultural Statistics at a glance, Directorate of Economics and Statistics. pp. 104-105.

[4] Dean M, Guerrini P and Paci F. 2006. Compatibility of the antagonistic bacterium *Bacillus subtilis* BSF4 with fungicides and insecticides. Giornate Fitopatologiche, Riccione (RN), 27.437-442.

[5] Johnson L.F and Curl E.A. 1977. Methods for research on the ecology of soil borne plant pathogens. Burgess Publishing Company. Minneapolis. 27-35.

[6] Morton D.J and Stroube W.H. 1995. Antagonistic and stimulatory effects of soil microorganisms upon *Sclerotium rolfsii*. Phytopahtology. 45: 417-420.

[7] Khan M.A and Gangopadhyay. 2008. Efficacy of *Pseudomonas fluorescens* in controlling root rot of chickpea caused by *Macrophomina phaseolina*. Journal of Mycology and Plant Pathology. 38 (3): 580-587.

[8] Kishore G.K, Pande S and Podile A.R. 2005. Biological control of collar rot disease with broad-spectrum antifungal bacteria with groundnut. Canadian Journal of Microbiology. 51: 123-132.

[9] Parmer N and Dadarwal K.R 1997. *Rhizobacteria* from the rhizosphere and rhizoplane of chickpea. Indian Journal of Microbiology. 37(4): 205-210.

[10] Rangaswamy G and Mahadevan A. 1999. Diseases of crop plants in India. (4th edition) Prentice Hall of India Pvt. Ltd. New Delhi, pp. 607.

[11] Rangeshwaran R and Prasad R.D. 2000. Isolation and evaluation of rhizospheric bacteria for biological control of chickpea wilt pathogens. Journal of Biological Control. 14: 9-15.

[12] Rangeshwaran R, Raj, J and Sreerama Kumar P. 2008. Identification of endophytic bacteria in chickpea (*Cicer arietinum* L.) and their effect on plant growth. Journal of Biological Control. 22 (1): 13-23.

[13] Sajeena A, Salalrajan F, Seetharaman K and Mohan Babu R. 2004. Evaluation of biocontrol agents against dry root rot of blackgram (*Vigna mungo*). Journal of Mycology and Plant Pathology. 34 (2): 341-343.

[14] Uppala S.S, Beena S, Chapala M.M and Bowen K.L. 2009. Bioefficacy of endophytes in the management of leaf blight disease of amaranth. Proceedings of First Asian PGPR Congress for Sustainable Agriculture. 21-24.

[15] Vinod Kumar, Anuj Kumar and Khadarwar R.N. 2007. Antagonistic potential of fluorescent Pseudomonads and control of charcoal rot of chickpea caused by *Macrophomina phaseolina*. Journal of Environmental Biology. 28 (1): 15-20