

Original Research Article

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In vitro Efficacy of *Bacillus* spp. against *Fusarium oxysporum* f. sp. *ciceri*, the Causal Agent of Fusarium wilt of Chickpea

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ABSTRACT

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Fusarium wilt of chickpea is a widely distributed and the most destructive disease caused by *Fusarium oxysporum* f. sp. *ciceri*. The main objective of this experiment is to identify the effective *Bacillus* isolates against *Fusarium oxysporum* f.sp. *ciceri* under *in vitro* condition. A total of five *Bacillus* spp. isolates (TB1, TB2, TB3, TB4 and TB5) were isolated from rhizosphere region of tomato and eight *Bacillus subtilis* isolates (BS4, B, KP5, KK, BSD3, C1, G1, and B2) were collected from Department of plant pathology TNAU, Coimbatore. Totally 13 *Bacillus* spp. isolates were used for determining their efficacy against *Fusarium oxysporum* f. sp. *ciceri*. The *Bacillus* spp. isolates showed variations in their antagonistic activity against *F. oxysporum* f. sp. *ciceri* mycelial growth. Among them the isolate B (*B. subtilis*) showed maximum per cent inhibition of 53.00 % followed by B2 (*B. subtilis*) (50.10 %), *Bacillus subtilis* G1 (48.97%) and *Bacillus* spp.TB1 (47.77%).

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse in the world, after beans and peas (Vishwadhar and Gurha, 1998). Chickpea (*Cicer arietinum* L.) is otherwise called as Bengal gram, gram or Spanish pea. They are mainly cultivated in rabi season on marginal lands under rain fed conditions (Shiyani *et al.*, 2001). There are two types of chickpea cultivated globally, first one is desi type another one is kabuli type. Kabuli type shows a larger cream-colored seed with a thin seed coat, desi type is the one type of chickpea has a smaller, reddish brown-colored seed with a thick seed coat. The general world production of chickpea consists of about 75% of desi and 25% of kabuli types.

Totally 172 pathogens are reported in chickpea crop (*Cicer arietinum* L.) in different parts of the world (Nene *et al.*, 1996).

The biotic causal agent of chickpea include 67 plant pathogenic fungi, 3 bacteria and 22 plant viruses are reported in chickpea (Nene *et al.*, 1996). Among them, important disease of chickpea is *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Nene *et al.*, 1996). It a soil borne pathogen which causes blocking of xylem vessels and wilting (Bateman *et al.*, 1996). *Fusarium* wilt is the most destructive soil borne disease of chickpea in India (Dileep kumar, 1999). At

national level it is causing yield losses up to 60 per cent (Singh *et al.*, 2007).

Materials and Methods

Isolation of pathogen

Fusarium oxysporum f. sp. *ciceri* was isolated by tissue segment method on potato dextrose agar medium. The Infected portions from chickpea stem regions were separately collected from farmer's field. They were split vertically for observing presence of vascular discoloration. The infected tissue bits from stem region were cut and surface sterilized using 10 per cent sodium hypochlorite solution for 5 – 10 min and subsequently washed three times with sterile distilled water. Then, they were placed in Petri dishes containing potato dextrose agar (PDA) medium and incubated under laboratory conditions at $25 \pm 2^{\circ}$ C for seven days. In order to obtain pure culture, single spore isolation was performed on PDA medium and maintained as a stock culture for further studies.

Collection and isolation of bacterial antagonist

The *Bacillus* isolates (TB1, TB2, TB3, TB4 and TB5) were isolated from the rhizosphere soil of tomato. One gram of rhizosphere soil sample was transferred to sterile test tubes containing 9 ml of sterile distilled water. After they were kept under shaker for 15 minutes, then the suspension mixture was serially diluted in sterile distilled water. One ml of each 10^{-5} and 10^{-6} dilutions of bacterial antagonists was pipetted out and poured into sterile Petri plates. Later, 15ml of nutrient agar media was poured, rotated gently in clockwise and anti-clockwise direction and incubated at room temperature. The isolates of biocontrol agent *B. subtilis* like BS4, B, KPB5, KK, BSD3, C1, G1, and B2 isolates

were also obtained from the culture collection section, Department of Plant Pathology. The pure culture of *B. subtilis* isolates were maintained on NA slants at 4° C for further studies (Plate 1).

In vitro testing of *Bacillus* spp. against *F. oxysporum* f. sp. *ciceri* (Dual plate method)

Endophytic bacterial strains were tested for their antagonistic activity against mycelial growth of *F. oxysporum* f.sp. *ciceri* by following the dual culture technique (Dennis and Webster, 1971). Mycelial disc (8mm diameter) of seven days old culture of *F. oxysporum* f. sp. *ciceri* was placed at one side of the Petri plate containing PDA medium at 10 mm away from the periphery. Bacterial cultures were streaked onto the medium exactly opposite to the mycelial disc 10 mm away from the periphery. The plates were incubated under room temperature ($30 \pm 2^{\circ}$ C) for 10 days. The effect of the antagonistic organisms against *F. oxysporum* f. sp. *ciceri* was assessed based on the inhibition zone observed.

The per cent reduction over control was calculated by using the following formula

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

Where,

C - Mycelial growth of pathogen in control

T - Mycelial growth of pathogen in dual plate technique.

Results and Discussion

The results revealed that all the isolates were effective in inhibiting the mycelial growth of *F. oxysporum*. f. sp. *ciceri*. Among these

isolates, isolate B recorded the maximum per cent inhibition of 53.00 %, followed by B2 (50.10 %), *Bacillus* G1 (48.97 %) and TB1 (47.77 %). The control recorded maximum mycelial growth of 90.00 mm (Table 1; Plate 2).

In the present study, among the thirteen *Bacillus* spp. isolates tested, the isolates B and B2 were found to show more than 50 percent inhibition over control against the chickpea wilt pathogen *F. oxysporum* f. sp. *ciceri* under *in vitro* condition.

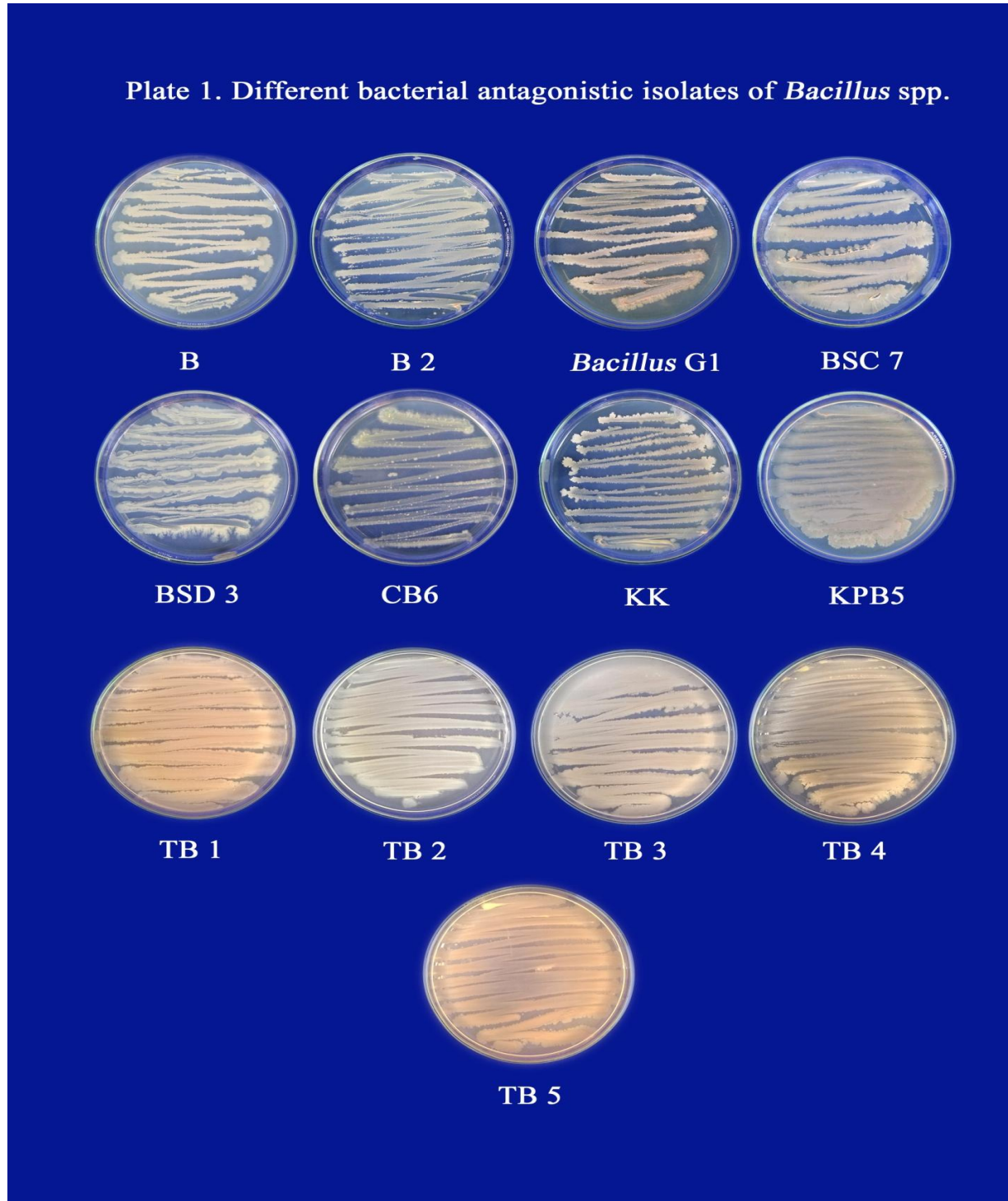


Plate 2. *In vitro* screening of *Bacillus* spp. against *F. oxysporum* f.sp. *ciceri*

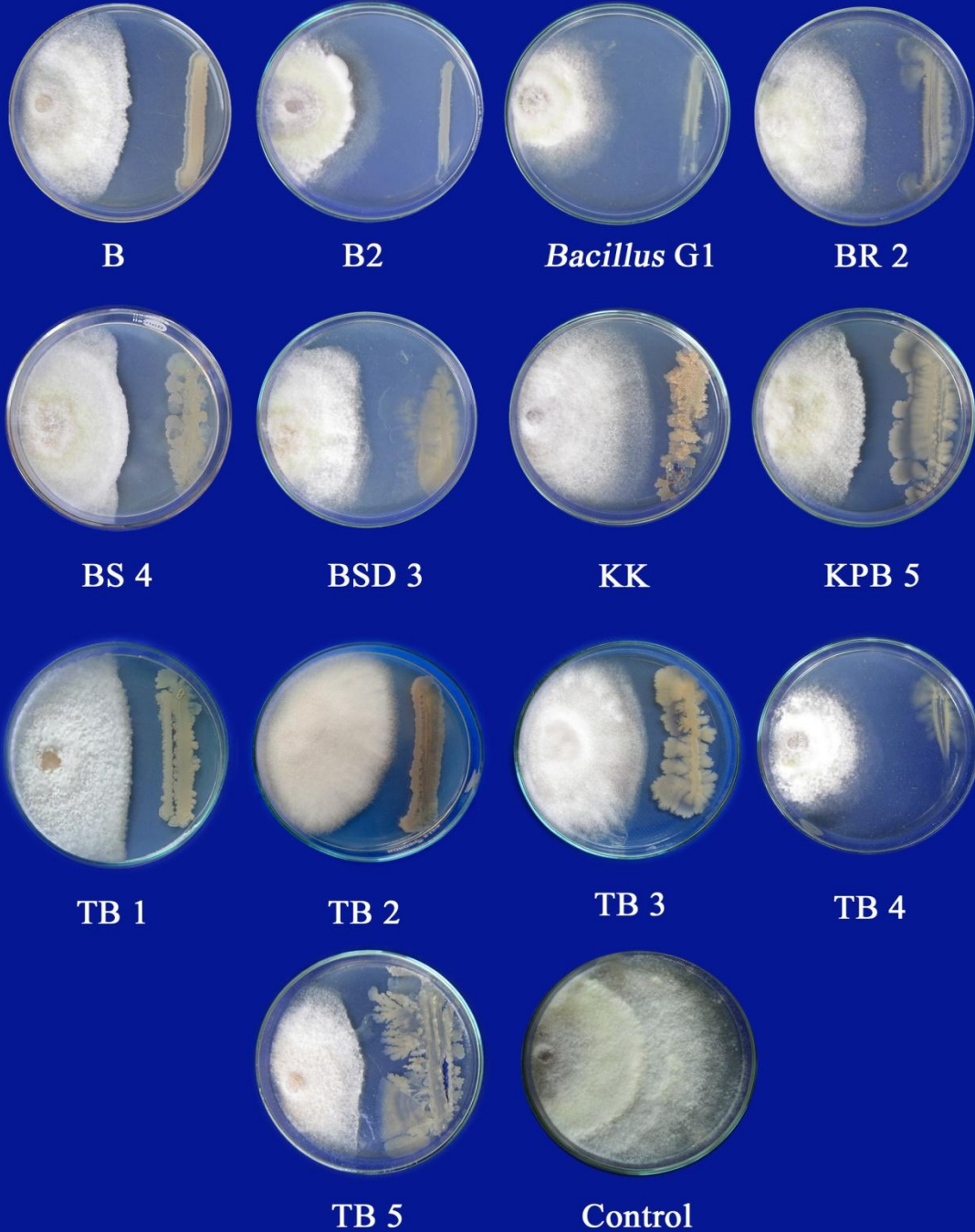


Table.1 *In vitro* screening of *Bacillus* spp. against *F. oxysporum* f.sp. *ciceri*

S. No	Isolate	Radial mycelial growth (mm)*	Percent Inhibition over control
1.	Bs4	50.37 ^{ab}	44.00 ^{bc}
2.	B	42.33 ^a	53.00 ^a
3.	KPB5	48.97 ^{ab}	45.60 ^{abc}
4.	KK	55.47 ^{abc}	38.37 ^{cd}
5.	BSD3	48.80 ^{ab}	45.80 ^{abc}
6.	C1	49.57 ^{ab}	44.90 ^{bc}
7.	<i>Bacillus</i> G1	45.90 ^a	48.97 ^{ab}
8.	B2	44.90 ^a	50.10 ^{ab}
9.	TB1	42.00 ^a	47.77 ^{ab}
10.	TB2	62.00 ^{bc}	31.10 ^{de}
11.	TB3	65.33 ^c	27.43 ^e
12.	TB4	55.33 ^{abc}	38.53 ^{cd}
13.	TB5	60.00 ^{bc}	33.33 ^{de}
14.	Control	90.00 ^d	0.00 ^f

*Mean of three replications

Means followed by a common letter are not significantly different at 5% level by DMRT.

The results are in similarity with the experimental finding of other workers. Zaim *et al.*, (2013) tested *Bacillus* spp against two *F. oxysporum* f. sp. *ciceri* isolates (*Foc* 1 and *Foc*2) by dual culture method. The isolates Rb29, Rb6, Rb12, Rb4, and Rb15 were the most effective and caused growth inhibition of *Foc*1 and *Foc*2 above 50% due to the production of volatile metabolites by the rhizobacteria. Karimi *et al.*, (2012) screened 6 isolates of *Bacillus* against *Fusarium oxysporum* f. sp *ciceri* under *in vitro*.

Among these isolates, *B. subtilis* B28 isolate showed the highest inhibition percentage (51.16%). The inhibitory action may be due to the production of secondary metabolites by the bacterial antagonists and production of antibacterial compounds *viz.*, surfactin, iturin and bacilliomycin (Lambert *et al.*, 1987; Sessitsch *et al.*, 2004). With these evidences, it is predicted that *Bacillus* isolates might have played a major role in inhibiting the growth of *F. oxysporum* f.sp *ciceri* by producing the secondary metabolites and antibacterial compounds.

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