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Biological Management of *Fusarium* Wilt in Chickpea (*Cicer arietinum* L.) Caused by *Fusarium oxysporum* f. sp. *ciceris*

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ABSTRACT

In the present study, the three rhizobacterial strains (CPs3, CBs5 and Pf1) and fungal antagonists (CTs2 and Tv1) were evaluated against *Fusarium* wilt of chickpea under glasshouse and field conditions. Among all the treatments the strain CPs3 (*Pseudomonas chlororaphis*) has recorded highest germination (100%) and yield parameters *viz*., yield of 1194.4 kg/ ha with 13 (Number of pod bearing branches/plant), 32.3 (Pods/plant) and 33.0 g of 100 seeds weight with lowest incidence of wilt at 14.3% with disease reduction 80.7% (glasshouse) and 21.67% with 70.18% in the field conditions when compared to other biocontrol agents. The highest population of 8.2 $x10^5$ cfu/ g of soil and followed by Pf1 (*Pseudomonas fluorescens*) recorded 7.5 $x10^5$ cfu/g of soil. The strain CPs3 (*Pseudomonas chlororaphis*) had better growth promoting traits and management of the wilt disease in chickpea with superior root colonization ability.

Key words: Chickpea, CBs5, CPs3, Germination, *Pseudomonas chlororaphis.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important cool season legume crops in India. It belongs to leguminosae family with widely cultivated around 50 countries about 90 per cent of cultivated area occupied by Asia, Africa, Central and South America (Jimenez-Diaz *et al*., 2015). India, accounts for 75% of world's chickpea production on 13.98 million ha area with production 137.3 lakh tonnes and productivity 982 kg/ha (Thaware, *et al*., 2016). In Tamil Nadu, chickpea was cultivated in an area of 6820 hectares with a production of 4177 tonnes and a productivity of 645 Kg / ha. Currently, the production and cultivated acreage was drastically reduced due to several abiotic and biotic factors like, pest and diseases (Pande *et al*., 2011).

Under favourable conditions, especially *Fusarium* wilt is playing a vital role in yield loss upto 100% in production (Chand and Khirbat, 2009). Normally high usage of chemical fungicides made changes in soil microbiome and pathogens also develop fungicidal resistance and suppress host defense mechanisms (Jimenez-Diaz *et al*., 2015; Raju *et al*., 2008). So, the alternative possible progress for management of the wilt disease is through biocontrol agents specifically rhizobacteria otherwise called as "Plant growth promoting rhizobacteria" (PGPR) (Datta *et al*., 2011). It consists of a vast group of *Pseudomonas* spp., *Azospirillum, Azotobacter* (Ahamad *et al*., 2008), *Bacillus* spp. (Cakmakci *et al*., 2007), *Serratia* spp. (Gyaneshwar *et al*., 2001), *Burkholderia* (Govindarajan *et al*., 2006), *Klebsiella* (Govindarajan *et al*., 2007) and *Beijerinckia* (Thuler *et al*., 2003). Numerous modes of action have been postulated and demonstrated for antagonistic effects of PGPR in controlling soil borne diseases with organic amendments (Kala *et al*., 2016), which include synergistic effects observed with a combination of antifungal compounds and competition of nutrients against soil borne pathogens and botanicals (Reena *et al*., 2018).

So, the present study was carried out *to* evaluate the effect of biocontrol agents and fungicides on the management of chickpea wilt under greenhouse and field conditions.

MATERIALS AND METHODS

Selection of biocontrol agents: The effective biocontrol agents of rhizobacteria and fungal antagonist *viz*., *Pseudomonas chlororaphis* (CBs3) - [MH628219], *Bacillus subtilis* (CBs5) - [MH746091] and fungal antagonist *Trichoderma harzianum* (CTs2) - [MH744120] were selected to based on their inhibitory potential against with *F. oxysporum* f. sp. *ciceris* under *in vitro* conditions. The isolates *Pseudomonas fluorescens* (Pf1) and *Trichoderma asperellum* (Tv1) and carbendazim 0.1% was used as check for management of wilt in chickpea under glasshouse and field conditions.

Management of wilt under glasshouse and field conditions: During *rabi* 2017-18, talc based bio formulation

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of biocontrol agents were used for management of wilt on chickpea cv. CO4. The seeds were soaked in double the volume of sterile distilled water containing the talc-based formulation 10g/kg of seeds (CPs3, CBs5 and Pf1) and 4g/kg of seeds (CTs2 and Tv1). After 24 hrs, the suspension was drained off and the seeds were dried under shade for 30 min and sown. Carbendazim at the rate of 2g /kg of seeds was applied as seed treatment. In the glasshouse and field conditions, biocontrol agents were applied through soil application @ 5g/kg of soil and 2.5 kg/ha mixed with 22.5 kg of FYM at 30 days before sowing in the field. Carbendazim (0.1%) was applied as seed treatment (2g / kg of seeds) and soil drenching (0.1%) at 14 days after sowing. Salicylic acid @ 3mM conc. was used as seed treatment (3-hours seeds were soaking before sowing). Biometric observations *viz.*, Number of pod bearing branches / plant, Number of pods / plant, 100 seeds weight, percent wilt incidence and yield data were recorded. The experiment was conducted in randomized block design (CRD and RBD) and replicated thrice. The observation on wilt incidence was observed. The per cent disease incidence was assessed using the following formula (Dasgupta *et al.*, 2015).

Per cent disease Incidence =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Assessment the viable population of biocontrol agents from treated field (cfu/g of soil): The population dynamics was enumerated on the application time and 35 days after sowing. The soil samples were collected and estimated by plating the rhizosphere soil using dilution plate technique. Potato dextrose agar and nutrient agar medium was used for plating fungi at a dilution of 10^3, 10^5, 10^6 (fungi and bacteria). All the plates were incubated at room temperatures. Counts were made in all the dilution in three replicate plates and fungal colonies developed on the agar plates were counted 5-7 days after incubation and the number of colony forming units (cfu) were calculated and expressed per gram of soil (Smitha *et al.*, 2017).

RESULTS AND DISCUSSION

Management of wilt under glasshouse and field conditions: Totally five biocontrol agents *viz.*, (CPs3, CBs5, CTs2, Pf1 and Tv1) were used in the study for management of the wilt disease in chickpea cv. CO4. All the treatments were significantly reduced the wilt incidence, among them the strain CPs3 (*Pseudomonas chlororaphis*) significantly reduced the wilt incidence compared than other biocontrol agents. The treatment of CPs3 (*Pseudomonas chlororaphis*) was more effective than other biocontrol agents for germination and recorded the maximum germination 100 per cent and followed by Pf1 (*Pseudomonas fluorescens*) recorded 88.3%. The results revealed that the carbendazim (0.1%) recorded the least wilt incidence of 10.6 per cent, which was followed by the biocontrol agent CPs3 (*P. chlororaphis*) with 14.3% with disease reduction of 85.6% and 80.7% in glasshouse conditions (Table 1; Fig 1).

Under field conditions, CPs3 (*Pseudomonas cholroraphis*) has recorded the highest yield of 1194.4 kg/ha with 13 (Number of pod bearing branches/plant), 32.3 (Pods/plant) and 33.0 g of 100 seeds weight followed by

Table 1: Management of *Fusarium* wilt in chickpea (cv. CO4) under glasshouse conditions (*Rabi*, 2017-18).

Treatment details (Seed treatment, Soil application and Soil drenching)	Germination (%)	Percent disease incidence (%)	Per cent disease reduction over control (%)
T1. Pf1(*Pseudomonas fluorescens*) Seed treatment with 10g / kg of seeds + soil application of 5g / kg of soil	88.3^{b}	28.3^{b}(32.15)	61.8^{b}
T2. Pf1 (*Pseudomonas fluorescens* - Liquid) Seed treatment with 10 ml / kg of seeds + soil application of 5g / kg of soil	72.1^{e}	35.3^{de}(36.47)	52.4^{de}
T3. Tv1 (*Trichoderma asperellum*) Seed treatment with 4g / kg of seeds + soil application of 5g / kg of soil	83.3^{c}	27.0^{b}(31.19)	63.6^{b}
T4. CPs3 (*Pseudomonas chlororaphis*) Seed treatment with 10g / kg of seeds + soil application of 5g / kg of soil	100.0^{a}	14.3^{a}(22.24)	80.7^{a}
T5. CBs5 (*Bacillus subtilis*) Seed treatment with 10g / kg of seeds + soil application of 5g / kg of soil	72.1^{e}	33.6^{cd}(35.46)	54.7^{cd}
T6. CTs2 (*Trichoderma harzianum*) Seed treatment with 4g / kg of seeds + soil application of 5g / kg of soil	78.3^{d}	38.6^{e}(38.44)	47.9^{e}
T7. Carbendazim 0.1% (Seed treatment 2g/ kg of seeds + Soil drenching @ 14 DAS)	83.3^{c}	10.6^{a}(19.05)	85.6^{a}
T8. Salicylic acid (Seed treatment @ 3mM (3- hours seeds soaking before sowing)	72.1^{e}	30.6^{bc}(33.62)	58.7^{bc}
T9. Control	50.0^{f}	74.3^{f}(59.57)	0.00^{f}

Values are mean of three replications.

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

*Values in the parenthesis are arcsine transformed values.

A. Over all pot culture view

B. Best Treatment of glasshouse condition

Fig 1: Management of *Fusarium* wilt of chickpea (cv. CO4) under glasshouse conditions.

Table 2: Management of *Fusarium* wilt of chickpea (cv. CO4) under field conditions (*Rabi*, 2017-18).

Treatments details (Seed treatment + Soil application)	Per cent disease incidence (%)	Per cent reduction over control (%)	Number of pod bearing branches / Plant	Number of pods / Plant	100 seeds weight (g)	Yield (kg / ha)
T1. Pf1 (*P. fluorescens*) Seed treatment with 10g/ kg of seeds + soil application 2.5kg /ha of field	32.00^{c}(34.43)	55.96	11.0^{bc}	24.7^{c}	30.6^{c}	1172.5^{c}
T2. Pf1 (*P. fluorescens*-Liquid) Seed treatment with 10 ml / kg of seeds+ soil application 2.5kg /ha of field	34.67^{cde}(36.05)	52.29	8.7^{d}	13.7^{f}	26.3^{de}	1121.3^{d}
T3. CPs3 (*P. chlororaphis*) Seed treatment with 10g / kg of seeds + soil application 2.5kg /ha of field	21.67^{b}(27.72)	70.18	13.0^{a}	32.3^{a}	33.0^{a}	1194.4^{a}
T4. CBs5 (*B. subtilis*) Seed treatment with 10g / kg of seeds + soil application 2.5kg /ha of field	33.67^{d}(35.46)	53.66	10.0^{cd}	23.0^{c}	25.6^{e}	1113.2^{e}
T5. Tv1 (*T. asperellum*) Seed treatment with 4g / kg of seeds+ soil application 2.5kg /ha of field	36.67^{e}(37.25)	49.53	9.7^{cd}	19.7^{d}	23.0^{f}	1057.0^{f}
T6. CTs2 (*T. harzianum*) Seed treatment with 4g / kg of seeds+ soil application 2.5kg /ha of field	42.33^{f}(40.58)	41.75	10.3 ^{c}	18.7 ^{d}	28.0 ^{cd}	1052.0^{g}
T7. Carbendazim (0.1%) Seed treatment with 2g/kg of seeds + soil drenching @ (14 DAS)	18.33^{a}(25.34)	74.77	12.0^{ab}	27.7^{b}	27.3^{b}	1180.0^{b}
T8. Salicylic acid Seed treatment @ 3mM (3- hours seed soaking before sowing)	33.67^{cd}(35.45)	53.66	8.7^{d}	15.7^{e}	22.3^{f}	1017.0^{h}
T9. Control (Untreated)	72.67^{g}(58.48)	0.00	7.0^{e}	13.7 ^{f}	18.3^{g}	859.5^{i}

Values are mean of three replications.
Means followed by a common letter are not significantly at 5 % level by DMRT.
*Values in the parenthesis are arscine transformed values.

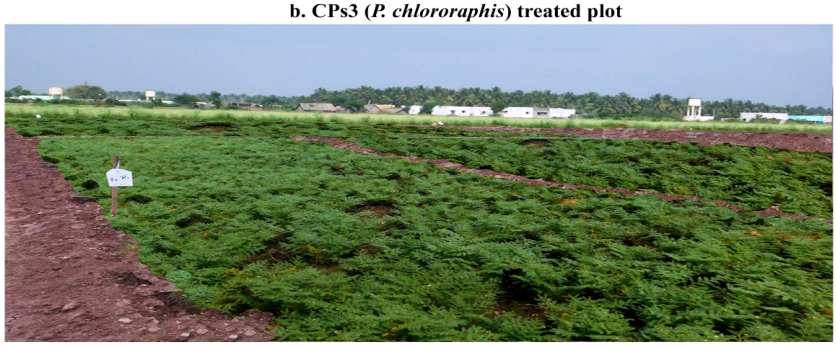
carbendazim 0.1% (1180.0 kg/ha) respectively. The control was recorded with least yield of 859.5 kg/ha. The lowest incidence recorded in carbendazim 0.1% (18.3%) followed by *Pseudomonas chlororaphis* (CPs3) at 21.67% with 70.18% disease reduction (Table 2; Fig 2). These results were related to Dasgupta *et al*. (2015) who reported that seed treatment and soil application in combined application resulted in better productivity in chickpea. Shahzaman *et al*. (2016) documented that siderophore producing *Pseudomonas* sp. isolates were promoted the plant growth, pod formation, invidual weight of seeds and biomass by the soil application in glasshouse conditions on chickpea. Inam-Ul-Haq *et al*. (2015) reported that three rhizospheric PGPR strains *viz*., RH-31, RH-32 and RH-33 (*Paenibacillus illinoisensis, Bacillus subtilis* and *Pseudomonas psychrotolerans*) increased the growth promotion and reduce the wilt from 22 to 67% under glasshouse conditions. Karimi *et al*. (2012) reported that *Pseudomonas aeuroginosa*, *Bacillus subtilis* provided better wilt management and growth promotion in chickpea under glasshouse and field conditions.

Assessment the viable population of biocontrol agents from treated field (cfu/g of soil): Among all the treatments, CPs3 (*Pseudomonas cholroraphis*) recorded the highest population of 8.2 x10^5 cfu/ g of soil and followed by Pf1 (*Pseudomonas fluorescens*) recorded 7.5 x10^5 cfu/g of soil and the least population load was recorded 2.7 x 10^3 cfu/g of soil by CTs2 (*Trichoderma harzianum*) on 35 days after sowing on cv. CO4 rhizosphere (Table 3; Fig 3).

These results are in conformity with those findings reported by earlier workers. Landa *et al*. (1997) reported that seed + soil application of PGPR strains *viz*., *Bacillus* sp. (RGAF 6a, RGAF 7 and RGAF 51) showed highest cfu/ g of soil *viz*., 5.9, 6.28 and 6.27 under chickpea field against wilt. Smitha *et al*. (2017) reported that *Bacillus subtilis* strain

a. Field layout of village (Chinna vedampatti)

b. CPs3 (*P. chlororaphis*) treated plot



c. Control (untreated)

Fig 2: Management of *Fusarium* wilt of chickpea (cv. CO4) under field conditions during *Rabi*, 2017-18.

Table 3: Population dynamics of biocontrol agents from treated chickpea field (*Rabi*, 2017-18).

Biocontrol agents (PGPR & Fungal antagonist)	Dilution factors	Number of. cfu / g of soil (Days after sowing)	
		0 DAS	35 DAS
Pf1 (*Pseudomonas fluorescens*)	10^5	2.6^b	7.5^b
Pf1 (*Pseudomonas fluorescens*) -L	10^5	2.0^c	7.1^c
CPs3 (*Pseudomonas chlororaphis*)	10^5	2.9^a	8.2^a
CBs5 (*Bacillus subtilis*)	10^6	2.1^c	4.1^d
Tv1 (*Trichoderma asperellum*)	10^3	2.9^a	3.2^e
CTs2 (*Trichoderma harzianum*)	10^3	2.9^a	2.7^f

Values are mean of three replications.
Means followed by a common letter are not significantly at 5 % level by DMRT.
*Values in the parenthesis are arscine transformed values.

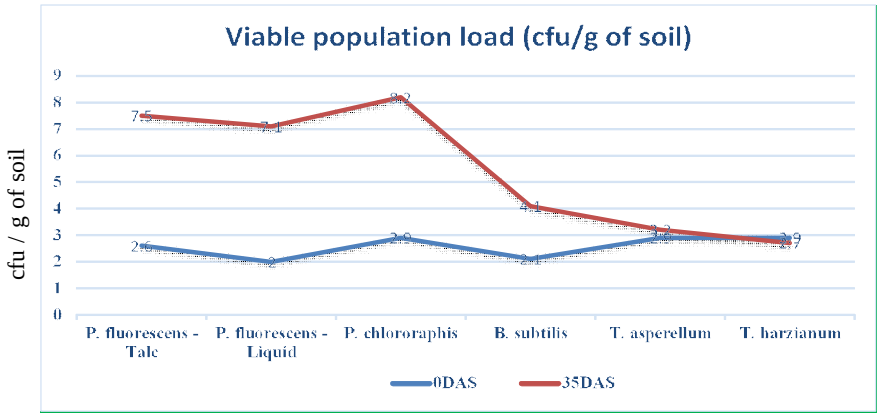


Fig 3: Population dynamics of biocontrol agents from treated chickpea field (*Rabi*, 2017-18).

CaB5 showed that population density of 51x10^6 cfu/g of soil 90 days after sowing from chickpea field. Maleki *et al.* (2010) reported that strain CV6 (*Pseudomonas fluorescens*) from cucumber rhizosphere act as a good root colonizer at a rate of 8.03 cfu/g of soil.

CONCLUSION

Rhizobacterial strains showing a potential role in the growth promotion traits and act as a good colonizer with chickpea rhizobiome with enhanced and balanced the nutrition uptake ability in the chickpea crop especially in black soil at soil pH >6.9. Adequate population of rhizobacteria were suppressed the invasion of pathogen to crop during ramification stage to podding stage in pulse crops. So, rhizobacteria was used to better management for soil borne diseases and yield induction.

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