Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2646

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 838-843 (2017)

Research Article

OPEN ACCESS

Efficacy of *Bacillus subtilis* Isolate K18 Against Chickpea Wilt *Fusarium oxysporum* F. Sp. *ciceri*

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Received: 12.04.2017 | Revised: 23.05.2017 | Accepted: 1.06.2017

ABSTRACT

The rizhospheric bacteria improve plant growth and development as well as impart tolerance against biotic and abiotic stress. Chickpea is vital source for protein in developing countries and called poor's men's meat, but it productivity is hampered by wilt diseases caused by Fusarium oxysporum f. sp. ciceris (Foc). The rizhospheric bacteria potentially act as biocontrol agent where it helps to reduce environmental pollution caused due to use of chemical control measures. In present study Bacillus subtilis isolate K18 (BS-K18) found effective antagonist against wilt pathogen Foc under in vitro condition was evaluated under laboratory study. The BS-K18 seed treatment effectively reduced the onset of disease symptoms in susceptible genotype JG-62 up to 7 days after transplanting in Foc sick soil. Apart from improving disease tolerance the BS-K18 showed PGPR effect on both resistance and susceptible genotype where shoot length, root length, number of root plant^{-1}, fresh weight and root volume were significantly improved as compare to control chickpea genotypes. These promising antagonist isolates, BS-K18 with PGPR activities could be further evaluated in field study for its commercial exploitation.

Key words: *Bacillus subtilis, Antagonist, Biocontrol, Fusarium oxysporum f. sp. ciceris*

INTRODUCTION

Rhizosphere is a potential place for complex plant-microbe and microbe-microbe interactions[1]. Thus, rhizosphere region is an extremely complex habitat for microbes known as Plant Growth Promoting Rhizobacteria (PGPR), plant beneficial bacteria. Many PGPR helps plant to impart resistance to biotic stresses by antagonism mechanism i.e. interference with plant pathogen growth, survival, infection or plant attack[2]. Antagonistic bacteria can inhibit plant pathogens by different mechanisms like inhibition of the pathogen by antibiotics and toxins, competition for space and nutrients, parasitism by extracellular cell-wall-degrading enzymes, mycophagy, etc[1,3].

Cite this article: Suthar, K.P., Patel, R.M., Singh, D. and Khunt, M.D., Efficacy of *Bacillus subtilis* Isolate K18 Against Chickpea Wilt *Fusarium oxysporum* F. Sp. *ciceri*, *Int. J. Pure App. Biosci.* **5(5):** 838-843 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2646

Chickpea (*Cicer arietinum* L.) is pivotal source of protein for vegetarian diet called poor men's meat; however the productivity of chickpea is far below its potential[4]. One of the major reasons for the low productivity of cultivated chickpea is its narrow genetic bases and its sexual incompatibility with other wild species of *Cicer* in natural inter specific cross. Furthermore, various biotic (*Fusarium* wilt, *Aschochyta* blight, nematodes and pests) and abiotic (heat, salinity, drought and cold) stresses severely reduce the yield[5]. Among the biotic stresses, the *Fusarium* wilt and *Ascochyta* blight are the most important fungal diseases causing serious yield losses. *Fusarium* wilt caused by *Fusaruim oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato (*Foc*) is one of the most destructive vascular diseases of chickpea first reported from India by Butler in 1918 [6] which cause yield loss up to 90 % annually under epidemic condition[7]. *Fusarium* wilt is primarily managed by resistance breeding programs but pathogenic variability and mutability leading to breakdown of naturally selected resistance, are the main hurdles for plant breeders[8]. The fungicides are used for wilt management however, the degradation of fungicides is very difficult and it's accumulation in food chains had higher toxic level in animals and also lead to environment pollution [9]. Therefore, Integrated Disease Management (IDM) strategy is need of time which includes minimum use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce the pathogen inoculums[10], further the crop rotation, pathogen-free seed, removal of plant debris and fungicide seed treatment are several disease management strategies that have been employed for the control of wilt in chickpea. Biological control of *Fusarium* wilt is a potential component of IDM where the principle of bacteria or fungal antagonist has been exploited for the control of disease. The application of microorganisms to control diseases, which is a form of biological control, is an environment friendly approach. Different scientists have realized the potential of rhizospheric microorganism in control of *Foc* in chickpea[11]. The PGPR's such as *Pseudomo*nas and *Bacillus* strains, are the major root colonizers[12,13] and can elicit plant defenses[14]. Different mechanisms have been reported for their performance as antagonist of *Fusarium* such as production of antibiotics, siderophore, hydrogen cyanide hydrogen, competition for nutrition and space, inducing resistance, inactivation of pathogen's enzymes and enhancement of root and shoot development[15]. Realizing the need of novel microbial biocontrol agent against the *Fusarium* wilt of chickpea, the present study is undertaken to evaluate potential antagonist reported in our previous study, *Bacillus subtilis* isolate K18 (*BS*-K18) for wilt management in chickpea.

MATERIALS AND METHODS

Plant material

The seeds of resistance (WR-315) and susceptible (JG-62) chickpea variety were procured from International Crops Research Institute for the Semi Arid Tropics (ICRISAT) Patancheru, Andhra Pradesh, India was used in present study. Prior to setting of each experiment, healthy and uniform seeds of above varieties were tested for viability. The seeds were surface sterilized with 0.01 % mercuric chloride ($HgCl_2$) for 1 min followed by rinsing with autoclaved double distilled water (DDW) four times, to remove all traces of $HgCl_2$. The seeds were then allowed to germinate in autoclaved sand up to 9 days, the 9 days old seedling were transferred to *Foc* sick or normal bags as per treatments.

PGPR isolate

The highly effective antagonist *Bacillus subtilis* isolate K18 (*BS*-K18) against *Fusarium oxysporum* f. sp. *ciceris* found in previous study where it reported 75% inhibition of *Foc* under *in vitro* condition was used in present study. It was preserved on agar slant at 4°C and glycerol stocks were prepared for long term preservation of isolates.

In vitro experiment

The *Foc* sick soil was prepared by mixing field soil and farm yard manure (FYM) in the proportion of 1:1 and sterilized in autoclave. Sorghum grains inoculated with pathogen *Fusarium oxysporum* f. sp. *ciceri* which had microbial load 2.5 X 10^7 cfu g^{-1} in sorghum grain media was then added to the soil in the proportion of 1:10 (Sorghum grain Inoculums + Sterilized soil mixture). The bags were filled with these mixtures @ 2.5 kg per bag as a sick soil.

RESULTS AND DISCUSSION

Antagonistic microbes have the potential to inhibit plant pathogenic microorganisms by different mechanisms in eco-friendly manner. Isolation of such important microorganisms was done from soil samples collected from healthy chickpea rhizospheric soil in previous study where *Bacillus subtilis* isolate K18 exhibited higher antagonist potential (75%) as well as plant growth promoting character *viz*., capacity to fix nitrogen and Zinc solubilization (Fig. 1).

The seed treatment with microbial antagonist (*BS*-K18) improved shoot and root growth of resistant var. WR-315 and susceptible var. JG-62 (Table 1). In case of disease symptoms, seed treatment with *BS*-K18 showed less intensity of disease as compared to control in susceptible var. JG-62 at 7 days after transplanting (DAT), besides, these it also improved the root growth (Fig. 2). The *BS*-K18 seed treatment under *Foc* stress condition reported higher shoot length (8.0 cm) as compared to *Foc* stress condition alone (7.65 cm) in resistance var. WR-315. In case of susceptible var. JG-62, the same trends were observed where *BS*-K18 seed treatment under *Foc* stress condition improved shoot length (8.50 cm) compared to *Foc* stress condition alone (7.30 cm). The *Foc* infect the root system of chickpea, in present study *BS*-K18 seed treatment improved the root growth of chickpea seedlings. The *BS*-K18 seed treatment under *Foc* stress increased root length in resistance and susceptible variety that is 8.60 and 6.20 cm, respectively as compared to *Foc* stress alone where it was 4.48 and 3.52 cm. The number of root $plant^{-1}$ was also improved by *BS*-K18 seed treatment under *Foc* stress condition in both varieties. The maximum 41 roots $plant^{-1}$ was observed in susceptible var. JG-62 for *BS*-K18 seed treatment under *Foc* stress condition whereas it was 40 roots $plant^{-1}$ in var. WR-315 under same condition. The *BS*-K18 seed treatment alone was observed to improve root volume in var. WR-315 and var. JG-62 that was 650 and 580 µl, respectively. The *Foc* stress drastically reduced the root volume, whereas the seed treatment with *BS*-K18 improved the root volume in both varieties. The fresh weight of plant give idea about its healthy growth and development, the *BS*-K18 seed treatment under *Foc* stress condition found effective in increasing the fresh weight of plant under *Foc* stress. The seed treatment under *Foc* stress reported higher fresh weight in var. WR-315 (8.36 g) and var. JG-62 (7.85 g) as compared to *Foc* stress alone where it was 7.11 and 6.35 g, respectively. Overall, *BS*-K18 was found effective against *Foc* and it also showed PGPR activities on chickpea seedling.

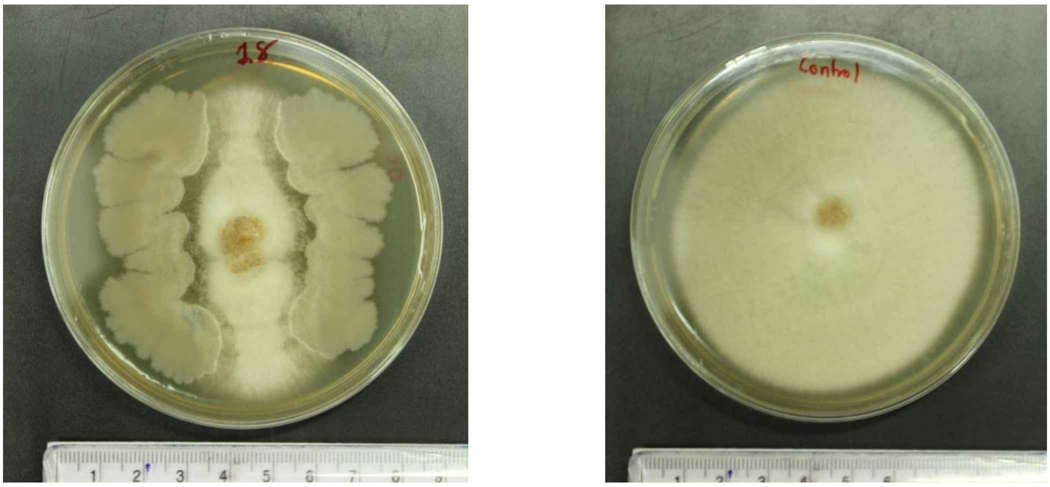
The microorganisms present in rhizosphere improve plant growth as well as protect plant from soil borne pathogens. This protection is mainly associated with a strong antagonistic activity in the rhizosphere and in planta, as well as with the induction of structural and biochemical barriers that adversely affect pathogen growth and development[16]. Moradi *et al.*, reported that *B. subtillis*, *T. harzianum* treatments in liquid and seed coating inoculation methods significantly reduced *wilt* disease severity (about 40%) either alone or in combination. They observed that although the combination of these bio-control agents was effective in controlling *Fusarium* wilt disease but did not differ significantly from bio-control treatments individually[17]. The potentiality of locally isolated bioagents *(Trichoderma Harzianum, Trichoderma viride and Aspergillus niger)* was tested against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt under pot experiments[18.] They found that the bioagents at different concentrations *viz.* (2%, 4%, 6%, 8% w/w) were effective in controlling wilt disease in susceptible variety *viz.* JG-62, further *T.*

harzianum and T. viride at 8% and *A. niger at* 10% concentration (w/w), inhibited the wilt incidence upto 100%. Landa, 2005 found that *B. subtilis* GB03 and *P. fluorescens* RG 26, applied either alone or each in combination with nonpathogenic *F. oxysporum* Fo 90105, was the most effective treatment in suppressing *Fusarium* wilt of chickpea[19]. Raju, 2005 reported that the lowest disease (pigeon pea wilt) incidence (6.6%), and the highest number of nodules per plant (23.3), fresh weight per plant (6.3g), and dry weight per plant (2.2g) were obtained with *T. viride* + carbendazim[20].

Table 1: Effect of *B. subtilis* isolate K18 on physiological characteristic of chickpea variety during *Foc* stress at 7 DAT

Sr. No.	Treatment	Shoot length (cm)	Root length (cm)	No. of roots Plant^{-1}	Fresh weight (g)	Root volume (µl)
1.	V_1SoDo	7.00	4.50	22	6.35	440
2.	V_1SiDo	8.50	8.02	30	8.42	650
3.	V_1SoDi	7.65	4.48	18	7.11	385
4.	V_1SiDi	8.00	8.60	40	8.36	635
5.	V_2SoDo	9.00	4.60	28	7.45	380
6.	V_2SiDo	9.00	6.70	32	7.22	580
7.	V_2SoDi	7.30	3.52	06	6.35	210
8.	V_2SiDi	8.50	6.20	41	7.85	415
Mean		**8.29**	**5.83**	**27**	**7.39**	**461**

V_1: Resistant var. WR-315
V_2: Susceptible var. JG-62
SoDo: Without *B. subtilis* seed treatment and normal soil transplanted
SoDi: Without *B. subtilis* seed treatment and wilt sick soil transplanted
SiDo: *B. subtilis* seed treated and normal soil transplanted
SiDi: *B. subtilis* seed treated and wilt sick soil transplanted



A **B**

Fig. 1: A. Plate of *BS*-K18 dual culture against *Foc* after 7 days incubation; . Control plate of *Foc* after 7 days incubation

WR-315

SoDo **SiDo** **SoDi** **SiDi**

JG-62

SoDo **SiDo** **SoDi** **SiDi**

Fig. 2: Influence of *BS*-K18 on root growth of chickpea verities under *Foc* infected soil at 7 DAT

SoDo: Without *B. subtilis* seed treatment and normal soil transplanted
SiDo: *B. subtilis* seed treated and normal soil transplanted
SoDi: Without *B. subtilis* seed treatment and wilt sick soil transplanted
SiDi: *B. subtilis* seed treated and wilt sick soil transplanted

CONCLUSION

Apart from antagonistic activity many biocontrol agents also posses PGPR activities, which exert plant growth promotion by direct or indirect mechanisms that could be useful for the eco-friendly plant disease management in sustainable agriculture. In present laboratory study, *Bacillus subtilis* isolate K18 (*BS*-K18) improved wilt tolerance in susceptible chickpea var. JG-62, further it improved plant root and shoot growth in resistance as well as susceptible variety. This potential antagonist with PGPR activities could be exploited for wilt management in chickpea.

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