Microbial Pathogenesis 157 (2021) 104961

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Streptomyces consortia-mediated plant defense against *Fusarium* wilt and plant growth-promotion in chickpea

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ARTICLE INFO

Keywords:
Streptomyces
Chickpea
Biocontrol
Consortium
Fusarium wilt
Plant growth promotion

ABSTRACT

Three strains of *Streptomyces griseus* (CAI-24, CAI-121 and CAI-127) and one strain each of *Streptomyces africanus* (KAI-32) and *Streptomyces coelicolor* (KAI-90) were reported by us as biocontrol agents against *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), and as plant growth-promoters (PGP) in chickpea. In the present study, the combined effect of these *Streptomyces* strains as a consortium were assessed for their biocontrol potential against *Fusarium* wilt and PGP in chickpea. Based on their compatibility, biocontrol ability and PGP performance, two consortia were assembled, consortium-1 having all the five strains of *Streptomyces* sp. and consortium-2 having the two promising strains (CAI-127 and KAI-32). Under greenhouse conditions, consortium-1 and consortium-2 were found to reduce the *Fusarium* wilt disease incidence by 55% and 74%, while under field conditions, these were by 86% and 96% in year-1 and by 54% and 69% in year-2, respectively, when compared to the positive control (only FOC treated). Shoot samples treated with consortia + FOC contained significantly enhanced antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase and phenylalanine ammonia-lyase, when compared to the positive control (only FOC treated) or the negative control samples (neither FOC nor consortia treated). When the consortia were evaluated for their PGP traits under field conditions in two chickpea cultivars, JG11 and ICCV2, and in two consecutive years, nodule number was found to enhance up to 25%, nodule weight up to 49%, leaf area up to 37%, leaf weight up to 43%, root weight up to 23%, shoot weight up to 35%, seed weight up to 30%, seed number up to 29%, total dry matter up to 22% and grain yield up to 22% over the un-inoculated control plants. This study had demonstrated that the selected consortium of *Streptomyces* spp. has a greater potential for biological control of *Fusarium* wilt disease and PGP in chickpea.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume grown in more than 50 countries in rain-fed areas across the world. In 2018, India alone had produced about 66% of the global chickpea production [1]. Major constraints to chickpea production are biotic stresses such as pod borers, aphids, leaf miner, dry root rot caused by *Rhizoctonia bataticola*, collar rot caused by *Sclerotium rolfsii*, *Ascochyta* blight caused by *Ascochyta rabiei*, *Botrytis* grey mold caused by *Botrytis cinerea* and *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC). Among these, FOC causes major yield losses of up to 100% under favorable conditions for disease build up [2,3]. FOC is primarily a soil-born pathogen but, it can also be transmitted through seeds. This diverse mode of FOC makes it a more devastating pathogen as it transmits the disease to successive generations too. *Fusarium* wilt of chickpea is usually managed by advancing sowing date, solarization of the soil and treating seeds with fungicides, but with limited success [4]. The use of resistant cultivar is the most efficient and economical control measure but the availability of *Fusarium* wilt resistance is overwhelmed by the presence of 8 pathogenic races of FOC [5]. Therefore, efforts need to be taken to develop environment-friendly methods such as biological control for the management of *Fusarium* wilt in chickpea.

Biological control of *Fusarium* wilt in chickpea has been reported using bacterial and fungal antagonists such as *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., non-pathogenic isolates of *Fusarium oxysporum* and *Streptomyces* spp. [2,6–8]. These antagonists were reported to be effective not only to manage *Fusarium* wilt but also to promote plant growth and yield in chickpea. Recent reports suggest that there is an increasing trend to use bio-products based on microbial consortia rather than single microorganisms with the aim to exploit their complementary

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https://doi.org/10.1016/j.micpath.2021.104961
Received 9 February 2021; Received in revised form 30 April 2021; Accepted 7 May 2021
Available online 24 May 2021

interactions. Typically, a microbial consortium is composed of compatible beneficial microbial strains with different modes of action to provide a broad spectrum of usage. Microbial consortia have been reported to be superior when compared to single strains [9–11].

Earlier, we reported five strains of *Streptomyces* spp. to have antagonistic potential against *Fusarium* wilt in chickpea [12] and PGP potential in rice, sorghum and chickpea under field conditions [13,14]. These strains namely, *S. tsusimaensis* CAI-24, *S. caviscabies* CAI-121, *S. setonii* CAI-127, *S. africanus* KAI-32 and *Streptomyces* sp. KAI-90, were identified based on the taxonomic assignment of 16S rRNA sequences against the 16s rRNA database available then. However, when these strains (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) were re-sequenced by whole genome sequencing in the year 2020, their taxonomy was re-assigned to *S. griseus*, *S. griseus*, *S. griseus*, *S. africanus* and *S. coelicolor*, respectively [15]. Among the three strains of *S. griseus*, CAI-127 was found to have more antagonistic potential against FOC and produce more indole acetic acid, siderophore and hydrocyanic acid than CAI-24 and CAI-121 [12]. The main objective of this study was to further evaluate the combined effect of the five strains of *Streptomyces*, as a consortium, for their antagonistic potential against *Fusarium* wilt and PGP potential in chickpea.

2. Materials and methods

2.1. Streptomyces and other biocontrol strains used in this study

Five strains of *Streptomyces*, namely *S. griseus* CAI-24, *S. griseus* CAI-121, *S. griseus* CAI-127, *S. africanus* KAI-32 and *S. coelicolor* KAI-90, previously reported to have biocontrol potential against *Fusarium* wilt and PGP potential in chickpea [12,14,15] were selected for the present study. Commercially available biocontrol agents such as *Trichoderma harzianum* and *Pseudomonas fluorescens* were acquired from TSTANES Pvt. Ltd., Coimbatore, Tamil Nadu, India.

2.2. Compatibility of the five Streptomyces strains

This study was carried out with two different combinations of the selected five *Streptomyces* strains. These were consortium-1 containing all the five *Streptomyces* strains; and consortium-2 containing the two best antagonistic strains against *Fusarium* wilt (CAI-127 and KAI-32; based on Gopalakrishnan et al., [12]). Compatibility studies were carried out *in vitro* by qualitative (cross streak plate) and quantitative methods (plate count) on actinomycetes isolation agar (AIA) and starch casein broth (SCB), respectively, as described by Oljira et al. [16] and Lopes et al. [17] with minor modifications. Briefly, in the qualitative method, the selected *Streptomyces* strains were streaked horizontally and vertically on AIA Petri plates and incubated for 7 days at 28 °C. The Petri plates were observed for any zone of inhibition at the end of 7 days. For the quantitative method, the selected strains were inoculated individually in SCB and incubated at 28 °C for 5 days. At the end of incubation, 0.5 ml from all the selected strains were inoculated into a single fresh SCB and further incubated in an orbital shaker at 28 °C for 5 days. The contents of the flask were serially diluted up to 10^{-5} dilution and 0.1 ml was spread plated on AIA Petri plates. After 5 days of incubation, the Petri plates were observed for any zone of inhibition.

2.3. Antagonistic activity of the Streptomyces consortia

2.3.1. Under greenhouse conditions

Two *Streptomyces* consortia, consortium-1 comprising all the five strains i.e. CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 and consortium-2 comprising the only two best strains i.e. CAI-127 and KAI-32, were evaluated for their antagonistic potential against *Fusarium* wilt in chickpea under greenhouse conditions in pots. A total of six treatments were tested, namely consortium-1, consortium-2, *T. harzianum* (commercial biocontrol agent 1) and *P. fluorescens* (commercial biocontrol agent 2), positive control (treated with only FOC) and negative control (treated with only water) with two doses (5% and 10%) of FOC inoculum in the pots. The experiment had 6 replications. FOC inoculum was mass-multiplied as per the protocols of Pande et al. [18] on sand maize media (maize granules 10 g, sand 90 g and sterilized water 20 ml). Pot mixture (800 g) was prepared by mixing Vertisols, sand and farm-yard manure at 3:2:1 (w/w) and was filled in 5-inch plastic pots followed by inoculation with FOC inoculum (at 5% and 10% of pot weight i.e., 40 g pot^{-1} and 80 g pot^{-1}, respectively). Consortium-1 and consortium-2 (grown separately in SCB for 5 days) were inoculated additionally (10 ml pot^{-1}; 10^8 CFU ml^{-1}) along with the FOC in their respective treatments. Biocontrol agents *T. harzianum* and *P. fluorescens* were also inoculated (10 g pot^{-1}; 10^6 CFU g^{-1}) along with the FOC in their respective treatments. The treatmental contents of the pot were mixed thoroughly with the potting mixture and the pots were covered with polythene sheets. The whole set-up was incubated at 28 ± 2 °C for 15 days to develop *Fusarium* wilt sick conditions. Two weeks later, the seeds of chickpea cultivars JG62 (susceptible to *Fusarium* wilt; acquired from Legumes Pathology, ICRISAT) were surface sterilized with 2.5% sodium hypochlorite solution for 5 min and rinsed with sterilized water (8 times). The surface-sterilized seeds were sown (six seeds in each pot) and one week later the seedlings were thinned to retain three seedlings per pot. Plants were irrigated once in every two days with 30 ml of sterilized distilled water. Incidence of *Fusarium* wilt disease (number of plants with wilt symptoms to the total number of plants in a pot) was recorded at a regular interval until 20 days after sowing (DAS).

2.3.2. Under field conditions

The two *Streptomyces* consortia, consortium-1 and consortium-2, commercial biocontrol agents *T. harzianum* and *P. fluorescens* were evaluated for their antagonistic potential against FOC under field conditions. Similar to the greenhouse experiment, a total of six treatments were tested in the field experiment including consortium-1, consortium-2, *T. harzianum*, *P. fluorescens*, positive control (treated with only FOC) and negative control (treated with only water). The field experiment was conducted in cement tanks (75 cm wide x 75 cm deep) filled with Vertisols and buried in the Vertisols field, with three replications. The experiment was performed in two consecutive years during the 2019−20 and 2020–21. FOC and the test organisms were mass-multiplied as explained earlier and incorporated at 5%, as per the treatments, in the top 15 cm soil layer of the cement tanks and covered with lids for 15 days. At the end of incubation, lids were opened and seeds of the chickpea cultivars, JG62 and JG11, susceptible and resistant cultivars of *Fusarium* wilt, were sown in each cement tank. A total of 30 seeds for each cultivar were sown in each cement tanks. The incidence of *Fusarium* wilt disease was recorded at a regular interval until 48 DAS.

At 30 DAS, the shoot samples of all the six treatments of both JG62 and JG11 cultivars were collected in liquid nitrogen and stored at −80 °C until analysed for antioxidant properties such as superoxide dismutase (SOD; [19]), catalase (CAT; [20]), ascorbate peroxidase (APX; [21]), guaiacol peroxidase (GPX; [22]), glutathione reductase (GR; [23]) and phenylalanine ammonia lyase (PAL; [24]).

2.4. Plant growth-promotion traits of the consortia

To assess the PGP traits of the *Streptomyces* consortia, consortium-1 and consortium-2 were evaluated on two popular cultivars of chickpea, JG11 and ICCV2 (acquired from chickpea breeding, ICRISAT), under field conditions. The three treatments tested include consortium-1, consortium-2 and un-inoculated control. The experiments were carried out during the 2018−19 and 2019−20 post-rainy cropping seasons at ICRISAT, Patancheru (17°30' N; 78°16' E; altitude 549 m). Soils at the experimental site were classified as Vertisols (having 26% sand, 52% clay and 21% silt) with an organic carbon content of 0.4–0.6% and alkaline pH of 7.7–8.5 [14]. The top 15 cm of rhizosphere soil was found

to contain 24.7, 8.6 and 298 mg kg^{-1} soil of available NPK. The research fields were kept fallow in the rainy season with these experimental trials following in the post-rainy season. The fields were prepared into broad bed and furrows, with beds 1.2 m wide flanked by 0.3 m furrows. Di-ammonium phosphate (DAP; 18 kg N ha^{-1} and 20 kg P ha^{-1}) was applied on the surface and incorporated in both the seasons before sowing. The size of the plots, for both the seasons, were 4 m × 3 ridges (rows). The experiment was laid out in a randomized complete block design (RCBD) with three replicates.

The five strains of *Streptomyces* were cultured individually on SCB at 28 °C for 5 days and later mixed as consortium-1 and consortium-2 just before planting. The seeds were treated with consortium-1 and consortium-2 (containing 10^8 CFU ml^{-1}) for 45–50 min and sown immediately by hand on October 11, 2018 in the first year and October 12, 2019 in the second year, in rows 30 cm apart at a depth of 4–5 cm to achieve an estimated plant density of at least 26 plants m^{-2}. Plants were inoculated with respective consortia (1000 ml; 10^8 CFU ml^{-1}) once in every 15 days on the soil close to the plant until the flowering stage. The plants that were not inoculated with consortia served as control. No pesticide was sprayed during the cropping period, as no serious insect pests or phytopathogens attacks were observed. The crop was harvested manually at maturity. At 30 DAS in both seasons, nodule number, nodule weight, leaf area, leaf weight, root weight and shoot weight were recorded. At crop maturity, pod dry weight, pod number, seed weight, seed number, total dry matter and grain yield were also recorded.

3. Results

3.1. Compatibility of the five Streptomyces strains

In the quantitative method, both the *Streptomyces* consortia, consortium-1, and consortium-2 were found to be compatible as no inhibition or lysis was noticed at the junctions of the strains, even after 7 days of incubation (Fig. 1). In the qualitative method, compatibility was clearly observed only in consortium-2, owing to the morphological difference of the two strains, one being milky white (CAI-127) while another being grey white (KAI-32) (Fig. 1). In consortium-1, even though there was no inhibition or lysis observed, it was not possible to differentiate the strains owing to their morphological similarity.

3.2. Antagonistic activity of Streptomyces consortia against Fusarium wilt in chickpea

3.2.1. Under greenhouse conditions

When the two *Streptomyces* consortia, consortium-1 and consortium-2, were evaluated for their antagonistic potential against *Fusarium* wilt, 55% and 74% reduction of disease incidence, respectively, were observed at 5% FOC concentration and 13% and 42% reduction of disease incidence, respectively, at 10% FOC concentration, when compared to the FOC positive control (Fig. 2). The two commercial biocontrol agents, *T. harzianum* and *P. fluorescens* were able to reduce the disease incidence by 35% and 29%, respectively at 5% FOC concentration, while 10% and 10%, respectively at 10% FOC concentration when compared to the FOC positive control. At 10% FOC concentration, only consortium-2 was found to reduce the disease incidence to satisfactory level (up to 42%) whereas consortium-1, *T. harzianum* and *P. fluorescens* were not able to reduce the disease incidence (Table 1).

3.2.2. Under field conditions

In the field, when the *Fusarium* wilt susceptible cultivar JG62 was used, consortium-1, consortium-2, *T. harzianum* and *P. fluorescens* were found to reduce the *Fusarium* wilt disease incidence by 86%, 96%, 78% and 53%, respectively in year-1 and 54%, 69%, 36% and 17%, respectively in year-2, when compared to the FOC positive control (Table 1; Fig. 3). *Fusarium* wilt disease symptoms were not found in the resistant cultivar JG11.

At 30 DAS, the shoot samples of JG62 treated with FOC + consortium-1 and FOC + consortium-2 exhibited enhanced antioxidant properties of all the parameters that were tested including SOD (up to 3% and 38%), CAT (up to 36% and 59%), APX (up to 12% and 7%), GPX (up to 14% and 63%), GR (up to 30% and 55%) and PAL (up to 68% and 86%), respectively over the shoot samples treated with only FOC control. The shoot samples of JG11 treated with FOC + consortium-1 and FOC + consortium-2 also exhibited enhanced antioxidant properties of

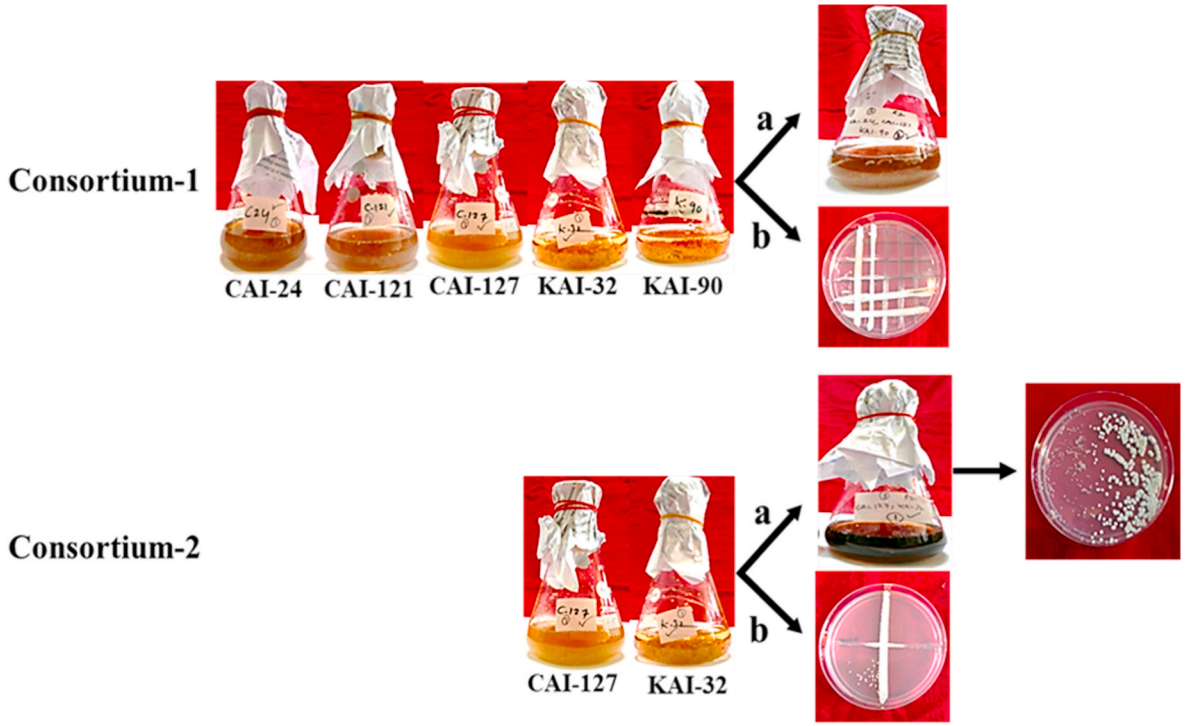

Fig. 1. Compatibility of the five *Streptomyces* strains in different consortia combinations. (a) By quantitative method (b) By qualitative method.

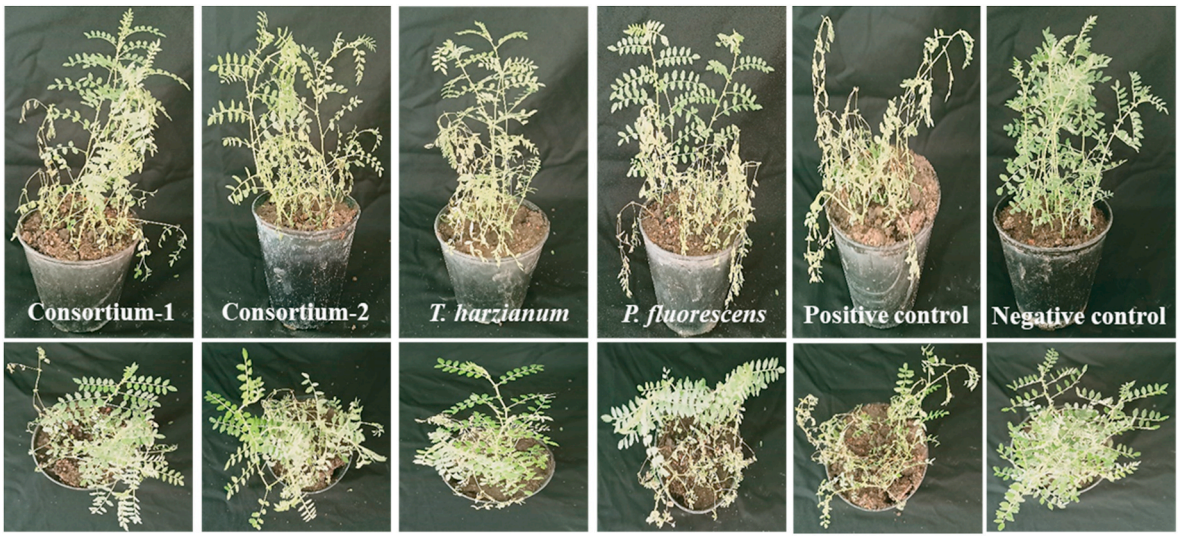

Fig. 2. Evaluation of *Streptomyces* consortium-1 and consortium-2 for their antagonistic potential against *Fusarium* wilt (5% FOC) in chickpea under greenhouse conditions, in comparison with commercially available biocontrol agents (*T. harzianum* and *P. fluorescens*) and controls at 20 days after sowing.

Table 1
Evaluation of *Streptomyces* consortium-1 and consortium-2 for their antagonistic potential against *Fusarium* wilt in chickpea under both greenhouse and field conditions.

Treatments	% disease incidence in the greenhouse trial (at 15 DAS)		% disease incidence in the field trials (at 48 DAS; at 5% FOC)	
	At 5% FOC	At 10% FOC	Year-1 (2019–20)	Year-2 (2020–21)
Consortium-1	40	87	11	40
Consortium-2	23	58	3	27
T. harzianum	58	90	18	56
P. fluorescens	63	90	38	73
Pos. control	89	100	81	88
Neg. control	0	0	1	22
Mean	46	71	25	49
SE±	6.3***	14.8**	4.8***	3.7***
LSD (5%)	19.8	46.5	15.0	11.7
CV%	24	36	33	13

DAS = Days after sowing; Pos. control = positive control; Neg. control = Negative control; FOC = *Fusarium oxysporum* f. sp. *ciceri*; SE = standard error; LSD = least significant differences; CV = coefficient of variation; ** = statistically significant at 0.01; *** = statistically significant at 0.001.

all the parameters that were tested including SOD (up to 75% and 71%), CAT (up to 21% and 51%), APX (up to 43% and 67%), GPX (up to 33% and 60%), GR (up to 76% and 94%) and PAL (up to 89% and 93%), respectively, over the shoot samples treated with only FOC control. The antioxidants were produced more in the *Fusarium* wilt resistant cultivar over the susceptible cultivar. In the case of commercial biocontrol agents, *T. harzianum* and *P. fluorescens* treatments, the results were inconclusive. (Table 2).

3.3. Plant growth-promoting traits of consortia under field conditions

Both consortium-1 and consortium-2 were found to positively enhance various PGP traits on both the tested cultivars of chickpea, JG11 and ICCV2, and on both the years. At 30 DAS, on JG11, the consortium-1 and consortium-2 were found to significantly enhance the nodule number by 5—19% and 7—22%, nodule weight by 6—18% and 33—49%, leaf area by 23—32% and 23—25%, leaf weight by 10—23% and 10—18%, root weight by 5—6% and 4—23%, and shoot weight by 13—20% and 5—20%, respectively over the un-inoculated control plants. Similar results were also found on ICCV2 as well; where the consortium-1 and consortium-2 were found to significantly enhance the nodule number by 8—20% and 4—25%, nodule weight by 4—11% and 2—17%, leaf area by 18—37% and 17—28%, leaf weight by 11—43% and 20—34%, root weight by 3—4% and 3—17%, and shoot weight by 4—35% and 20—22%, respectively, over the un-inoculated control plants (Table 3).

At crop maturity, on JG11, the consortium-1 and consortium-2 were found to enhance the pod weight by 4—12% and 2—4%, pod number by 2—5% and 4—12%, seed weight by 11—12% and 4—6%, seed number by 1—13% and 10—11%, total dry matter by 9—12% and 11—14% and grain yield by 8—15% and 12—15%, respectively over the un-inoculated control plants. Similar results were also observed on ICCV2, where the consortium-1 and consortium-2 were found to enhance the pod weight by 8—32% and 2—38%, pod number by 11—31% and 8—23%, seed weight by 11—30% and 8—25%, seed number by 9—29% and 4—24%, total dry matter by 1—22% and 3—7% and grain yield by 2—22% and 3—12%, respectively, over the un-inoculated control plants (Table 4).

4. Discussion

Microbial consortia are gaining importance over individual strains in managing phytopathogens and improving crop yield. Earlier, we reported that a few individual strains of *Streptomyces* (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90), to work against *Fusarium* wilt as biocontrol agents and also to exhibit PGP potentials in chickpea under field conditions [12,14]. In the present study, we attempted to evaluate the combinations of these selected five strains of *Streptomyces* in managing *Fusarium* wilt and PGP potentials in chickpea. This includes consortium-1 containing all the five *Streptomyces* strains; and consortium-2 containing the two best antagonistic strains against *Fusarium* wilt (CAI-127 and KAI-32; following Gopalakrishnan et al., [12]). In earlier study, CAI-127 and KAI-32 were reported to produce superior PGP traits such as indole acetic acid, siderophore and hydrocyanic acid [12]. In the present study, both the tested combinations showed good compatibility but a clear compatibility was noticeable only in consortium-2 (CAI-127 and KAI-32) due to their apparent morphological differences.

The *Streptomyces* consortia were further evaluated for their biocontrol potential against *Fusarium* wilt in the greenhouse and field conditions. In the greenhouse, at 5% FOC concentration, a maximum reduction of disease incidence was observed in consortium-2 (74%) than

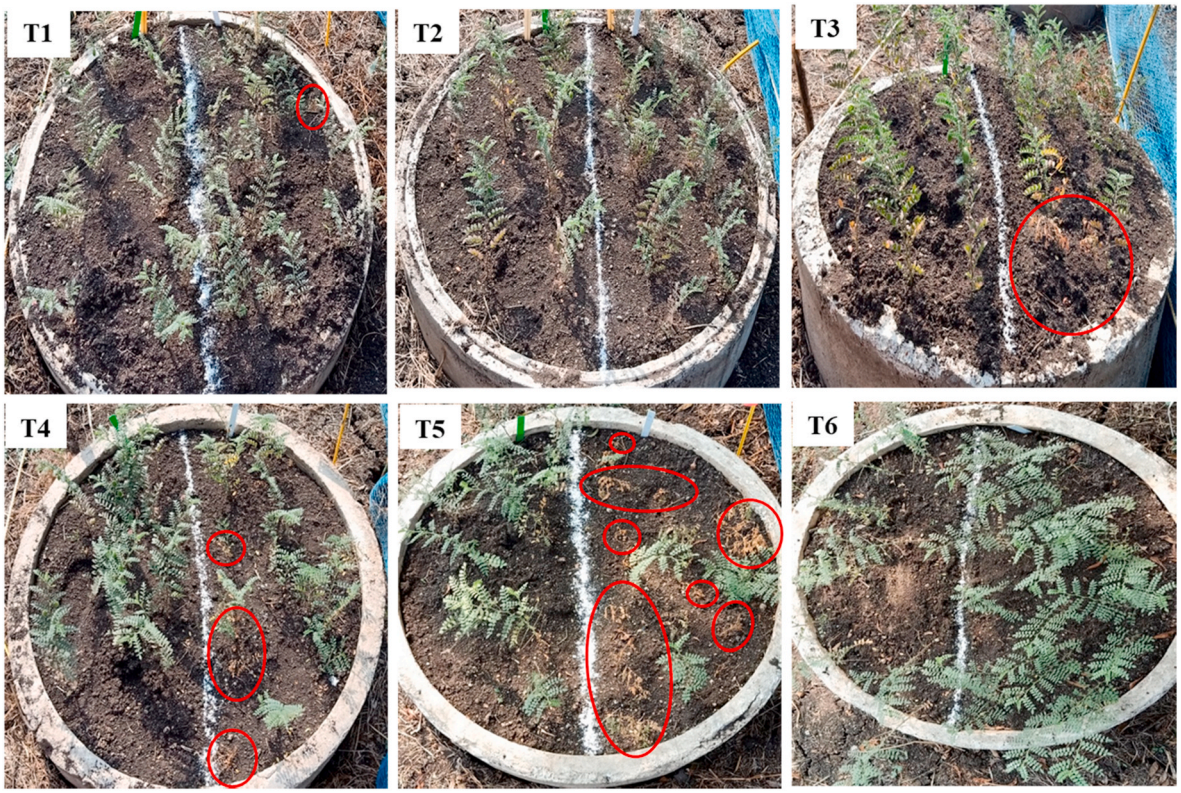

Fig. 3. Evaluation of *Streptomyces* consortium-1 and consortium-2 for their antagonistic potential against *Fusarium* wilt in chickpea under field conditions, in comparison with commercially available biocontrol agents (*T. harzianum* and *P. fluorescens*) and positive and negative controls. Left side of the ring shows JG11 (resistant) and right side JG62 (sensitive) cultivars of chickpea. T1 = Consortium-1; T2 = Consortium-2; T3 = *T. harzianum*; T4 = *P. fluorescens*; T5 = Positive control (FOC treated); T6 = Negative control.

Table 2
Antioxidant parameters of chickpea genotypes (JG62 and JG11) against *Fusarium* wilt by selected biocontrol agents.

Treatments	SOD	CAT	APX	GPX	GR	PAL
	JG62					
Consortium-1	246	224	1.78	0.07	0.68	0.004
Consortium-2	386	352	1.68	0.16	1.06	0.009
T. harzianum	144	96	1.38	0.12	1.86	0.002
P. fluorescens	101	134	2.73	0.06	1.51	0.003
Pos. control	238	144	1.57	0.06	0.48	0.001
Neg. control	244	36	0.17	0.02	0.50	0.001
Mean	227	164	1.55	0.08	1.01	0.003
SE±	15.7***	21.0***	0.050***	0.013***	0.233**	0.0007***
LSD (5%)	49.3	66.0	0.156	0.040	0.736	0.0023
CV%	12	22	6	27	40	37
	JG11					
Consortium-1	632	68	0.42	0.06	0.95	0.002
Consortium-2	531	111	0.73	0.10	3.49	0.004
T. harzianum	287	22	0.31	0.06	0.35	0.002
P. fluorescens	218	48	0.10	0.06	0.05	0.001
Pos. control	155	54	0.24	0.04	0.23	0.000
Neg. Control	343	0	0.07	0.04	0.23	0.000
Mean	361	51	0.31	0.06	0.88	0.001
SE±	30.6***	12.2***	0.058***	0.009**	0.221***	0.0008*
LSD (5%)	96.5	38.5	0.184	0.028	0.696	0.0024
CV%	15	42	32	26	43	93

Note: Pos. control = positive control; Neg. control = Negative control; SE = standard error; LSD = least significant differences; CV = coefficient of variation; The units for the antioxidant parameters are as follows: SOD = superoxide dismutase, mol units mg^{-1}; CAT = catalase, $unit^{-1}$ min^{-1} g fresh weight; APX = ascorbate peroxidase, $unit^{-1}$ min^{-1} g fresh weight; GPX = guaiacol peroxidase, $unit^{-1}$ min^{-1} g fresh weight; GR = glutathione reductase, $unit^{-1}$ min^{-1} g fresh weight; PAL = phenylalanine ammonia lyase, units ml^{-1} enzyme.· * = Statistically significant at 0.05; ** = Statistically significant at 0.01; *** = Statistically significant at 0.001.

consortium-1 (55%). This was true even at 10% FOC concentration, where consortium-2 showed maximum reduction (42%) followed by consortium-1 (13%) over FOC positive control. The higher biocontrol ability of consortium-2 might be due to a greater survival in the field conditions, colonization in the rhizosphere of chickpea and control of the pathogen. The efficacy of consortium-2 was decreased with increasing FOC concentration to 10%. Similarly, *T. harzianum* and *P. fluorescens* biocontrol efficacy was also reduced with the increasing

Table 3
Plant growth-promoting ability of consortia on JG11 and ICCV2 cultivars of chickpea under field conditions- at 30 DAS.

Treatment	Year 1 (2018/19)						Year 2 (2019/20)					
	Nodules number (plant^{-1})	Nodule weight (g plant^{-1})	Leaf area (cm^{-2} plant^{-1})	Leaf weight (g plant^{-1})	Root weight (g plant^{-1})	Shoot weight (g plant^{-1})	Nodules number (plant^{-1})	Nodules weight (g plant^{-1})	Leaf area (cm^{-2} plant^{-1})	Leaf weight (g plant^{-1})	Root weight (g plant^{-1})	Shoot weight (g plant^{-1})
JG11												
Cons-1	26**	30	301*	1.36***	0.22	2.28	44*	132	276**	1.78**	0.24*	1.49**
Cons-2	27**	49**	301*	1.51***	0.27**	2.49***	45*	185***	249**	1.51**	0.24*	1.26
Control	21	25	232	1.23	0.21	1.99	42	124	187	1.37	0.23	1.20
Mean	25	35	278	1.37	0.23	2.26	44	147	237	1.55	0.24	1.32
LSD (5%)	3.10	10.3	59.6	0.058	0.027	0.353	1.8	9.4	47.2	0.150	0.009	0.115
CV (%)	6	13	10	2	5	7	2	3	9	4	2	4
ICCV2												
Cons-1	26*	45**	507**	2.58	0.33	4.08	40*	143**	315**	2.21**	0.24**	2.36***
Cons-2	28*	48**	576**	2.87*	0.39**	4.93***	38	140**	240	1.92**	0.24**	1.96***
Control	21	40	415	2.31	0.32	3.93	37	137	199	1.27	0.23	1.53
Mean	25	44	499	2.59	0.35	4.31	39	140	251	1.80	0.24	1.95
LSD (5%)	4.1	2.6	59.9	0.376	0.034	0.193	2.5	2.6	45.5	0.433	0.004	0.135
CV (%)	7	3	5	6	2	2	3	1	8	11	1	3

* = Statistically significant at 0.05, ** = Statistically significant at 0.01, *** = Statistically significant at 0.001; CV = Coefficients of variation; LSD = Least significant difference; Cons-1 = Consortium-1; includes CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 strains; Cons-2 = Consortium-2; includes CAI-127 and KAI-32 strains.

Table 4
Yield parameters of consortia on JG11 and ICCV2 cultivars of chickpea under field conditions -at crop maturity.

Treatment	Year 1 (2018/19)						Year 2 (2019/20)					
	Pod dry weight (g plant^{-1})	Pod number (plant^{-1})	Seed weight (g plant^{-1})	Seed number (plant^{-1})	Total dry matter (t ha^{-1})	Grain yield (t ha^{-1})	Pod dry weight (g plant^{-1})	Pod number (plant^{-1})	Seed weight (g plant^{-1})	Seed number (plant^{-1})	Total dry matter (t ha^{-1})	Grain yield (t ha^{-1})
JG11												
Cons-1	28.71**	96	24.06**	108*	6.75*	3.14*	27.52**	81	21.80**	89	5.22	3.02
Cons-2	27.98	95	22.69**	105*	6.68*	3.01*	25.25	91*	20.17	98* 5.56* 3.25*		
Control	27.50	91	21.30	94	5.93	2.66	24.35	80	19.36	88	4.77	2.77
Mean	28.06	94	22.68	102	6.46	2.93	25.71	84	20.44	92	5.18	3.01
LSD (5%)	0.605	7.6	1.323	9.8	0.493	0.315	1.417	8.9	1.234	7.9	0.553	0.330
CV (%)	1	4	3	4	3	5	2	5	3	4	5	5
ICCV2												
Cons-1	35.29*	116*	27.96*	120*	5.77	2.80	27.64*	98**	21.63**	96**	4.83*	2.64*
Cons-2	33.08	112	26.91*	114*	5.92*	2.84*	30.20*	85**	20.39**	89**	4.03	2.31
Control	32.33	103	24.78	109	5.75	2.76	18.78	65	15.25	68	3.76	2.04
Mean	33.57	110	26.55	114	5.82	2.80	25.54	81	19.09	84	4.21	2.33
LSD (5%)	2.157	9.11	1.711	4.0	0.137	0.052	6.222	11.6	3.179	1.8	0.626	0.360
CV (%)	3	4	3	2	1	1	11	6	7	6	7	5

Single plant observation (pod dry weight, number of pods, seed weight and number of seeds) and net plot observations (total dry matter and grain yield) at final harvest. * = Statistically significant at 0.05, ** = Statistically significant at 0.01; CV = Coefficients of variation; LSD = Least significant difference; Cons-1 = Consortium-1; includes CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 strains; Cons-2 = Consortium-2i includes CAI-127 and KAI-32 strains.

FOC concentration. Thus, the biocontrol potential of beneficial microbes are seemingly threshold-dependent and biocontrol potential decrease with increased pathogen load is a well-known dynamics [25].

Also, both the consortia were tested against 5% FOC in field conditions for two consecutive years. A significant reduction of disease incidence was noted in consortium-2 (up to 96%) followed by consortium-1 (up to 86%), *T. harzianum* (up to 78%) and *P. fluorescens* (up to 53%). The efficacy of the selected bioagents was found greater in year-1 compared to year-2. This trend was similar in both consortium-1 and consortium-2. Several factors that could have influenced the host-pathogen-antagonist interactions and efficacy of the introduced antagonist such as native microflora, pH, temperature, moisture and inorganic and organic constituents of the soils [25,26]. In the current study, perhaps any one or more than one of such factors might have enhanced the efficacy of the bioagents. Further, the ability of the selected *Streptomyces* strains in reducing the *Fusarium* wilt disease incidence was far higher by the consortia (up to 86%) as reported in the current study, than their individual applications (up to 19%) as reported earlier by us [12]. Similar results were also observed by others [27] where they used a consortium of *Serratia marcescens*, *Pseudomonas fluorescens*, *Rahnella aquatilis* and *Bacillus amyloliquefaciens* for managing the *Fusarium* wilt of chickpea over the individual strains [27]. Nagpal et al. [28] also reported better management of *Fusarium* wilt in two chickpea cultivars PBG-7 and PBG-1 by a consortium of *Mesorhizobium* sp. and endophytic bacteria. The enhanced antagonistic potential of biocontrol agents in a consortium over its individual use was even reported against various other phytopathogens and crops. For example, a consortium of *Pseudomonas* (PHU094), *Trichoderma* (THU0816) and *Rhizobium* (RL091) was shown to alleviate collar rot of chickpea caused by *Sclerotium rolfsii* more potently than individual microbes [29]. Similarly, a consortium of fluorescent *Pseudomonas*, *Trichoderma harzianum* and *Glomus intraradices* was shown to inhibit *Fusarium* wilt in tomato by 50% more than single strain application [30]. Sundaramoorthy et al. [31] also reported a synergistic effect of two *Bacillus subtilis*; EPCO16 and EPC5 with a strain of *Pseudomonas fluorescens* Pf1, in controlling chilli wilt caused by *Fusarium solani* more effectively over single agents. Hence, it is concluded that consortia may work more potently over the individual bio-agents to combat the phytopathogens.

It is widely known that plant immune system gets triggered by the pathogen attack or beneficial microbe(s) colonization. In the present

investigation, the ability of consortium in inducing host plant resistance against FOC was evaluated from the shoot samples of cultivars JG62 (*Fusarium* wilt susceptible) and JG11 (*Fusarium* wilt resistant) of chickpea. The results clearly showed that antioxidant enzymes such as SOD, CAT, APX, GPX, GR and PAL were significantly enhanced in consortium-1 and consortium-2 + FOC samples over FOC only positive control. Among the two consortia, the consortium-2 showed a greater induction of all the antioxidant enzymes compared to consortium-1. The higher production of antioxidants in consortium-2, containing the strains CAI-127 and KAI-32, might be due to their better adoption and colonization in the chickpea roots under field conditions. Other factors that could have influenced is the host-pathogen-consortium-2 interactions, native microflora, pH, temperature, moisture and inorganic and organic constituents of the soils [25,26]. In a similar approach to exploit synergistic advantages, Vijayabharathi et al. [32] had reported an enhanced activity of antioxidant enzymes (such as PAL, SOD and CAT), by treating with a consortium of endophytic *Streptomyces* sp. AUR2, AUR4 and ARR4 aimed at reducing the disease incidence of *Botrytis* Grey Mold in chickpea. Yet another study had reported that these antioxidant enzymes were to get activated when chickpea plants were treated with a consortium of fluorescent *Pseudomonas* (PHU094), *Trichoderma* (THU0816) and *Rhizobium* (RL091) against *Sclerotium rolfsii* [29]. Many other reports are also available highlighting greater beneficial effects of microbial consortium over application of single strains [28,29,33,34].

The *Streptomyces* strains, used in the present study, were earlier reported to significantly enhance PGP traits, when evaluated individually under field conditions [14]. In the present study, the consortia (consortium-1 and consortium-2) were found to be even better in enhancing PGP and yield traits in both JG11 and ICCV2 cultivars over the untreated control plants. The enhancement was found even more in consortium-2 than consortium-1. Therefore, the specific combination of microbes in a consortium is a noteworthy criterion in maximizing its potential towards growth promotion and yield enhancement. For example, enhanced nodulation and nitrogen fixation were observed by Vijayabharathi et al. [32] when they used a consortium of *Streptomyces* and *Mesorhizobium ciceri* over individual constituent strains in five different cultivars of chickpea. The efficiency of beneficial microbial consortia over their single application was well realized in chickpea for plant growth promotion and for alleviating stress conditions. Baliyan et al. [35] observed an enhanced grain yield and biological yield of chickpea by 9.86% and 3.49%, respectively after treating with a consortium of *Bacillus altitudinis* MRN-16 and *Pseudomonas chlororaphis* MRN-52. Also, a consortium of phosphate solubilizing bacteria *Bacillus* sp. RM-2 + fungus *Aspergillus niger* S-36 in one study and halotolerant + drought-tolerant bacterial strains *Azotobacter chroococcum*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Bacillus pumilus* in another study, significantly enhanced the chickpea growth parameters than their individual application [36,37]. Similarly, an increase in maize growth parameters like root length, stem length, plant height, numbers of leaves and seeds weight was reported by different consortium combinations of six PGP microbes namely *Bacillus subtilis*, two *Pseudomonas* sp., *Streptomyces globisporus*, *Streptomyces griseoflavus* and *Streptomyces heliomycin* over their individual application [38]. Therefore, it is conclusive that the application of bio-agents as a consortium is more beneficial and prominent over the individual cultures in enhancing plant growth.

5. Conclusion

It is concluded that the antagonistic potential and growth promotion ability of the five selected *Streptomyces* strains such as CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 were enhanced more when applied as a consortium than individually in chickpea. Among the two consortia, consortium-2, having CAI-127 and KAI-90 strains, showed a consistently greater reduction in FOC disease incidence, over consortium-1 as well as other commercial bioagents. The induction of antioxidant enzymes such as SOD, CAT, APX, GPX, GR and PAL in chickpea were significantly greater in consortium-2 in comparison with FOC positive control. Therefore, it is concluded that consortium-2 can be an effective growth promoter and biocontrol agent against *Fusarium wilt* in chickpea. However, there is a need to further evaluate the biocontrol potential of these consortia in different fields to know their effectiveness in varied environmental conditions. Further, the secondary metabolites of these consortia can unravel many novel biocontrol and growth-promoting metabolites, which can be identified and characterized to serve as biopesticides and bio-fertilizers.

Credit author statement

Sravani Ankati: Methodology, Investigation, Formal analysis, Data Curation, Writing Original Draft, Writing Review and Editing and Visualization. **Vadlamudi Srinivas:** Methodology, Investigation and Data Curation. **Sambangi Pratyusha:** Writing Review and Editing. **Subramaniam Gopalakrishnan:** Conceptualization, Methodology, Software Validation, Investigation, Formal analysis, Resources, Data Curation, Writing Original Draft, Writing Review and Editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Subramaniam Gopalakrishnan reports financial support was provided by Biotechnology Industry Research Assistance Council (BIRAC).

Acknowledgments

This work was undertaken as a part of the CGIAR Research Program on Grain Legumes Dry Land Cereals. ICRISAT is a member of CGIAR Consortium. The authors also thank the Biotechnology Industry Research Assistance Council (BIRAC) for supporting this work under the Small Business Innovation Research Initiative (SBIRI) with ref. No: BT/BIPP/1154/44/18-SBIRI. The authors thank Mr. PVS Prasad for his significant contributions in both greenhouse and field experiments.

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