



Biocontrol Activity of Endophytic *Bacillus subtilis* Strains against Wilt Complex in Chickpea (*Cicer arietinum* L.) under Sick Plot and Field Conditions

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ABSTRACT

Background: The wilt complex caused by several soil-borne fungal pathogens is a serious threat to chickpea production. Biocontrol with bacterial bioagents is an emerging alternative to chemical control. However, the literature on the biocontrol of wilt complex in chickpea by endophytic bacteria is scarce.

Methods: Experiments were conducted in *rabi* 2022-23 and 2023-24 under sick plot of *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri* and *rabi* 2023-24 under field conditions to evaluate the effect of endophytic *Bacillus subtilis* strains TRO4 and CLO5 on biocontrol of wilt complex in chickpea.

Result: The experimental results showed that in sick plots of *rabi* 2022-23, the seed treatment with chemical fungicide (carbendazim) has the lowest percent disease incidence (PDI) while seed treatment with *B. subtilis* TRO4 has a higher yield (kg/ha) ($p < 0.05$). In sick plots of *rabi* 2023-24, the seed treatment with *B. subtilis* TRO4 has the lowest PDI, better plant growth parameters and higher yield (kg/ha) ($p < 0.05$). In the field experiment of *rabi* 2023-24, the seed treatment with *B. subtilis* TRO4 (@10 mL/kg) resulted in the lowest PDI, better plant growth parameters and higher yield (kg/ha) ($p < 0.05$).

Key words: *Bacillus subtilis*, Bacterial endophyte, Biocontrol, Chickpea, *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, Wilt complex.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the major food legume in South Asia and globally, it is grown third-largest after common bean and field pea (Jendoubi *et al.*, 2017). India is the largest producer with 65 % of global production. It is a major pulse crop in India with a share of 46 % followed by pigeon pea, black gram, green gram, lentil, pea and others. Madhya Pradesh is the major producer of chickpea with a share of 38 % followed by Maharashtra, Rajasthan, Andhra Pradesh and Uttar Pradesh (Parmer and Gohel, 2024a). Though several diseases occur in chickpea, the wilt complex disease is caused by soil-borne fungal pathogens which comprise, wilt caused by *Fusarium oxysporum* f. sp. *ciceri*, collar rot by *Sclerotium rolfsii* and wet root rot by *Rhizoctonia solani* are considered as economically important pathogens in chickpea disease management (Rudresh *et al.*, 2005). The biocontrol ability of *Trichoderma* spp. against soil-borne fungal pathogens causing wilt complex in chickpea has been well established (Rudresh *et al.*, 2005; Khan *et al.*, 2014; Jaisani *et al.*, 2016; Pandey *et al.*, 2017; Parmer and Gohel, 2024a). The rhizosphere bacteria (*Bacillus*, *Paenibacillus* and *Pseudomonas*) showed biocontrol activity against wilt complex in chickpea (Smitha *et al.*, 2017; Suthar *et al.*, 2017; Elbouzaoui *et al.*, 2022; Mageshwaran *et al.*, 2022). Of the different strategies of biocontrol, bacterial endophytes are the potential bio-agents as they provide localized protection to host plants against the invasion of pathogenic fungi and bacteria because they compete with the pathogens in the same niches (Santos *et al.*, 2018).

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The mechanism of antagonism against plant pathogens is related to the production of siderophores, hydrogen cyanide, chitinase, antibiotics, Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (ASR) (Fatima *et al.*, 2022; Mageshwaran *et al.*, 2022). Though several reports were made on the isolation of bacterial endophytes from different crop plants, few attempts were made to evaluate bacterial endophytes on suppression of

wilt complex pathogens in chickpea under field conditions. The present study is aimed to evaluate the endophytic *B. subtilis* strains against the soil-borne pathogens causing wilt complex in chickpea.

MATERIALS AND METHODS

Microorganisms

The endophytic bacteria *B. subtilis* strains TRO4 (isolated from tomato root) and CLO5 (isolated from chickpea leaf) were obtained from Microbial Technology Laboratory, ICAR-NBAIM, Mau and their respective NAIMCC accessions numbers are B-02794 and B-02790. The bacterial cultures were grown on nutrient agar (NA) or nutrient broth (NB) (Himedia Pvt. Ltd., Mumbai, India). The cultures were grown at $28\pm 2^\circ\text{C}$ for 48 h. The culture plates were preserved at 4°C till further use.

Preparation of bacterial inoculum

The inoculum of endophytic bacteria was prepared by inoculating the culture (from agar plates) to the flask containing 100 mL of sterilized NB (1X) and incubated for 24 h under shaking conditions (125 rpm) at $28\pm 2^\circ\text{C}$. The load of the bacterial inoculum used in the experiment was 1×10^8 CFU mL⁻¹.

Effect of bacterial endophyte on biocontrol of wilt complex in chickpea under Sick plot conditions

The sick plot facility available at ICAR- National Bureau of Agriculturally Important Microorganisms, Kushmaur, Mau was utilized to conduct the experiments in chickpea (var. JG-62) during *rabi* 2022-23 and 2023-24. There were four treatments *viz.*, T₁- control (uninoculated); T₂- seed treatment with TRO4; T₃- seed treatment with CLO5; T₄- seed treatment with carbendazim @2g/kg. For seed bacterization, the seeds were suspended in respective 24 h bacterial inoculums for 30 min before sowing. The sick plots used were *Rhizoctonia solani* (RS), *Scerotium rolfsii* (SR) and *Fusarium oxysporum* f. sp. *ciceri* (FO). The set of treatments was applied to each plot size of 20 m². There were five replications in each treatment. Therefore, each plot was divided into 20 subplots of 1 m².

At 45 days after sowing, the observations such as Percent Disease Incidence (PDI), seed yield (kg/ha) were recorded for *rabi* 2022-23 and 2023-24, while germination percentage, root and shoot length (cm), fresh biomass weight (g), peroxidase (Units/g) and phenol content (µg/g) were recorded for *rabi* 2023-24. The PDI was determined according to Mageshwaran *et al.* (2022).

Effect of bacterial endophyte on biocontrol of wilt complex in chickpea under Field conditions

The experiment was conducted at a farmer field, Arepally village, Warangal which is adjacent to the Regional Agricultural Research Station (RARS), Warangal during *Rabi* 2023-24, to study the efficacy of endophytic bacteria against wilt complex disease of chickpea (var. JG-11). The field selected for the study was recurrent and endemic to

the wilt complex of chickpea. The standard package of practices of chickpea from sowing to the harvesting stage of the crop was followed uniformly in all the treatments.

The treatment details are as follows: T₁ (Un-inoculated control); T₂- (TRO4 2 mL/kg); T₃- (TRO4 5 mL/kg); T₄- (TRO4 10 mL/kg); T₅- (CLO5 2 mL/kg); T₆- (CLO5 5 mL/kg); T₇- (CLO5 10 mL/kg); T₈- (Carbendazim @ 2 g/kg). The treatments were imposed before the sowing of seeds through seed treatment of endophytic bacteria followed by air drying the seeds under the shade for about 30-40 min. The seeds which were not treated with endophytic bacteria were kept as an un-inoculated control. There were three replications per treatment. A plot size of 200 m² per replication was maintained. The observations on percent germination of the seeds, PDI, root and shoot length and yield of chickpea were recorded. The PDI was recorded for Rhizoctonia rot and Sclerotial wilt at 25 DAS while *Fusarium* wilt was observed in both flowering and pod stages.

Statistical analysis

The data collected in this study were analyzed in a randomized block design (RBD) using a one way analysis of variance (ANOVA; WASP.1; ICAR research complex, Goa). For all analyses, the differences were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Sick plot experiments

The effect of endophytic *Bacillus subtilis* strains TRO4 and CLO5 on the percent disease incidence of wilt complex and seed yield (kg/ha) in chickpea (var. JG-62) in sick plot conditions is presented in Table 1 and 2, respectively. The experimental results showed that in sick plots of *rabi* 2022-23, the seed treatment with chemical fungicide (carbendazim) has the lowest PDI and the corresponding values observed in RS, SR and FO sick plots were 0, 0.92 and 2.38, respectively (Table 1). In *rabi* 2023-24, the lowest PDI was observed in seed treatment with TRO4 and CLO5 in RS sick plot (0.02), CLO5 in SR sick plot (8.93) and TRO4 in FO sick plot (0.46). In a similar study, Rudresh *et al.* (2005) stated that *T. harzianum* PDBCTH10 and *T. virens* PDBCTV12 showed biocontrol against wilt complex pathogens *R. solani*, *S. rolfsii* and *F. oxysporum* f. sp. *ciceri* in chickpea. In *rabi* 2022-23, the seed yield (kg/ha) was higher in seed treatment with chemical fungicide (carbendazim) (2333) in RS sick plot, TRO4 in SR sick plot (3467) and FO sick plot (3867). In 2023-24, higher seed yield (kg/ha) was obtained in seed treatment with chemical fungicide (carbendazim) (2757) in the RS sick plot, CLO5 in the SR sick plot (1633) and TRO4 in the FO sick plot (3607). Thus, the results showed, that the seed treatment with chemical fungicide (carbendazim) or *B. subtilis* strains TRO4/CLO5 has the lowest PDI, better plant growth parameters and higher yield (kg/ha) compared to uninoculated control ($p < 0.05$). The seed bio-priming with endophytic bacterial bioagent(s) impart multiple benefits to the host

plant. They colonize the host plant with immediate vicinity of the invasive pathogens and compete with the pathogens for nutrients and space. Besides, they are capable of inducing systemic resistance and host defense properties through the synthesis of pathogenesis-related proteins and enzymes, secrete antimicrobial substance and produce siderophore and hydrogen cyanide (HCN), thereby suppressing the invading soil-borne pathogens (Mageshwaran *et al.*, 2022). The previous studies showed that different modes of applications of biocontrol agents viz., seed bio-priming, soil application and combined application of seed bio-priming and soil application have significantly reduced the incidence of wilt complex and increased the seed yield in chickpea. *B. subtilis* CaB5, a talc-based formulation application by seed treatment @ 10 g/kg of seed, basal application @ 2.5 kg/ha followed by soil application at 45DAS reduced the wilt complex by 60-70% (Smitha *et al.*, 2017). In a similar study, *Bacillus subtilis* isolate K18 (BS-K18) showed improved plant growth parameters (root and shoot length, germination percentage) in *F. oxysporum* f. sp. *ciceri* challenged chickpea plants (Suthar *et al.*, 2017).

The effect of bacterial endophytes on growth parameters (Germination percentage, root and shoot length (cm), fresh biomass (g), peroxidase (Units/g) and phenol content (µg/g)) of chickpea (var. JG-62) is presented in Fig 1 (A-F). The results showed the germination percentage

was higher in chickpea seeds treated with TRO4 (more than 80 %) compared to control (uninoculated) in RS, SR and FO sick plots (Fig 1A). The root and shoot length (cm) and fresh biomass (g) were higher in chickpea seeds treated with chemical (carbendazim) compared to control (Fig 1 B, C, D). The peroxidase activity (U/g) was higher in chickpea seeds treated with *B. subtilis* TRO4 in RS, SR and FO sick plots and the corresponding values were 0.37, 0.39 and 0.36, respectively (Fig 1E). Similarly, the phenol content (µg/g) was higher in chickpea seeds treated with *B. subtilis* TRO4 in RS, SR and FO sick plots and the corresponding values were 38.3, 39.3 and 43.1, respectively (Fig 1F). The consortium of four different bacterial strains (*Serratia* sp. IN-1, *Serratia* sp. IS-1, *Enterobacter* sp. IN-2, *Enterobacter* sp. IN-6) improved the root morphology traits of chickpea and suppressed the pathogen, *F. oxysporum* f. sp. *ciceri* infestation (Fatima *et al.*, 2022). Parmer and Gohel, 2024b stated that the phenol content of resistant cultivars/bio-primed susceptible cultivars was higher compared to diseased ones which illustrates the role of phenol in providing resistance against the infestation of root pathogens. The bio-primed chickpea plants have reduced accumulation of superoxide radicals, enhanced activity of plant defense enzymes viz., peroxidase, phenol oxidase, phenyl ammonia lyase and improved root architecture (Fatima *et al.*, 2022; Mageshwaran *et al.*, 2022).

Table 1: Effect of endophytic *Bacillus subtilis* strains on per cent disease incidence of wilt complex in chickpea (var. JG-62) in sick plot conditions.

Treatment details	R.S sick plot		S.R sick plot		F.O sick plot	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
T1 - Un-inoculated	18.35 ^a	3.65 ^a	15.85 ^a	87.52 ^a	15.63 ^a	3.33 ^a
T2- Seed treatment with <i>B. subtilis</i> TRO4	3.57 ^c	0.02 ^c	4.98 ^c	11.62 ^c	6.94 ^b	0.46 ^c
T3- Seed treatment with <i>B. subtilis</i> CLO5	12.04 ^c	0.02 ^c	8.71 ^b	8.93 ^c	15.22 ^a	1.93 ^b
T4- Chemical treatment (Carbendazim @ 2 g/kg of seed)	0 ^d	0.12 ^b	0.92 ^d	37.06 ^b	2.38 ^c	1.56 ^b
CV	14.43	11.51	8.56	6.84	7.53	7.77

Treatment values followed by the same alphabet do not differ significantly at p<0.05. CV: Coefficient of variation.

Table 2: Effect of endophytic *Bacillus subtilis* on strains seed yield (kg/ha) of chickpea (var. JG-62) in sick plot conditions.

Treatment details	R.S sick plot		S.R sick plot		F.O sick plot	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
T1 - Un-inoculated	1066 ^c	1700 ^c	2250 ^d	533 ^c	2100 ^c	2033 ^c
T2- Seed treatment with <i>B. subtilis</i> TRO4	2333 ^a	2500 ^{ab}	3467 ^a	1500 ^a	3867 ^a	3067 ^a
T3- Seed treatment with <i>B. subtilis</i> CLO5	2333 ^a	2307 ^b	3117 ^b	1633 ^a	2100 ^c	2400 ^{bc}
T4- Chemical treatment (Carbendazim @ 2 g/kg of seed)	2000 ^b	2757 ^a	2800 ^c	933 ^b	2500 ^b	2867 ^{ab}
CV	6.60	7.93	4.38	7.73	4.30	10.25

Treatment values followed by the same alphabet do not differ significantly at p<0.05. CV: Coefficient of variation.

Field experiment

The effect of seed bio-priming of endophytic *B. subtilis* strains TRO4 and CLO5 on percent germination, PDI, root

and shoot length and yield of chickpea (var. JG-11) were recorded (Table 3). The PDI of Rhizoctonia rot was not recorded due to non-occurrence of the disease. The

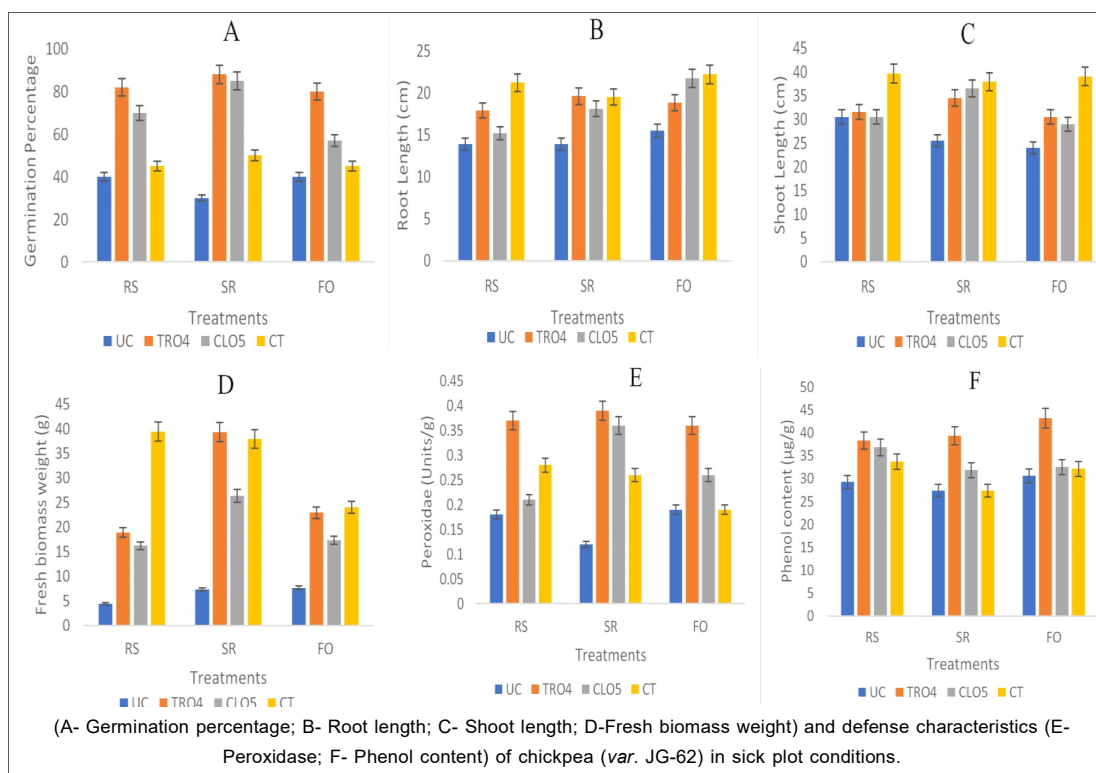


Fig 1: Effect of endophytic *Bacillus subtilis* on plant growth.

Table 3: Effect of endophytic *Bacillus subtilis* strains on suppression of wilt complex in chickpea (var. JG-11) in field conditions (rabi, 2023-24).

Treatment details	Per cent wilt disease incidence (PDI%)						Yield (kg/ha)
	Germination percentage	Sclerotial wilt	Fusarium wilt (F)	Fusarium wilt (Pod)	Root length (cm)	Shoot length (cm)	
T1- Un-inoculated Control	90.18 (73.0) ^{ab}	5.81(13.92) ^a	6.77 (15.06) ^a	12.59 (20.77) ^a	8.7	32.5	1187 ^d
T2- <i>Bacillus subtilis</i> TRO4@2mL/kg of seed	73.72 (59.23) ^c	3.65 (10.99) ^{bc}	2.81 (9.64) ^{bcd}	4.61 (12.39) ^d	9.7	40.2	1581 ^{bc}
T3- <i>Bacillus subtilis</i> TRO4@5mL/kg of seed	73.72 (59.2) ^c	4.29 (11.93) ^b	3.16 (10.23) ^{bc}	4.97 (12.87) ^d	9.5	35.8	1565 ^{bc}
T4- <i>Bacillus subtilis</i> TRO4@10mL/kg of seed	93.59 (78.86) ^a	1.52 (7.06) ^e	2.45 (8.99) ^d	3.15 (10.22) ^e	10.0	40.3	1982 ^a
T5- <i>Bacillus subtilis</i> CLO5@2mL/kg of seed	82.69 (65.63) ^{bc}	2.62 (9.3) ^d	2.59 (9.25) ^{cd}	6.92 (15.24) ^c	10.8	36.3	1500 ^{bc}
T6- <i>Bacillus subtilis</i> CLO5@5mL/kg of seed	71.95 (58.14) ^c	3.77 (11.6) ^{bc}	3.51 (10.98) ^b	5.22 (13.20) ^d	8.8	35.7	1346 ^{cd}
T7- <i>Bacillus subtilis</i> CLO5@10mL/kg of seed	76.44 (61.05) ^d	4.14 (11.72) ^{bc}	2.88 (9.76) ^{bcd}	9.92 (18.35) ^b	11.1	41.3	1696 ^b
T8- Carbendazim @2g/kg of seed	91.00 (73.01) ^{ab}	3.47 (10.72) ^c	3.09 (10.11) ^{bcd}	9.51 (17.95) ^b	10.1	37.4	1429 ^{cd}
CV	7.8	5.87	6.67	5.11	NS	NS	9.78

Treatment values followed by the same alphabet do not differ significantly at $p < 0.05$. The values in the parentheses are arc sign transformed. CV: Coefficient of variation. NS: Non-significant.

germination percentage was recorded higher (93.59) in TRO4 @ 10 mL/kg of seed and lower (71.95) in CLO5 @ 5 mL/kg of seed. The PDI of sclerotial wilt at 25 DAS was recorded higher (5.81) in un-inoculated control and lower (1.52) in TRO4 @ 10 mL/kg of seed. The PDI of *Fusarium* wilt (flowering) was observed lower (2.45) in TRO4 @ 10 mL/kg of seed and higher in un-inoculated control (6.77). Similarly, the PDI of *Fusarium* wilt (pod) was found higher in the un-inoculated control (12.59) and lower in TRO4 @ 10 mL/kg of seed (3.15). The seed yield (kg/ha) was higher (1982) in TRO4 @ 10 mL/kg among the treatments and the lowest yield was observed in the un-inoculated control (1187). The inoculation of endophytic bacterial bioagent(s) through seed bio-priming offers benefits to the host plant directly and indirectly through multiple plant growth-promoting traits. The direct benefits include fixation of atmospheric nitrogen, solubilization of nutrients, secretion of phytohormone and antimicrobial metabolites, synthesis of ammonia, siderophore, HCN, etc. which are helpful in plant growth and yield and also provide protection against invading pathogens. The indirect benefits to the host plant include induction of systemic resistance through the synthesis of PR proteins and phenols, peroxidase etc., thus offering multiple resistance mechanism against the biotic stress (Fatima *et al.*, 2022; Mageshwaran *et al.*, 2022). In a similar study, the soil application of *T. harzianum*, *T. hamatum* and *T. asperellum* reduced the wilt incidence by 25-56% and increased the yield by 12-28% (Khan *et al.*, 2014). Seed bio-priming for 10 h with suspension of talc-based formulation of *T. asperellum* (2×10^8 CFU/g) followed by soil application of *T. asperellum* enriched FYM (10 g/kg of FYM) @ 100 m² resulted in 51 -70% of disease reduction and 41-51 % increase in grain yield (Parmar and Gohel, 2024). The combined application of seed bio-priming and soil application of *T. asperellum* or *T. harzianum* reduced 46-78 % incidence of wilt complex and increased the grain yield by 13-27 % (Pandey *et al.*, 2017). In a similar study seed bio-priming along with soil application of *T. asperellum* (@100 g/m²) resulted in better seedling emergence (97%) and the highest vigor index (3496) (Jaisani *et al.*, 2016). Though several reports have been made on the biocontrol of wilt complex in chickpea by *Trichoderma* spp., the present study highlights the potential of the endophytic bacterium, *B. subtilis* TRO4 in wilt complex management. In a similar study, the commercial bio-formulation containing bacterial bioagent, *B. subtilis* (Biocure-X) has been reported for effective management of wilt complex in chickpea (Mohiddin and Khan, 2019).

CONCLUSION

In the present study, the effect of endophytic *B. subtilis* strains TRO4 and CLO5 on biocontrol of wilt complex in chickpea was evaluated under sick plot and field conditions. The results showed that under sick plot conditions, the

PDI of wet root rot, collar rot and wilt was found lower in seeds treated with a chemical (carbendazim) followed by *B. subtilis* strains TRO4 and CLO5 in comparison to un-inoculated control in RS, SR and FO sick plots. While, the seed yield, peroxidase activity and phenol content were higher in seeds treated with TRO4. In field conditions, seed treatment with *Bacillus subtilis* TRO4 @ 10 mL/kg of seed performed better against the wilt disease complex of chickpea and also showed higher germination percentage and seed yield 1982 kg/ha. Thus, the study concludes that the seed bio-priming with endophytic bacterium, *B. subtilis* strain TRO4 @ 10 mL/kg of seed is found to be the best for sustainable management of wilt complex in chickpea.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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