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Short Communication

EFFICACY OF *BACILLUS* SP. TO AUGMENT CHICKPEA GROWTH UNDER BIOTIC STRESS CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *CICERIS*

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ABSTRACT
The experiment was conducted during the year 2013-14 in glasshouse of Schools of Biotechnology, Punjab Agricultural University, India to study the influence of antagonistic *Bacillus* sp. isolate, B-I, as seed bioinoculant alone and in consortium with *Mesorhizobium ciceris* on seedling emergence, growth parameters and wilt incidence in pathogen inoculated chickpea plants. Seed bacterization with isolate B-I reduced the wilt incidence in bacterized pots (36%) as compared to non-bacterized plants (74%). Co-inoculation with *Mesorhizobium ciceris* further reduced the wilt incidence (26%) and provided defense against the pathogen which was significantly higher than that recorded with fungicides (46%). Inoculation with isolate B-I alone and in co-inoculation with *Mesorhizobium* recorded 94 and 96% seedling emergence respectively as compared to 89% in control. Chlorophyll content affected by B-I inoculation alone and in combination with *Mesorhizobium* was also at par to control (0.87 and 0.90 mg/g leaf tissue respectively). In pathogen challenged plants, *Bacillus* sp. isolate alone increased the fresh weight by 29.2 % and in combination with *Mesorhizobium* by 49.9% over uninoculated control. Maximum increase in root and shoot length was also recorded with dual inoculation. Co-inoculated treatment (B-I + *Mesorhizobium)* recorded maximum shoot and root length (21.5 cm and 15.9 cm respectively).

KEY WORDS: Antagonism, plant growth promotion, wilt incidence.

INTRODUCTION
Chickpea (*Cicer arietinum* L.) is one of the most important food legumes grown globally. In addition to having high protein content (20-22%), chickpea is rich in fiber, minerals (phosphorus, calcium, magnesium, iron and zinc) and β-carotene. Its ability to improve soil fertility by fixing atmospheric nitrogen has further popularized its cultivation. However, the sustainability of chickpea based agriculture in the Indian continent and other countries in Asia is severely threatened by a number of biotic and abiotic factors. Among the several biotic factors, attack by pathogenic microorganisms is the major constraint affecting the productivity of chickpea. As soil-borne fungi, *Fusarium oxysporum* f. sp. *ciceris* (Padwick) (Foc) causal agent of fusarium wilt has emerged as a major threat to chickpea production (Ghosh *et al.,* 2013). The most relevant and potential method of disease management and maximizing crop productivity worldwide to date has been the use of fungicides. But, the need for greater sustainability in agriculture and rising public concern about the harmful environmental effects of fungicides has necessitated the development of alternate disease control strategies. Non-pathogenic soil *Bacillus* species are found to be promising agents for disease management due to their abundance and persistence in soil as a result of the formation of resistant endospores and their antimetabolites (Kumari *et al*., 2015). This greatly facilitates post-culture conditioning as bacterial suspensions can be converted to easy to handle powder formulations without the impressive bacterial mortality observed with non-sporulating bacteria (Lolloo *et al.*, 2010). These soil inhabiting bacteria not only act as potential biocontrol agents through competition for resources, parasitism, systemic induced resistance and antibiosis (Martinez *et al.*, 2006) but are also known to promote plant growth through increased mineral nutrient, improving plant tolerance to abiotic stresses by producing enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and producing phytohormones such as indole-3-acetic acid (IAA) and gibberellins. The present investigation was taken up to study the efficacy of antagonistic *Bacillus* sp. in managing wilt and promoting growth in *Fusarium oxysporum* inoculated chickpea plants under glasshouse condition. The antagonistic rhizobacteria B-I, used in present investigation was obtained from our previous study and was found to represent *Bacillus* sp. (Kumari and Khanna, 2016a). The pathogen, *Fusarium oxysporum* was obtained from Department of Plant Breeding and Genetics, PAU, Ludhiana

and *Mesorhizobium ciceris* from Department of Microbiology, PAU, Ludhiana. The experiment was conducted during the year 2013-14 in glasshouse of Schools of Biotechnology, Punjab Agricultural University, India to study the influence of antagonistic *Bacillus* sp. B-I as seed bioinoculants alone and in consortium with *Mesorhizobium ciceris*. Medium black clayey soil collected from chickpea field, Punjab Agricultural University, Ludhiana was autoclaved 3x (1h, 121°C) at 24 hrs-intervals and 1 kg sterilized soil amended with microconidial suspension (1000 conidia mL^{-1}) of *Fusarium oxysporum* @ 50 ml/kg was filled per pot. Susceptible variety JG-62 of chickpea was procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Uniform sized chickpea seeds were surface-sterilized with 0.1% mercuric chloride for 30 s, then washed 5-6 times with sterile distilled water and dried under stream of sterile air. Seeds were then soaked in 10 ml of bacterial inoculum containing 3×10^8 cfu mL^{-1} for 3-4h, thereafter dried and sown @ 10 seeds /bag. Each treatment was replicated 7 times. For dual inoculation, *Bacillus* sp. culture and *Mesorhizobium* inoculum was mixed in 1:1 ratio. Growth parameters and wilt incidence was recorded at different intervals.

Seed bacterization with bacterial isolates recorded reduced incidence of wilt in *Fusarium oxysporum* inoculated chickpea plants. Table 1 summarizes the wilt incidence at 10 days interval in all the treatments and also reveals the efficacy of *Bacillus* isolate, B-I, to be used as biocontrol agent. Isolate, B-I, reduced the wilt incidence in bacterized pots (36%) as compared to non-bacterized plants (74%).

TABLE 1: Effect of seed bacterization with bioantagnists on wilt incidence in chickpea

Treatments	Wilt incidence (%)			
	20th day	25th day	30th day	35th day
B-I+R +Foc	4.29	7.15	10.01	26
B-I+Foc	12.86	16.86	25.72	36
Foc +fungicide	11.4	22.8	38.6	46.0
Foc (Control)	18.6	28.6	50.0	74.0

R-*Rhizobium;* Foc- *Fusarium oxysporum* f.sp. *ciceris*

TABLE 2: Effect of seed bacterization on % germination and photosynthetic efficiency of chickpea plants challenged with *Fusarium oxysporum*

Treatments	% germination	Chlorophyll content (mg/gm)	
		20th day	30th day
B-I+R+Foc	96	0.90	0.81
B-I+Foc	94	0.87	0.77
Foc +fungicide	90	0.66	0.42
Foc control	89	0.61	0.45
CD @5%		0.14	0.13

R-*Rhizobium;* Foc- *Fusarium oxysporum* f. sp. *ciceris*

However, co-inoculation of bacterial antagonist with *Mesorhizobium ciceris* depicted a cumulative effect and further reduced the wilt incidence (26%) and provided defense against the pathogen which was significantly higher than that recorded with fungicides (46%). It has been reported that even if mixtures of PGPR strains do not always results in additive or synergistic effects of possible advantage, but different strains may have different mechanisms and their combinations may provide a spectrum of activity which may have beneficial effect on plant health and productivity (Raupach and Kloepper, 2000). Hahm *et al.* (2012) also reported that the degree of disease suppression was significantly increased where dual culture treatment was used. The reflection of seed bacterization with antagonistic bacteria on plant growth was also observed. Inoculation with isolate B-I recorded 94% seedling emergence as compared to 89% in control. Co-inoculation with *Mesorhizobium* further enhanced the germination percentage to 96 %. Higher germination in pathogen challenged plants may be due to ACC-deaminase production by rhizobacterial isolates which is induced under stress condition (Kumari and Khanna, 2016b) including biotic stress and provides initial stimulus for plant growth by reducing the level of stress induced ethylene (Selvakumar *et al* 2012). A perusal of Table 3 shows inoculation with *Bacillus* sp. significantly increased chlorophyll content over uninoculated control. Chlorophyll content affected by B-I inoculation alone and in combination with *Mesorhizobium* was at par over control (0.90 and 0.87 mg/g leaf tissue respectively) after 20 days of germination. After 30 days, decrease in photosynthetic ability was observed with all the treatments, however, B-I inoculation again surpassed others in terms of chlorophyll content. Less chlorophyll content in pathogen-challenged plants as compared to not challenged plants may be attributed to the chlorosis induced by the pathogen.

Seed treatment with isolate, B-I, significantly increased plant biomass (Table 3) and root –shoot length (Table 4) compared to uninoculated control. In pathogen challenged plants, *Bacillus* sp. isolate alone increased the fresh weight by 29.2 % and in combination with *Mesorhizobium* by 49.9% over uninoculated control. Maximum increase in root and shoot length was also recorded with dual inoculation. Co-inoculated treatment (B-I + *Mesorhizobium)* recorded maximum shoot and root length (21.5 cm and 15.9 cm respectively). Higher increase in growth parameters with co-inoculation signified secretion of some plant growth promoting hormones such as IAA, cytokinins etc by *Rhizobium* which possibly lead synergism between the two isolates. There are reports for production of plant growth regulators such as auxins, cytokinins and gibberellins like substances by rhizobia that stimulate and enhance plant growth (Hemissi *et al.*, 2011). Synergistic effect in use of dual cultures is well documented (Hahm *et al.*, 2012). The same synergistic response was evident in this study.

TABLE 3: Effect of seed bacterization with bioantagonists on biomass of chickpea plants challenged with *Fusarium oxysporum*

Treatments	Plant weight (gm)					
	15th day		25nd day		35nd day	
	Pfw	Pdw	Pfw	Pdw	Pfw	Pdw
B-I+R+Foc	4.43	1.07	5.89	1.14	6.97	1.45
B-I+Foc	3.94	0.79	5.46	1.41	6.01	1.30
Foc +fungicide	3.35	0.71	4.68	1.00	5.75	1.18
Foc	2.93	0.68	3.77	0.99	4.65	0.97
CD @5%	0.62	0.06	0.45	0.04	0.51	0.07

R-*Rhizobium;* Foc- *Fusarium oxysporum* f. sp. *ciceris*

TABLE 4: Effect of seed bacterization with bioantagonists on root and shoot length of chickpea plants challenged with *Fusarium oxysporum*

Treatments	Length (cm)					
	15th day		25th day		35nd day	
	Shoot	Root	Shoot	Root	Shoot	Root
B-I+R+Foc	15.4	12.7	19.4	15.3	21.5	15.9
B-I+Foc	14.8	11.2	18.8	13.5	21.2	14.4
Foc +fungicide	13.0	9.8	16.5	10.9	17.4	13.2
Foc	11.5	9.3	14.0	10.2	15.2	11.1
CD@5%	0.95	1.13	1.08	1.17	1.75	1.04

R-*Rhizobium;* Foc- *Fusarium oxysporum* f. sp. *ciceri*

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