Original Research Article

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

P. Murali Sankar¹*, S. Vanitha¹, A. Kamalakannan¹, P. Anantha Raju², P. Jeyakumar³ and T. Raguchander¹

¹Department of Plant Pathology, ²Department of Pulses, ³Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641003 India

*Corresponding author

**Abstract**

*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%.

**Keywords**

Rhizobacteria, *Pseudomonas* sp., Carbendazim, Siderophore and CPs3

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**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 pathogen (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). Trichodroma is a secondary opportunistic invader and colonized in rhizobione 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 to 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, $KCl$ - 0.5g, $NH_4Cl$ - 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:

\[
\text{Inhibition (\%)} = \frac{(R-r)}{R} \times 100
\]

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, 0.05 and 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.                   |
| S. No | Isolates | Biochemical tests | 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.    | Ramachandra 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        | 59.33e                        | 34.07e                             |
| 2.    | Thippampatti    | CPs2        | 52.67d                        | 41.47d                             |
| 3.    | Mukkonam        | CPs3        | 41.33a                        | 54.07a                             |
| 4.    | Modakkupatti    | CPs4        | 77.33g                        | 14.07g                             |
| 5.    | Valzavadi       | CPs5        | 66.00f                        | 26.66f                             |
| 6.    | Ramachandra Puram | CPs6      | 51.67d                        | 42.58d                             |
| 7.    | P.N.Palayam     | CPs7        | 80.67h                        | 10.36h                             |
| 8.    | Poolankinaru    | CPs8        | 77.33g                        | 14.07g                             |
| 9.    | Konnur          | CPs9        | 47.67e                        | 47.03e                             |
| 10.   | Coimbatore      | Pf 1        | 43.67b                        | 51.47b                             |
| 11.   | -              | Control     | 90.00f                        | 0.00f                              |

*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                         | 59.25b                             |
| 2.     | Thippampatti      | CBs2        | 55.67                         | 38.14d                             |
| 3.     | Mukkonam          | CBs3        | 43.33                         | 51.85c                             |
| 4.     | Modakkupatti      | CBs4        | 58.67                         | 34.81e                             |
| 5.     | Vazlavadi         | CBs5        | 34.33                         | 61.85a                             |
| 6.     | Ramachadrapuram   | CBs6        | 60.67                         | 32.58e                             |
| 7.     | P.N.palayam       | CBs7        | 56.33                         | 37.41d                             |
| 8.     | Poolankinaru      | CBs8        | 63.33                         | 29.63f                             |
| 9.     | Konnur            | CBs9        | 81.00                         | 10.00h                             |
| 10.    | Pannaikinaru      | CBs10       | 74.67                         | 17.03g                             |
| 11.    | -                 | Control     | 90.00                         | 0.00i                              |

*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)* | Percent inhibition over control (%) |
|--------|-----------|-------------|-------------------------------|------------------------------------|
| 1.     | Coimbatore| Tv1         | 29.00                         | 67.77a                             |
| 2.     | Gomangalam pudur | CTs1  | 55.33                         | 38.52g                             |
| 3.     | Thippampatti | CTs2 | 34.00                         | 62.22b                             |
| 4.     | Mukkonam   | CTs3        | 40.33                         | 55.18d                             |
| 5.     | Modakkupatti | CTs4 | 43.67                         | 51.47e                             |
| 6.     | Valzavadi  | CTs5        | 38.67                         | 57.03d                             |
| 7.     | Ramachadrapuram | CTs6 | 36.00                         | 60.00c                             |
| 8.     | P.N.Palayam | CTs7 | 51.33                         | 42.96f                             |
| 9.     | Poolankinaru | CTs8 | 61.33                         | 31.85h                             |
| 10.    | Konnur     | CTs9        | 76.00                         | 15.55l                             |
| 11.    | -          | Control     | 90.00                         | 0.00i                              |

*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 (%) | Percent inhibition over control (%) | Percent inhibition over control (%) |
| 1.    | Carbendazim (Bavistin)              | 20.67                            | 77.03<sup>a</sup>                   | 18.33                           | 79.63<sup>a</sup>                   | 12.00                           | 86.66<sup>a</sup>                   |
| 2.    | N-trichloromethylthio-4-cyclohexane1,2-dicarboximide (Captan) | 38.00                            | 57.77<sup>d</sup>                   | 28.67                           | 68.14<sup>c</sup>                   | 22.33                           | 75.30<sup>d</sup>                   |
| 3.    | Copper oxychloride (Kocide)         | 52.33                            | 41.85<sup>e</sup>                   | 33.00                           | 63.33<sup>e</sup>                   | 15.33                           | 82.96<sup>b</sup>                   |
| 4.    | Cymoxanil + Mancozeb (Curzate)     | 71.00                            | 21.11<sup>g</sup>                   | 40.67                           | 54.81<sup>f</sup>                   | 28.33                           | 68.52<sup>e</sup>                   |
| 5.    | Fenamidone + Mancozeb (Sectin)     | 85.33                            | 5.18<sup>h</sup>                    | 54.33                           | 39.63<sup>g</sup>                   | 30.33                           | 66.30<sup>f</sup>                   |
| 6.    | Fluopyram (Luna Experience)        | 33.00                            | 63.33<sup>c</sup>                   | 31.00                           | 65.55<sup>d</sup>                   | 27.33                           | 69.63<sup>e</sup>                   |
| 7.    | Iprovalicarb + Probineb (Melody Duo) | 59.00                           | 34.44<sup>f</sup>                   | 58.00                           | 35.55<sup>h</sup>                   | 32.33                           | 64.07<sup>g</sup>                   |
| 8.    | Isoprothiolane (Fujione)           | 24.33                            | 72.96<sup>b</sup>                   | 21.67                           | 75.92<sup>b</sup>                   | 20.33                           | 77.41<sup>c</sup>                   |
| 9.    | Control                             | 90.00                            | 0.00<sup>j</sup>                    | 90.00                           | 0.00<sup>j</sup>                    | 90.00                           | 0.00<sup>i</sup>                    |

*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
Plate 4 In vitro screening of different fungicides against 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 conditions (Table 6; Plate 3). Andradi 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 (Andradi et al., 2011)

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Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, India.

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