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EFFICACY OF *STREPTOMYCES* SPP. AND *BACILLUS* SPP. FOR THEIR ANTAGONISTIC POTENTIAL AGAINST DRY ROOT ROT (*Rhizoctonia Bataticola)*OF CHICKPEA

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ABSTRACT

Five strains of *Streptomyces* (AC-5, AC-6, AC-10, AC-18 and AC-19) and another five strains of *Bacillus* (BS-10, BS-15, BS-17, BS-19 and BS-20) were earlier reported by us as biological control agents against *Fusarium* wilt of chickpea, caused by *Fusariumoxysporum* f. sp. *ciceri* (FOC), and plant growth-promoting agents in chickpea. In the present study, the selected strains of *Streptomyces* and *Bacillus* were further characterized for their antagonistic potential against dry root rot of chickpea, caused by *Rhizoctoniabataticola*, by dual culture assay, blotter paper assay and in greenhouse. Both the *Streptomyces* and *Bacillus* strains inhibited three strains of *R. bataticola*(RB6, RB24 and RB115) in both dual culture and blotter paper assays. When these were further evaluated for their antagonistic potential against *R. bataticola*under greenhouse conditions, reduced incidence of dry root rot was observed in *Streptomyces* and *Bacillus* treatments compared to the pathogen control.Among the ten strains studied,AC-18and BS-20recorded the highest reduction of disease both at 30 days after sowing (DAS) (58% and 75%, respectively)and 45 DAS (36% and 48%, respectively), when compared to the pathogen control.This study indicates that the selected *Streptomyces* and *Bacillus* strains have the potential for biocontrol of dry root rot of chickpea.

KEYWORDS: Biocontrol, *Streptomyces* spp., *Bacillus* spp.,dry root rot, chickpea

INTRODUCTION

Chickpea (*Cicerarietinum* L.), the second most important grain legume crop after bean (*Phaseolus vulgaris* L.*),* is grown in more than 55 countries (FAOSTAT, 2016), of which India is the largest producer. Chickpea plays an important role in human diet, farm health and sustainable agriculture. Many of the poorest countries in the world derive 10–20 per cent of their total dietary protein from chickpea and/or other grain legumes (Akibode and Maredia, 2011). It is mostly grown under rain-fed conditions in arid and semi-arid regionsof the world. During the year 2016, in India, it was grown in about 8.39 million hectares with a total production of 7.82 million tons (FAOSTAT, 2016). The average yield of chickpea was about 0.93 t ha^{-1}(FAOSTAT, 2016) which is farlower than its potential yield of 4 t ha^{-1}. A number of constraints such as infertile and marginal lands, drought or excessive moisture, increasing temperature, weeds and buildup of virulent insect pests and fungal pathogens are responsible for this yield gap in chickpea.There are about 30 diseases reported in chickpea and of which root diseases such as wilt, black root rot and dry root rot, caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), *Fusarium solani* and *Rhizoctoniabataticola*,respectively, have greater importance.

Dry root rot is endemic in temperate and tropical regions of the world with the capacity to infect over 500 different host crops and causes yield losses up to 100% under favorable conditions. In chickpea, the onset of the disease appears as scattered drying of the plants. Affected plants are usually straw colored, but in some cases the lower leaves and stems show brown discoloration. The tap root appears black, rotten and devoid of most of the lateral and fine roots. The dead root become quite brittle and shows shredding of bark. Dark minute sclerotial bodies can be seen on the roots exposed or inside the wood.

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When the dry stem of the collar region is split vertically, sparse mycelium or minute sclerotia can be seen in the pith(Nene *et al*., 1991).Although many control measures are available to manage dry root rot, soil borne nature, persistence in soil and a wide host range of *R. bataticola* makes this disease difficult to control. The use of resistant cultivar is also not completely effective. Under such scenario, biocontrol can be an alternative strategy for management of this disease.

Biocontrol of soil and seed-borne plant pathogens has been addressed using bacterial and fungal antagonists. Strains of *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp. and *Streptomyces* spp. were shown not only effective to manage plant pathogens but also mobilize and acquire nutrients for the plants (Postma*et al*., 2003; Khan *et al*., 2004; Perner*et al*., 2006; Gopalakrishnan*et al*., 2011a; Gopalakrishnan*et al*., 2011b; Alekhya and Gopalakrishnan, 2017).Previously, we have reported the potential of a set of five strains of *Streptomyces* (AC-5, AC-6, AC-10, AC-18 and AC-19) and another set of five strains of *Bacillus* (BS-10, BS-15, BS-17, BS-19 and BS-20) for the biological control of *Fusarium* wilt of chickpea, caused by FOC, and plant growth-promotion (PGP) in chickpea (Anusha *et al*., 2018). The objective of this study was to further evaluate these *Streptomyces* and *Bacillus* strains for their antagonistic potential against dry root rot of chickpea caused by *R.bataticola*.

MATERIALS AND METHODS

Streptomyces spp. and *Bacillus* spp.strains

Five strains of *Streptomyces* spp., AC-5 (NCBI accession number: MF361862), AC-6 (NCBI accession number: MF359563), AC-10 (NCBI accession number: MF359746), AC-18 (NCBI accession number: MF359734) and AC-19 (NCBI accession number: MF359745), and another set of five strains of *Bacillus* spp., BS-10 (NCBI accession number: MF359733), BS-15 (NCBI accession number: MF359735), BS-17 (NCBI accession number: MF359737), BS-19 (NCBI accession number: MF370070) and BS-20(NCBI accession number: MF370069) previously reported by us to have capacity for the biocontrol of *Fusarium* wilt and PGP in chickpea (Anusha *et al*., 2018), were further studied in the present investigation.

Dual culture assay

The selected five strains of *Streptomyces* spp. (AC-5, AC-6, AC-10, AC-18 and AC-19) and another five strains of *Bacillus* spp. (BS-20, BS-19, BS-17, BS-15 and BS-10)were screened for their antagonistic activity against three strains of *Rhizoctonia bataticola* such as RB6, RB24 and RB115(acquired from legume pathology, ICRISAT Patancheru, Hyderabad, India) by dual culture assay on glucose casamino acid yeast-extract (GCY) agar as per the protocols of Gopalakrishnan *et al.*(2011b).

Blotter paper assay

The selected five strains of *Streptomyces* and another five strains of *Bacillus* were screened for their antagonistic potential against *R. bataticola* strain RB6 (the most virulent strain) by blotter paper assay.In brief, two-week-old seedlings of chickpea (BG212- susceptible to dry root rot) were dipped in the inoculum of *R. bataticola*RB6 (grown separately in potato dextrose broth (PDB) at 28 ±2 °C) for 30 min followed by test strains (grown separately in starch casein broth [SCB] for *Streptomyces* spp. and nutrient broth for *Bacillus* spp.) for another 30 min and placed side by side on a blotter paper (45 X 25 cm) in a plastic tray, so that only the roots were covered. Positive and negative controls were made by inoculating the plants only with pathogen (*R. bataticola* RB6) and sterile water, respectively. Ten plants per replicate and three replications were made for each treatment.Trays were transferred to incubators maintained at 30°C temperature with 12h photoperiod and regularly moistened with sterile deionized water for seven days. At the end of the incubation, the disease symptoms of the dry root rot (black-colored infection and microsclerotia on the root surface) were recorded on a 0"4 rating scale (0 represents no visible dry root rot symptom, while 4 represents maximum disease symptoms) and the percentage of infected roots in *Streptomyces* spp. and *Bacillus* spp. inoculated treatments compared with the control. Disease incidence (DI) was also calculated as per the following formula:

DI (%)=[Number of infected plants/Total number of plants] x 100

Greenhouse study

The five *Streptomyces* and *Bacillus* strains were evaluated individually for their antagonistic potential in dry root rot sick pots under greenhouse conditions. Dry root rot sick pots (pots infected with *R. bataticola*RB 6 inoculum) were prepared as per the protocols of Pande*et al*. (2012).Pot mixture was prepared by mixing Vertisol, sand and farm yard manure at 3:2:1 (w/w) and filled (800g) in 8 inch plastic pots. *R. bataticola* enriched soil (sick soil) was added into the above pots at 20% of pot weight(200 g pot^{-1}) two weeks before sowing. Sick soil was thoroughly mixed with the pot mixture and the pots were covered with polythene sheets. The whole set-up was incubated at 30±1°C for 15 days in order to get *R. bataticola* sick conditions. Two weeks later, the seeds of

chickpea variety BG212 were surface-sterilized (with 2.5% sodium hypochlorite solution for 2 min and rinsed 8 times with sterilized water) and treated with respective *Streptomyces*/ *Bacillus* strains. Each *Streptomyces*/ *Bacillus* strains were inoculated by seed treatment + soil application methods. Seed treatment was done by soaking the seeds in the respective *Streptomyces*/ *Bacillus* strains for 1 h while soil application was done by inoculating the potting mixture with *Streptomyces*/ *Bacillus* strains at the time of sowing (10 ml of well grown culture [10^8 CFU ml^{-1}]).Six seeds were sown at 2%3 cm depth in each pot. The experiment had six replications. Plants were irrigated once in two days with 20 ml of sterilized distilled water. Incidence of dryroot rot disease (number of plants showing disease symptoms to the total number of plants in a pot) was recorded on 30 and 45 days after sowing (DAS). Disease incidence was calculated using the method reported by Cao *et al*. (2011) with the following formula:

Disease incidence (%) = (Number of diseased plants/ Total number of plants) x 100

Statistical analysis

The data were subjected to analysis of variance (ANOVA) (GenStat 10.1 version 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) to evaluate the efficiency of biocontrol agent's application in the greenhouse studies. Significance of differences between the treatment means was tested at P = 0.01 and 0.05.

RESULTS

Dual culture assay

All the selected strains of *Streptomyces* and *Bacillus* inhibited all the three strains of *R. bataticola* RB6, RB24 and RB115. Inhibitory activity was found highest in AC-18 (22.0 mm),followed by AC-5 (21.8mm),BS-17 (16.3 mm) and BS-20(15.75) for *R. bataticola* RB6; AC-6 (17.0 mm) followed by AC-19 (15.3 mm), BS-19 (16.3)and BS-17 (15.5) for *R. bataticola* RB24; and AC-10 (22.0 mm) followed by AC-5 (21.0 mm), BS-20 (18.0) and by BS-17 (10.7) for *R. bataticola* RB115. Among the five *Streptomyces* strains, AC-5 followed by AC-10 recorded good antagonistic activity against all the three strains of *R. bataticola* whereas, BS-20 and BS-17 among *Bacillus* strains (Table 1).

Blotter paper assay

When the selected strains of *Streptomyces* and *Bacillus* were evaluated for their *in vivo* antagonistic activity against *R. bataticola* RB6 strain by blotter paper assay, significant reduction in disease symptoms was observed on 5th day in all the *Streptomyces* strains while the highest reduction was found in AC-19(rating 1.87; disease incidence 49%) followed byAC-18 (rating1.87; diseaseincidence48%) when compared to positive control (only RB6) (rating 3.8; disease incidence 92%). In case of *Bacillus* strains,significant reduction was found in two strains, BS-20 (rating 2.0; disease incidence 49%) followed by BS-19 (rating 2.67; disease incidence 69%) when compared to positive control (only RB6) (rating 3.8; disease incidence 90%) (Table 2).

Greenhouse study

Under greenhouse conditions, at 30 DAS, the selected *Streptomyces* strains were found to significantly reduce the dry root rot disease incidence over the RB6 (positive control). The lowest disease incidence was found in AC-18 (37%) followed by AC-19 (52%), AC-6 (58%), AC-5 (61%) and AC-10 (62%) when compared to RB6 (positive control; 89%), this was 58 per cent, 42 per cent, 35 per cent, 32 per cent and 30 per cent, reduction in disease incidence , respectively over RB115 (Table 3).In case of *Bacillus* strains, the lowest disease incidence was

recorded in BS-20 (23%) followed by BS-10(58%), BS-17 (59%), BS-15 (61%) and BS-19 (63%) when compared to RB6 (89%), this was 75 per cent, 35 per cent, 33 per cent, 31 per cent and 29 per cent, reduction in diseaseincidence, respectively over RB-6. Similar results were observed at 45 DAS for both *Streptomyces* and *Bacillus* strains but the reduction of disease incidence over control was less. For instance, up to 58 per cent reduction was found in *Streptomyces* strains at 30 DAS whereas it was only 36 per cent at 45 DAS; similarly, up to 75per centof disease incidence reduction was found in *Bacillus* strains at 30 DAS whereas it was only 48per cent at 45 DAS (Table 3).

DISCUSSION

Dry root rot caused by *R. bataticola* is emerging as one of the serious biotic constraint for chickpea production in India. Biological control can be one of the important strategy for managing this disease. Previously, we have reported the potential of a set of 10 strains of *Streptomyces* spp. and *Bacillus* spp. for biological control of *Fusarium* wilt and PGP in chickpea (Anusha *et al*., 2018). In the present study, these ten *Streptomyces* and *Bacillus* strains were further evaluated for their antagonistic potential against dry root rot of chickpea.

In the dual culture assay, all the selected strains of *Streptomyces* and *Bacillus* inhibited all the three strains of *R. bataticola* RB6, RB24 and RB115. Among the tested *Streptomyces* strains, AC-5 and AC-10 and among the *Bacillus* strains, BS-20 and BS-17 recorded highest antagonistic activity against all the three strains of *R. bataticola*. In the blotter paper assay, significant reduction of dry rot symptoms were observed in plants treated with *Streptomyces* and *Bacillus* strains. The highest reduction of disease incidence was found in AC-19 (rating 1.87; disease incidence 49%) and AC-18 (rating1.87; disease incidence 48%) for *Streptomyces* strains and BS-20 (rating 2.0; disease incidence 49%) and BS-19 (rating 2.67; disease incidence 69%) for *Bacillus* strains when compared to RB6 (pathogen control; rating 3.8; disease incidence 90%) (Table2). The inhibition of *R. bataticola* RB6, RB24 and RB115 by *Streptomyces* or *Bacillus* strains in dual culture array could be due to the production of hydrolytic enzymes or antibiotics which were dispersed medium. Bacteria are known to produce growth hormones such as auxins and hydrolytic enzymes such as cellulase and â-1,3-glucanase and help plants to inhibit pathogens (Pal *et al*., 2001; Correa *et al.,* 2004).The selected ten *Streptomyces* and *Bacillus* strains were found to produce indole acetic acid, â-1,3-glucanase, cellulase(except AC-5), protease (except AC-5, AC-10 and BS-10), lipase (except AC-5) and hydrocyanic acid (except AC-18 and BS-10) under *in vitro* conditions (Anusha *et al*., 2018). It is concluded that extra cellular enzymes and growth-promoting hormones, produced by the *Streptomyces* and *Bacillus* strains would have played a role in inhibition of *R. bataticola*.

In the present study, the strains BS-20 and BS-17 were found to show significant differences in their ability to inhibit mycelial growth of *R.bataticola*. Such variation among isolates in their ability to control the growth of pathogens has been reported by several researchers (Laha *et al*.,1992; Naik *et al*., 2000; Upmanyu*et al*., 2002; Singh *et al*., 2008) and highlights the need for selection of the best isolates for use as bio-control agents (Suriachandraselvan *et al*., 2004) against specific pathogens and under specific agro-climatic conditions. Further, difference in the inhibitory ability between isolates has been often been attributed to factors such as antibiosis, mycoparasitism, competition for space and nutrients and over growth (Ghaffar *et al*., 1964; Karunanithi *et al.,* 2000; Naik *et al*., 2000, 2009; Naik and Sen 1995).

In the present investigation, under greenhouse conditions, both at 30 DAS and 45 DAS, the selected *Streptomyces* and *Bacillus* strains were found to significantly reduce the dry root rot disease incidence up to 58% and 75%, respectively over the pathogen control. *Trichodermaharzianum* and *Pseudomonas fluorescence* were reported to control dry root rot in chickpea (Manjunatha *et al*.,2011 and 2013. Deepa *et al*., 2018). In the literature, according to our knowledge, there areno reports of usage of *Streptomyces* and *Bacillus* strains for biocontrol of dry root rot in chickpea.

In the present study, the usefulness of ten *Streptomyces* and *Bacillus* strains for biocontrol of dry root rot disease in chickpea were demonstrated by dual culture assay, blotter paper assay and at greenhouse conditions. However, field trial(s) needs to be conducted at multi-locations in order to demonstrate its usefulness under various soil and climatic conditions. Further, these strains need to be formulated as bio-inoculants and used for biocontrol of dry root rot in other crops also. The secondary metabolite(s) responsible for inhibition of *R. bataticola* needs to be identified and characterized.

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Ethical approval This article does not contain any studies with human participants or animal performed by any of the authors.

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Table1. Bioefficacy of *Streptomyces* and *Bacillus* strains against three strains of *Rhizoctonia bataticola*

Test strains	Inhibition zone (mm)		
	R. bataticola RB6	*R. bataticola* RB24	*R. bataticola* RB115
AC-5	21.8	14.3	21.0
AC-6	15.3	17.0	16.8
AC-10	18.5	15.3	22.0
AC-18	22.0	09.3	17.5
AC-19	19.0	15.3	17.5
BS-10	15.4	09.3	04.5
BS-15	12.2	11.3	07.6
BS-17	16.3	15.5	10.7
BS-19	15.0	16.3	6.2
BS-20	15.8	13.8	18.0
SE±	1.31	2.04	1.48
LSD at 5%	4.71	6.14	4.45

Table 2. Bioefficacy of *Streptomyces* and *Bacillus* strains against *Rhizoctonia bataticola* RB6 by blotter paper assay

Treatment	Visual rating		Disease incidence (%)	
	1st time	2nd time	1st time	2nd time
AC-5 + RB6	2.67	2.67	70	67
AC-6 + RB6	2.67	3.00	67	77
AC-10 + RB6	3.33	3.00	83	73
AC-18 + RB6	1.67	2.00	43	53
AC-19 + RB6	1.67	2.00	40	57
Only RB6 (Positive control)	4.00	3.67	93	90
Mean	2.67	2.72	66	69
SE±	0.28***	0.49NS	5.3***	4.0***
LSD (5%)	0.88	1.55	16.8	12.7
BS-10 + RB6	3.00	2.33	73	63
BS-15 + RB6	3.00	2.33	70	60
BS-17 + RB6	3.00	3.00	73	70
BS-19 + RB6	2.67	2.67	70	67
BS-20 +RB6	2.00	2.00	50	47
Only RB6 (Positive control)	4.00	3.67	100	90
Mean	2.94	3.67	73	66
SE±	0.53NS	0.28*	3.6***	3.9***
LSD (5%)	1.68	0.88	11.4	12.3

*= Statistically significant at 0.05, ***= Statistically significant at 0.001, NS= Not significant

Table 3. Evaluation of *Streptomyces* and *Bacillus* strains against *Rhizoctonia bataticola* RB6 under greenhouse conditions

Treatment	At 30 DAS		At 45 DAS	
	Disease incidence (%)	Percent reduction over control	Disease incidence (%)	Percent reduction over control
AC-5 + RB6	60.9	31.5	75.4	22.0
AC-6 + RB6	57.9	34.7	74.6	22.8
AC-10 + RB6	62.1	30.0	75.0	22.4
AC-18 + RB6	37.1	58.2	62.1	35.8
AC-19 + RB6	51.7	41.8	67.5	30.3
Only RB6 (Positive control)	88.8		96.7	
Mean	59.8		75.2	
SE±	2.91***		2.34***	
LSD (5%)	9.16		7.37	
BS-10 + RB6	57.5	35.2	74.2	23.3
BS-15 + RB6	61.3	31.0	72.9	24.6
BS-17 + RB6	59.2	33.3	65.0	32.8
BS-19 + RB6	62.9	29.1	73.8	23.7
BS-20 + RB6	22.5	74.6	50.0	48.3
Only RB6 (Positive control)	88.8		96.7	
Mean	58.7		72.1	
SE±	4.74***		3.85***	
LSD (5%)	14.92		12.13	

***= Statistically significant at 0.001