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Testing the Efficacy of Bio Control Agents and Fungicides against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* Conditions

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A B S T R A C T

Fusarium oxysporum f. sp. *ciceris* is a ubiquitous seed and soil borne pathogen in several crops. It survives in the soil without host from several years. To manage the wilt pathogen is such as a difficult in crop management. The rhizobacteria as grouped under two categories by the cultural, morphological and biochemical characters. Ten isolates (CPs1 to CPs9) were identified as *Pseudomonas* sp. by medium to strong production of KOH, HCN production and siderophore synthesis. Another ten isolates of (CBs1 to CBs10) identified as brown creamish and serrated margins with production of catalase and citrate. Under *in vitro* efficacy, *Pseudomonas* isolates CPs3 showed maximum inhibition per cent of 54.07% followed Pf1 at 51.47%. In *Bacillus* sp. isolate CBs5 showed maximum inhibition per cent of 61.85% and followed CBs1 recorded 59.25% and fungal antagonistic activity Tv1 showed maximum mycelial inhibition per cent of 67.77% followed by CTs3 at 62.22%. Carbendazim recorded maximum mycelial growth inhibition of 86.66%.

Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop in the world for its easy available form of edible proteins and vitamins. In India it is cultivated at cool winter (Rabi) season in semi-arid tropics by irrigated or rain-fed conditions (Nene *et al.*, 1984). India is largest producer of chickpea in world sharing 65.25 per cent in area and 65.49 per cent in production and is grown on 10.23 million ha area with production 9.88 million tonnes and productivity 967 kg/ha (Thaware *et al.*, 2017). Despite the production was reduced due to several biotic and abiotic factors. Chickpea is noticed to be more than 52 pathogens at cropping season (Nene *et al.*, 1984). Among these pathogens *F. oxysporum* f. sp. *ciceris* causing a potential yield loss for both in seed yield and seed weight by wilt about 10 to 15 per cent (Navas-Cortes *et al.*, 2000; Khilare *et al.*, 2009). Although many control measures have been developed for management the wilt disease in chickpea, the soil borne nature of

pathogen make control difficult. An extensive using of resistant cultivars, the pathogen races were breakdown the host resistance and probably attempted higher incidence throughout the world (Inam-Ul-Haq *et al.*, 2015). But cultural and chemical practices provided significantly reduction in the disease incidence and it should change the crop diversity due to highly influence of soil conservation by usage of chemicals (Nene and Reddy, 1987).

Out of these, several measures biocontrol agents were reported to an alternative potential tool for management of pathogens (El-Katatany *et al.*, 2003). Because, the crop rhizosphere holding Plant growth-promoting rhizobacteria (PGPR) is the initial barrier for invasion of soil borne pathogens attack (Weller, 1988; Joseph *et al.*, 2007). The Plant growth promoting rhizobacteria (PGPR) improve the plant growth by root colonization and derived a resistance against soil borne pathogens through initiate strongly nutrition competition, production of antibiotics, N2 fixation, extracellular hydrolytic enzymes, secondary metabolites such as hydrogen cyanide and induced systemic resistance (Khan *et al.*, 2009; Datta *et al.*, 2011). A large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter* (Ahmad *et al.*, 2008), *Bacillus, Beijerinckia*, *Klebsiella* and *Serratia* (Gyaneshwer *et al.*, 2001) have been reported to widely adapted soil antagonistic species for eco-friendly without harmful for respected host also (Kamala Devi, 2012). It is beneficial to wide crop diversity in agriculture production (Govindarajan *et al.*, 2006). These biocontrol agents were mostly depended on the host and soil ecosystem (Hervas *et al.*, 1997).

Rhizobacteria group of *Bacillus* and *P. chlororaphis* strongly antagonistic against with three races (0, 1 and 5) of *F. oxysporum* f. sp. *ciceris* by their antibiotics synthesis under *in vitro* conditions and suppressed the utilization of chemicals in field conditions also (Landa *et al.*, 1997). In fungal antagonist, *Trichoderma, Gliocladium, Candida, Ampelomyces, Coniothyrium* placed in potential role against several plant pathogens (*Liu et al., 1995*). *Trichodrma* is a secondary opportunistic invader and colonized in rhizobiome and changes plant metabolism by nutrient uptake from soil, plant growth and increase primary defense against pathogen invasion (Poddar *et al.*, 2004). Several species *Trichoderma* were notified strong antagonistic activity against soil borne pathogens *viz*., *F. oxysporum*, *F. solani*, *M. phaseolina* and *S. rolfsii*, by their generation of ROS, lytic enzymes and secondary metabolites (Singh *et al.*, 2009). Hossain *et al.*, (2013) reported that *T. harzianum* (T75) isolate completely inhibited the mycelial proliferation of *F. oxysporum* f. sp. *ciceris* in dual culture assay. Although each of these practices individually potential, yet none is completely viable when applied alone the problem still throughout the world. The chemical based control is most effective and reliable. No economical and eco-friendly strategies are available to combat this devasting soilborne pathogen. So, to using new chemical fungicides potential for control new races of pathogens management (Sharma *et al.*, 2010).

The present study was carried out for evaluate the bioefficacy of biocontrol agents from chickpea rhizosphere and fungicides against with *F. oxysporum* f. sp. *ciceris* under *in vitro*.

Materials and Methods

Isolation and identification of rhizobacteria

Rhizosphere colonizing twenty bacterial isolates was isolated from different rhizosphere soils of chickpea from major growing areas of Tamil Nadu. The soil particles tightly adhered with root portion of

chickpea were gently removed and suspended in 10ml sterile distilled water. After serial diluted (upto 10^6) one ml of suspension from each 10^3 to 10^6 dilutions transferred into sterile Petri plates containing Nutrient agar media (*Bacillus* sp.) and King's B media *(Pseudomonas* sp.) for the isolation of respectively by kept under 30ºC for 24 hours.

The further purification is done in the respective media and kept under laboratory conditions. The isolates were identified based on their phenotypical and morphological characters (Cakmakci *et al.*, 2007).

Biochemical characterization

The bacterial isolates were biochemical characterized on the basis of KOH test, HCN production, gelatine hydrolysis, siderophore production, catalase production, starch hydrolysis; citrate utilization and influence of NaCl for growth were checked as per the standard methods for followed for *Pseudomonas* and *Bacillus* sp. The reactions were referred as (+) - Positive; (-) - Negative (++) - Medium production; (+++) - Strong production (Schaad, 1992).

Isolation and identification of *Trichoderma* sp.

Soil samples were collected from chickpea rhizosphere of nine various locations. The soil particles were gently removed and suspended in 10ml sterile distilled water. After serial diluted (10^3) one ml of suspension was transferred into sterile Petri plates containing *Trichoderma* selective media (Glucose - 3g, K_2HPO_4 - 0.9g, $MgSO_4$ - 0.2g, KCl_2 - 0.5g, NH_4Cl_2 - 1.05g, Rose Bengal - 0.15g, chloromphenical - 0.25g, metalaxyl - 0.3g, Agar - 15g, D. water - 1lit). After three days the green coloured colonies are sub cultured in PDA containing Petri plates and kept under 4ºC for further studies (Thaware *et al.*, 2017).

In vitro efficacy of biocontrol agents

Antagonistic activity of the ten bacterial isolates of *Pseudomonas* sp. (CPs1 - CPs9 and Pf1 as check), *Bacillus* sp. (CBs1- CBs10) and fungal antagonist for ten *Trichoderma* sp. (CTs1 – CTs10) were against *F. oxysporum* f. sp. *ciceris* isolate (Foc4) evaluated based on dual culture technique (Landa *et al.*, 1997) and replicated thrice. Radial growth of the fungus was measured and percentage of growth inhibition was calculated using the formula:

Inhibition (%) = (R-r) / R x 100

Where, r is the radius of the fungal colony opposite the bacterial colony and R, is the maximum radius of the fungal colony in the absence of the bacterial colony (Kumari and Khanna, 2014).

In vitro efficacy of fungicides (Poisoned food technique)

The efficacy of eight different following fungicides *viz.*, Carbendazim (Bavistin), N-trichloromethylthio-4-cyclohexane 1,2-dicarboximide (Captan), Copperoxychloride (Kocide), Cymoxanil + Mancozeb (Curzate), Fenamidone + Mancozeb (Sectin), Fluopyram (Luna Experience), Iprovalicarb + Probieb (Melody Duo) and Isoprothiolane (Fujione) at different conc. of 0.025, 0.05and 0.1% were tested on the radial mycelial growth of the pathogen by poisoned food technique (Kumar and Mane, 2017). At first, the stock solution of above fungicides at different concentration was prepared and the required concentration of fungicides was mixed with PDA medium in sterile conical flask. The three different concentrations of the fungicides were poured individually in sterile petridish at 20 ml and allowed to solidify. Eight mm of mycelial disc of the pathogen (Foc 4) was placed in the centre and incubated at room temperature (28±2°C). The three replications have been

maintained for each concentration and untreated control was also maintained. The radial mycelial growth of the pathogen was observed at seven days after inoculation (Subhani *et al.,* 2011; Maitlo *et al.,* 2014).

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

Where,

C- Mycelial growth of pathogen in control
T- Mycelial growth of pathogen treated plates

Results and Discussion

Isolation and identification of rhizobacteria

The different isolates of rhizobacteria were isolated by dilution method and it was grouped and characterized by their cultural and morphological, ten isolates of *Pseudomonas* sp. were phenotypically appeared as pale yellow to white in colour and translucent slimy and *Bacillus* species were showed pale brown to dull white in coloured, serrated and wavy margin (Table 1).

These results revealed that (Kumari and Khanna, 2014) isolated 40 rhizobacterial isolates from chickpea and 20 isolates were confirmed as *Pseudomonas* sp. and 16 isolates reported as *Bacillus* sp. from chickpea. Landa *et al.,* (1997) reported that slimy and fluorescent growth of rhizobacteria was confirmed as seven isolates of *Pseudomonas* sp. from chickpea.

Biochemical characterization of rhizobacteria

The isolated bacterial antagonists were biochemical characterized *viz*., ten isolates exhibited medium production in KOH test and gelatine hydrolysis. In HCN production and siderophore production isolates *viz*., Pf1, CPs3 and CPs9 were showed strong production and confirmed as *Pseudomonas* sp. Another ten isolates of rhizobacteria were showed medium production in catalase test, starch hydrolysis, citrate utilization and growth in NaCl and confirmed as *Bacillus* sp. (Table 2). Hundred and fifty isolates of PGPR from rhizosphere of chickpea among these isolates, 35 belonged to *Pseudomonas* sp. and 40 under *Bacillus* sp. by biochemical reaction of hydrogen cyanide production, siderophore synthesis, catalase and nitrate reduction, (Joseph *et al.,* 2007). Karimi *et al.,* (2012) reported that six *Bacillus* isolates exhibited strong production of IAA synthesis and protease.

Isolation and identification of *Trichoderma* sp.

The dilution plate method, after three days, green coloured mycelial antagonistic fungi (CTs1 to CTs9) was isolated from nine different locations and sub cultured in PDA contained Petri dishes and identified through all the isolates were produced cylindrical numerous conidia (Table 3).

These results revealed that Hossain *et al.,* (2013) reported that 20 isolates of *Trichoderma* sp. (T-1 to T-77/2) were isolated and identified from various cultivars of chickpea.

In vitro efficacy of biocontrol agents

Pseudomonas sp.

Antagonistic activity among the 10 *Pseudomonas* sp. against *F. oxysporum* f. sp. *ciceris* (Foc4) in dual culture assay, all the isolates showed significant inhibition per cent from 10.36% to 54.07%. Out of these ten isolates, isolate CPs3 showed maximum inhibition per cent of 54.07% followed Pf1 at 51.47% (Table 4; Plate 1).

Table.1 Phenotypic and morphological characterization of antagonistic rhizobacteria from chickpea

S. No	Locations	Isolate code	Phenotypic characters	Morphological edge	Referred as
1.	Coimbatore	Pf1 (TNAU)	Creamy, translucent, slimy	Rounded	*P. fluorescens*
2.	Gomangalam pudur	CPs1	Creamy, light translucent, slimy	Rounded	*Pseudomonas* sp.
3.	Thippampatti	CPs2	Pale yellowish, translucent, slimy	Rounded	*Pseudomonas* sp.
4.	Mukkonam	CPs3	Milky white, translucent, slimy	Rounded	*Pseudomonas* sp.
5.	Modakkupatti	CPs4	Brownish, translucent, slimy	Rounded	*Pseudomonas* sp.
6.	Valzavadi	CPs5	Pale white, translucent, slimy	Rounded	*Pseudomonas* sp.
7.	Ramachadra Puram	CPs6	Creamy yellow, translucent, slimy	Rounded	*Pseudomonas* sp.
8.	P.N.Palayam	CPs7	Creamy white, translucent, slimy	Rounded	*Pseudomonas* sp.
9.	Poolankinaru	CPs8	Brownish, translucent, slimy	Rounded	*Pseudomonas* sp.
10.	Konnur	CPs9	Pale white, translucent, slimy	Rounded	*Pseudomonas* sp.
11.	Gomangalam	CBs1	Pale brown slimy	Serrated point margin	*Bacillus* sp.
12.	Thippampatti	CBs2	Pale brown slimy	Thick serrated margin	*Bacillus* sp.
13.	Mukkonam	CBs3	Pale white slimy	Wavy winged margin	*Bacillus* sp.
14.	Modakkupatti	CBs4	Pale brown thick slimy	Light serrated margin	*Bacillus* sp.
15.	Vazlavadi	CBs5	Pale brown powdery slimy	Serrated margin	*Bacillus* sp.
16.	Ramachadra puram	CBs6	Pale white slimy	Wavy branched margin	*Bacillus* sp.
17.	P.N.palayam	CBs7	Pale white slimy	Wavy branched margin	*Bacillus* sp.
18.	Poolankinaru	CBs8	Pale brown slimy	Wavy branched margin	*Bacillus* sp.
19.	Konnur	CBs9	Pale brown thick slimy	Wavy branched margin	*Bacillus* sp.
20.	Pannaikinaru	CBs10	Dull white slimy	Serrated margin	*Bacillus* sp.

Table.2 Identification and characterization of rhizobacteria from chickpea by biochemical characteristics

S. No	Isolates	Biochemical tests									
		Gram's Staining	KOH test	HCN production	Catalase test	Starch hydrolysis	Gelatine hydrolysis	Growth in 7% NaCl	Citrate utilization test	Siderophore production	Tentatively identified as
1	Pf1	Pink	++	+++	-	-	++	-	-	+++	*Pseudomonas fluorescens*
2	CPs2	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* sp.
3	CPs3	Pink	++	+	-	-	++	-	-	+++	*Pseudomonas* sp.
4	CPs4	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* sp.
5	CPs5	Pink	++	++	-	-	++	-	-	+	*Pseudomonas* sp.
6	CPs6	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* sp.
7	CPs7	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* sp.
8	CPs8	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* sp.
9	CPs9	Pink	++	+++	-	-	++	-	-	+++	*Pseudomonas* sp.
10	CPs10	Pink	++	+	-	-	++	-	-	+	*Pseudomonas* sp.
11	CBs1	Violet	-	-	++	++	-	+	++	-	*Bacillus* sp.
12	CBs2	Violet	-	-	++	++	-	+	+	-	*Bacillus* sp.
13	CBs3	Violet	-	-	++	++	-	+	++	-	*Bacillus* sp.
14	CBs4	Violet	-	-	+	++	-	+	+	-	*Bacillus* sp.
15	CBs5	Violet	-	-	++	++	-	+	++	-	*Bacillus* sp.
16	CBs6	Violet	-	-	++	++	-	+	+	-	*Bacillus* sp.
17	CBs7	Violet	-	-	++	++	-	+	++	-	*Bacillus* sp.
18	CBs8	Violet	-	-	++	++	-	+	++	-	*Bacillus* sp
19	CBs9	Violet	-	-	+	++	-	+	+	-	*Bacillus* sp.
20	CBs10	Violet	-	-	++	++	-	+	+	-	*Bacillus* sp.

(+) - Positive; (-) – Negative; (++) - Medium production; (+++) - Strong production

Table.3 Cultural and morphological characters of fungal antagonistic isolates of *Trichoderma* sp. from chickpea

S. No	Locations	Isolate code	Cultural characters	Morphological characters	Referred as
1.	Coimbatore	Tv1 (TNAU)	Dark greenish with white fluffy growth	Cylindrical conidia	*Trichoderma viride*
2.	Gomangalam pudur	CTs1	White with greenish, scattered growth	Cylindrical conidia	*Trichoderma* sp.
3.	Thippampatti	CTs2	Dark greenish adherent growth	Cylindrical conidia	*Trichoderma* sp.
4.	Mukkonam	CTs3	Dark greenish adherent growth	Cylindrical conidia	*Trichoderma* sp.
5.	Modakkupatti	CTs4	Dark greenish adherent growth	Cylindrical conidia	*Trichoderma* sp.
6.	Valzavadi	CTs5	Dull green with white adherent growth	Cylindrical conidia	*Trichoderma* sp.
7.	Ramachadra Puram	CTs6	White with greenish fluffy growth	Cylindrical conidia	*Trichoderma* sp.
8.	P.N.Palayam	CTs7	Brownish adherent growth	Cylindrical conidia	*Trichoderma* sp.
9.	Poolankinaru	CTs8	Greenish with white fluffy growth	Cylindrical conidia	*Trichoderma* sp.
10.	Konnur	CTs9	Creamy white with greenish fluffy growth	Cylindrical conidia	*Trichoderma* sp.

Table.4 Effect of different *Pseudomonas* sp. isolates against radial mycelial growth of *Fusarium oxysporum* f. sp. *ciceris in vitro*

S. No.	Locations	Isolate No.	Radial mycelial growth (mm) *	Percent inhibition over control (%)
1.	Gomangalampudur	CPs1	59.33^{e}	34.07^{e}
2.	Thippampatti	CPs2	52.67^{d}	41.47 ^{d}
3.	Mukkonam	CPs3	41.33^{a}	54.07^{a}
4.	Modakkupatti	CPs4	77.33^{g}	14.07^{g}
5.	Valzavadi	CPs5	66.00^{f}	26.66^{f}
6.	Ramachadra Puram	CPs6	51.67^{d}	42.58^{d}
7.	P.N.Palayam	CPs7	80.67^{h}	10.36^{h}
8.	Poolankinaru	CPs8	77.33^{g}	14.07^{g}
9.	Konnur	CPs9	47.67^{c}	47.03^{c}
10.	Coimbatore	Pf 1	43.67^{b}	51.47^{b}
11.	-	Control	90.00^{i}	0.00^{i}

*Mean of the three replications
Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table.5 Effect of different isolates of *Bacillus* sp. against radial mycelial growth of *Fusarium oxysporum* f. sp. *ciceris in vitro*

S. No.	Locations	Isolate No.	Radial mycelial growth (mm) *	Percent inhibition over control (%)
1.	Gomangalam pudur	CBs1	36.67	59.25[b]
2.	Thippampatti	CBs2	55.67	38.14[d]
3.	Mukkonam	CBs3	43.33	51.85[c]
4.	Modakkupatti	CBs4	58.67	34.81[e]
5.	Vazlavadi	CBs5	34.33	61.85[a]
6.	Ramachadra puram	CBs6	60.67	32.58[e]
7.	P.N.palayam	CBs7	56.33	37.41[d]
8.	Poolankinaru	CBs8	63.33	29.63[f]
9.	Konnur	CBs9	81.00	10.00[h]
10.	Pannaikinaru	CBs10	74.67	17.03[g]
11.	-	Control	90.00	0.00[i]

*Mean of the three replications
Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table.6 Effect of different isolates of *Trichoderma* sp. isolates against radial mycelial growth of *Fusarium oxysporum* f. sp. *ciceris in vitro*

S. No	Locations	Isolate. No	Radial mycelial growth (mm)*	Per cent inhibition over control (%)
1.	Coimbatore	Tv1	29.00	67.77[a]
2.	Gomangalam pudur	CTs1	55.33	38.52[g]
3.	Thippampatti	CTs2	34.00	62.22[b]
4.	Mukkonam	CTs3	40.33	55.18[d]
5.	Modakkupatti	CTs4	43.67	51.47[e]
6.	Valzavadi	CTs5	38.67	57.03[d]
7.	Ramachadra Puram	CTs6	36.00	60.00[c]
8.	P.N.Palayam	CTs7	51.33	42.96[f]
9.	Poolankinaru	CTs8	61.33	31.85[h]
10.	Konnur	CTs9	76.00	15.55[i]
11.	-	Control	90.00	0.00[j]

*Mean of the three replications
Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table.7 Effect of different fungicides against the radial mycelial growth of *F. o.* f. sp. *ciceris* (Foc 4) *in vitro* conditions

S. No	Fungicides	Concentrations					
		0.025%		0.05%		0.1%	
		Radial mycelial growth (mm)*	Percent inhibition over control (%)	Radial mycelial growth (mm)*	Percent inhibition over control (%)	Radial mycelial growth (mm)*	Percent inhibition over control (%)
1.	Carbendazim (Bavistin)	20.67	77.03[a]	18.33	79.63[a]	12.00	86.66[a]
2.	N-trichloromethylthio-4-cyclohexane1,2-dicarboximide (Captan)	38.00	57.77[d]	28.67	68.14[c]	22.33	75.30[d]
3.	Copper oxychloride (Kocide)	52.33	41.85[e]	33.00	63.33[e]	15.33	82.96[b]
4.	Cymoxanil + Mancozeb (Curzate)	71.00	21.11[g]	40.67	54.81[f]	28.33	68.52[e]
5.	Fenamidone + Mancozeb (Sectin)	85.33	5.18[h]	54.33	39.63[g]	30.33	66.30[f]
6.	Fluopyram (Luna Experience)	33.00	63.33[c]	31.00	65.55[d]	27.33	69.63[e]
7.	Iprovalicarb + Probineb (Melody Duo)	59.00	34.44[f]	58.00	35.55[h]	32.33	64.07[g]
8.	Isoprothiolane (Fujione)	24.33	72.96[b]	21.67	75.92[b]	20.33	77.41[c]
9.	Control	90.00	0.00[i]	90.00	0.00[i]	90.00	0.00[h]

*Mean of the three replications.
Means followed by a common letter are not significantly different at the 5% level by DMRT.

Plate.1 *In vitro* screening of different isolates of *Pseudomonas* sp. on mycelial growth of *F. oxysporum* f. sp. *ciceris*

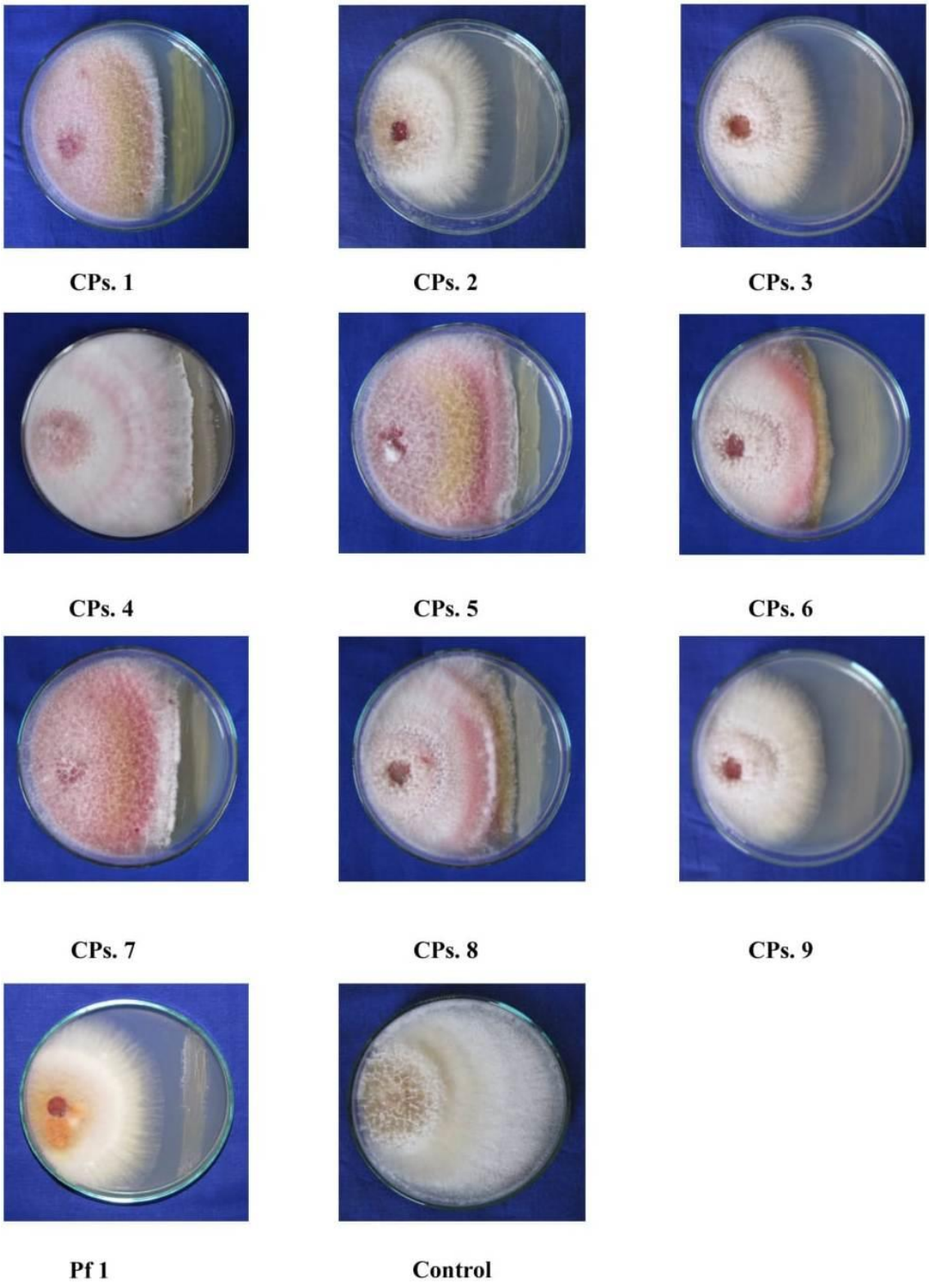


Plate.2 *In vitro* screening of different isolates of *Bacillus* sp. on mycelial growth of *F. oxysporum* f. sp. *ciceris*

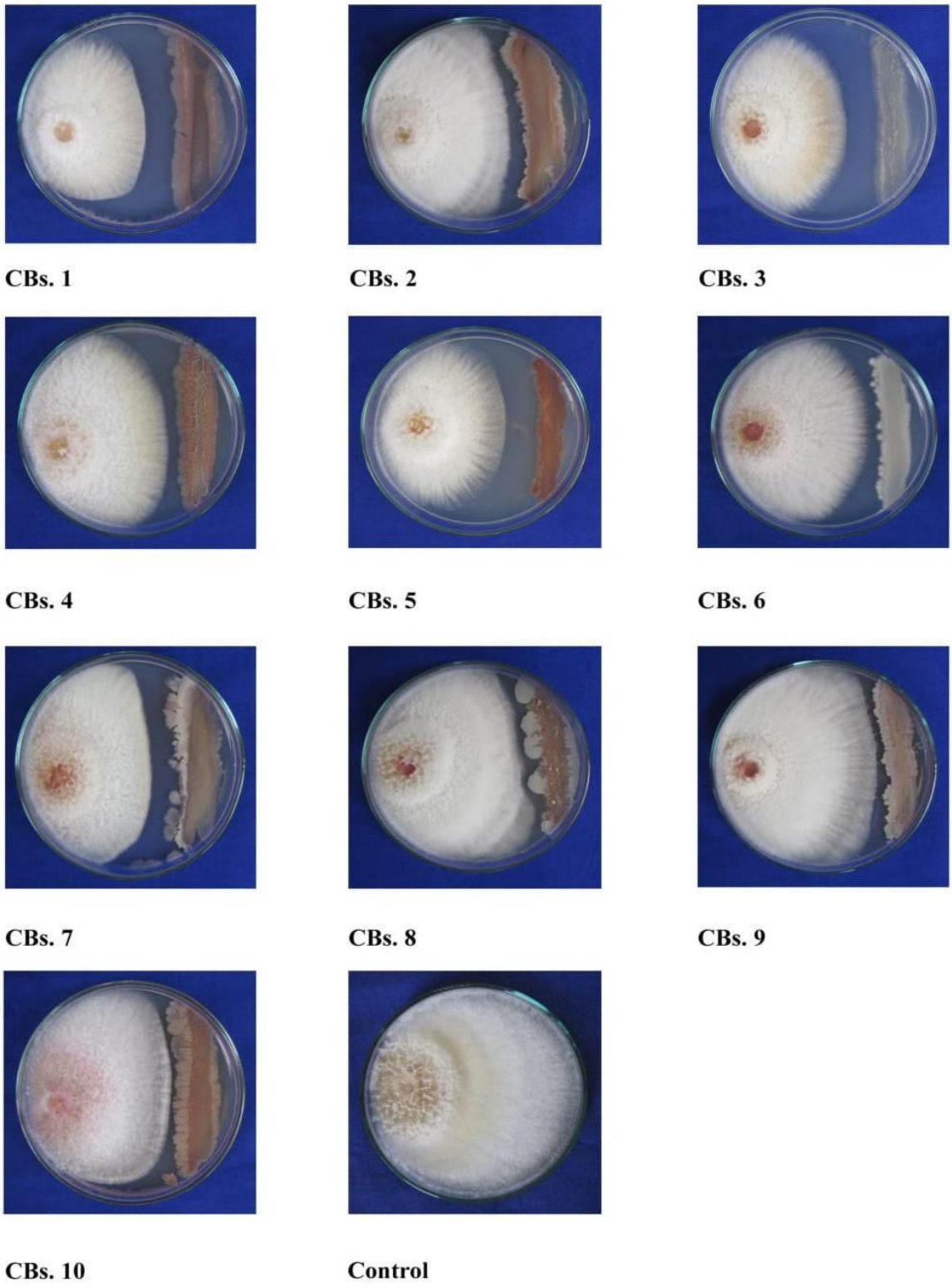
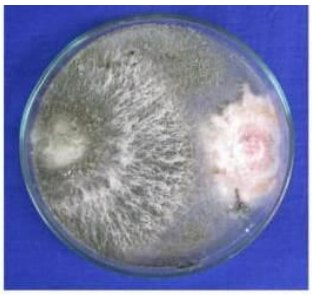


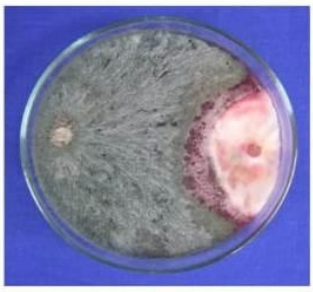
Plate.3 *In vitro* screening of different isolates of *Trichoderma* sp. on mycelial growth of *F. oxysporum* f. sp. *ciceris*

Tv 1

CTs. 1



CTs. 2



CTs. 3

CTs. 4

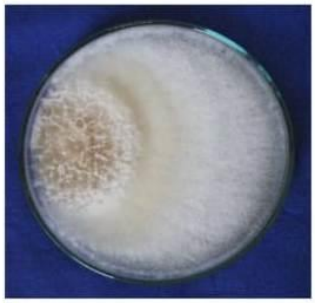
CTs. 5

CTs. 6

CTs. 7

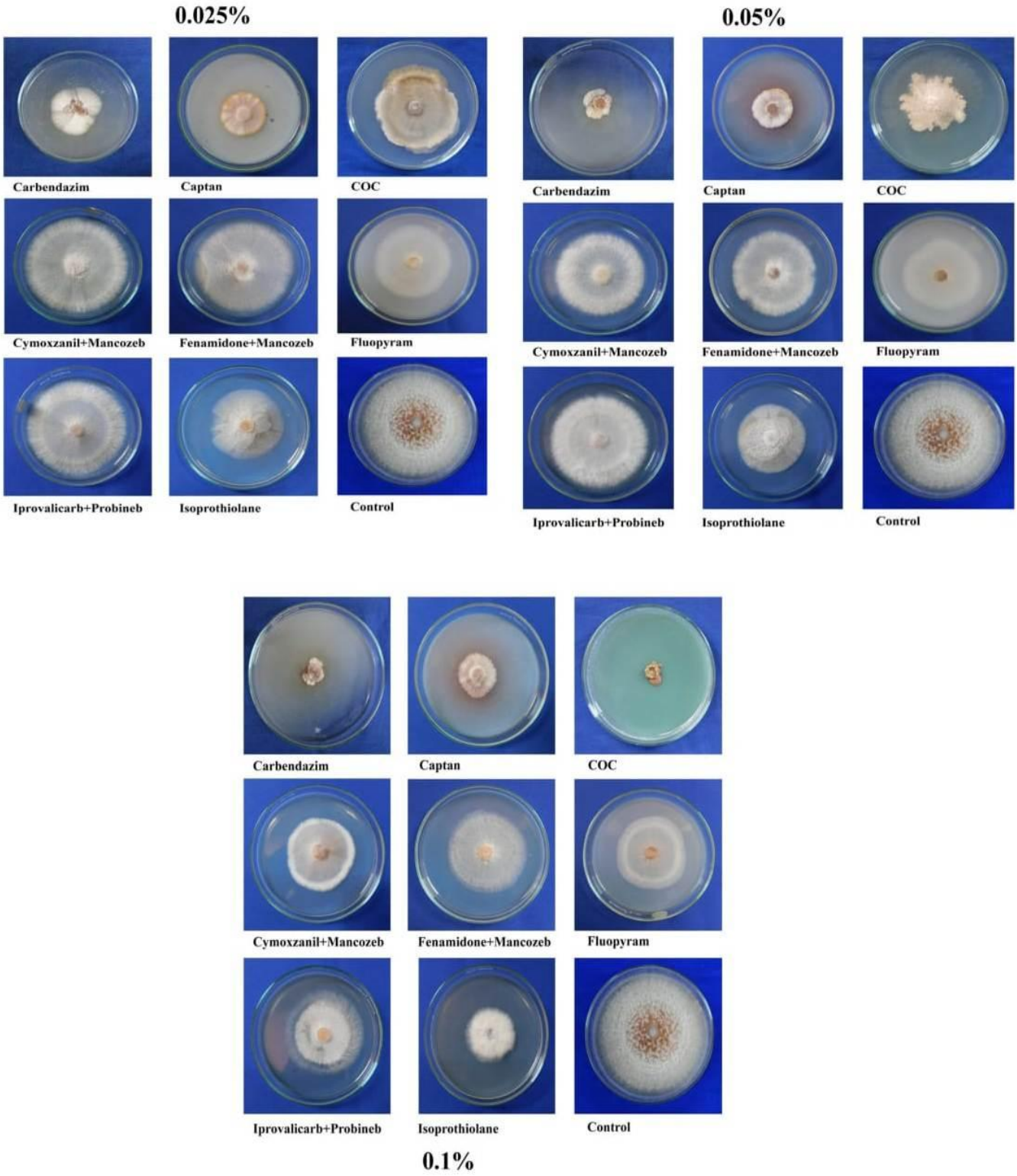
CTs. 8

CTs. 9



Control

Plate.4 *In vitro* screening of different fungicides against on mycelial growth of *F. oxysporum* f. sp. *ciceris*



Inam-Ul-Haq *et al.,* (2015) reported that three different species of rhizobacteria (RH-31, RH-32 and RH-33) among three isolates (RH-33) *P. psychrotolerans* showed maximum inhibition against soil borne pathogens of *M. phaseolina*, *F. oxysporum* and *F. solani*.

Bacillus sp.

All the ten isolates of *Bacillus* sp., showed significant antagonistic activity of inhibition per cent from 10.00 to 61.85%. Out of these isolates, CBs5 showed maximum inhibition per cent of 61.85% and followed CBs1 recorded 59.25% under *in vitro* conditions (Table 5; Plate 2). Karimi *et al.,* (2012) reported that three isolates of Bacillus sp. (B28, B6 and B40) showed maximum mycelial growth inhibition per cent from 49.7 -51.2 against *F. oxysporum* f. sp. *ciceris*

Trichoderma sp.

In fungal antagonistic activity all the ten *Trichoderma* isolates were significantly inhibited mycelial growth from 15.55 to 67.77% against *F. o*. f. sp. *ciceris*. Out of these ten isolates, Tv1 showed maximum mycelial inhibition per cent of 67.77% followed by CTs3 at 62.22% inhibition under dual culture under *in vitro* (Table 6; Plate 3). Andrabi *et al.,* (2011) reported that *T. viride* is exhibited superior antagonistic activity by maximum inhibition of mycelial growth per cent at (86.21%) compared than *T. virens* (85.29%) against *F. oxysporum* f. sp. *ciceris*.

In vitro efficacy of fungicides

Totally eight fungicides were used for checked the efficacy against *F. oxysporum* f. sp. *ciceris* isolate Foc4. All the fungicides were significantly reduced the mycelial growth of pathogen. Among these eight fungicides, Carbendazim (Bavistin) showed maximum mycelial growth inhibition of 86.66 per cent and followed by Copper oxychloride (Kocide) at 82.96 % at 0.1% concentration (Table 7; Plate 4). These results were similar to Maitlo *et al.,* (2014) reported that screened with fourteen fungicides against with *Foc* the two fungicides carbendazim and thiophanate-methyl were highly effective at all concentrations of 1-10000ppm. Carbendazim showed maximum inhibition of mycelial growth at 100, 200 and 500 ppm concentrations against *F. oxysporum* and *R. solani* under *in vitro* conditions (Andrabi *et al.,* 2011)

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