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Evaluation of compatible *Pseudomonas* isolates and effective fungicides against *Fusarium oxysporum* f. sp. *ciceri* (Pot culture)

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Abstract
Present research work entitled "Evaluation of compatible *Pseudomonas* isolates and effective fungicides against *Fusarium oxysporum* f. sp. *ciceri* in pot culture" was conducted at the Department of Plant Pathology, College of Agriculture, Latur (VNMKV, Parbhani), M.S. during the year 2019-20. Compatibility of isolates *P. fluorescens viz*., PF2 and PF5 were tested with five fungicides. PF2 and PF5 were compatible with Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and Tebuconazole 50% + Trifloxystrobin 25% WG and least compatibility was recorded with Carboxin 37.5% + Thirum 37.5% WP. The potential isolates *viz*., PF2 and PF5, effective fungicides *viz*., Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and their combinations evaluated using susceptible variety JG-62 against *F. oxysporum* f. sp. *ciceri* in pot culture. The results revealed that, highest disease control percentage recorded with Carbendazim 50% WP + PF5 (80.73%) followed by seed treatments with Carbendazim 50% WP + PF2 (79.13%), Carbendazim 25% + Mancozeb 50% WP + PF5 (72.72%), Carbendazim 25% + Mancozeb 50% WP + PF2 (68.79%), Carbendazim 50% WP (66.94%), Carbendazim 25% + Mancozeb 50% WP (62.99%) and PF5 (46.79%). The lowest disease control percentage was observed in PF2 (38.67%).

Keywords: Chickpea, compatibility, *Pseudomonas fluorescens*, *in vitro*, fungicide, *Fusarium oxysporum* f. sp. *ciceri*

Introduction
Chickpea (*Cicer arietinum* L.) is a leguminous annual plant in the family Fabaceae grown for its edible seeds and variously known as gram, bengal gram, chana and chole. It is world's third most important pulse crop and preferred food legume due to its high nutritional value, high yield potential and low cost of cultivation. The area under chickpea cultivation was 106 lakh ha with annual production of 111 lakh tonnes and productivity of 1056 kg/ha in India during 2019-20. Area and production of chickpea in Maharashtra during 2019-20 was 20.38 lakh ha and 17.29 lakh tonnes, respectively with productivity of 848.55 kg/ha, whereas in Marathwada region of Maharashtra chickpea was cultivated on an area of 10.59 lakh ha with production and productivity 7.96 lakh tonnes and 707.56 kg/ha, respectively during 2019-20 (Anonymous, 2020) [1]. Chickpea suffers from several diseases but wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most serious disease which causes heavy losses up to 10 per cent in yield (Dubey *et al*., 2007) [3]. Seed treatment with ecofriendly bioagent can be utilized as substitute for fungicides and in combination with ecofriendly tool in integrated disease management module with all economical manners to overcome the problem of pathogen. Use of biological control agents such as Plant Growth Promoting Rhizobacteria (PGPR) can be a suitable approach in control of wilt disease (Schmidt *et al*., 2004) [11]. Hence present investigation on evaluation of compatible *Pseudomonas* isolates and effective fungicides against *Fusarium oxysporum* f. sp. *ciceri* in pot culture.

Materials and Methods
Compatibility of potential isolates of *Pseudomonas* spp. (PF2 and PF5) with fungicides
The most efficient strains of *P. fluorescens* (PF2 and PF5) were used for compatibility study with effective fungicides (Table 1). *In vitro* compatibility of *P. fluorescens* with effective fungicides were evaluated, each at different concentration to access their compatibility with *P. fluorescens*, by employing paper disc / inhibition zone technique and using King's B as a basal culture medium. Autoclaved and cooled (45 ºC) King's B medium was poured (20 ml /plate) in petri plate and rotated the plates gently in clock-wise and anti-clockwise directions, for uniform spreading of medium in plate. After solidifying media 1ml of individual bacterial culture was spread on these medium with sterile glass spreader aseptically.

Whatman's filter paper (Whatman Filter paper No. 42) discs (1mm dia.) pre-sterilized in autoclave were soaked / impregnated for 5 min. in the test concentrations of the test fungicides separately.

A single disc was placed at centre on *P. fluorescens* seeded solidified King's B medium in petri plates. Four petri plates per treatment per concentration were maintained. The petri plates containing test bacterium seeded King's B medium and inoculated with Whatman's filter paper disc soaked in distilled water were maintained as untreated control. Both treated and untreated petri plates were incubated at 28±2 ºC.

Table 1: Compatibility of effective fungicides with potential isolates of *Pseudomonas* spp.

Treatments	Fungicides	Concentration (ppm)
T_1	Carbendazim 50% WP	1000
T_2	Tebuconazole 25.9% EC	1000
T_3	Carboxin 37.5% + Thirum 37.5% WP	2000
T_4	Tebuconazole 50% + Trifloxystrobin 25% WG	2000
T_5	Carbendazim 25% + Mancozeb 50% WP	2000
T_6	Control	-

Observations

Observations on zone of inhibition was recorded after 72 hrs. of the incubation, to know the effect of fungicides on *P. fluorescens*. The per cent growth inhibition of test *P. fluorescens* strain were calculated by following formula

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition.

C = Growth of test isolates in control in mm

T = Growth of test isolates in treatment in mm

Evaluation of compatible isolates of *Pseudomonas* and fungicides in control of wilt of chickpea in pot culture

Evaluation of effective isolates of *Pseudomonas* with effective and compatible fungicides in control of chickpea wilt was studied using sick pot culture technique. Two most effective *Pseudomonas* spp. were selected for pot culture study. Clean seed of chickpea crop (Variety – JG-62) were treated with effective and compatible fungicide and then inoculated with most antagonism strain of *Pseudomonas* suspension. After 2 hrs. of fungicide treatment *Pseudomonas* methyl cellulose suspension were uniformly sprinkled over fungicide treated seed and dried in shade for one hour. Fungicide alone, combined fungicide – *Pseudomonas* spp. and non treated seeds were sown in pots. The experiment of combined evaluation of effective isolates of *Pseudomonas* with effective and compatible fungicides in control of chickpea wilt were executed as per following details

Experimental Detail

Design: CRD

Replications : Three

Treatments : Nine

Variety : JG-62 (Susceptible)

Seed treatment details

1. *Pseudomonas* spp. (PF2)
2. *Pseudomonas* spp. (PF5)
3. Carbendazim (1 gm/kg of seed)
4. Carbendazim + Mancozeb (2.5 gm/kg of seed)
5. Carbendazim (1 gm/kg of seed) + *Pseudomonas* spp. (PF2)
6. Carbendazim (1 gm/kg of seed) + *Pseudomonas* spp. (PF5)
7. Carbendazim + Mancozeb (2.5 gm/kg of seed) + *Pseudomonas* spp. (PF2)
8. Carbendazim + Mancozeb (2.5 gm/kg of seed) + *Pseudomonas* spp. (PF5)
9. Control

Observations

Observation were recorded in respect of plant height, dry wt, and total per cent disease incidence by following formula,

$$\text{Germination (\%)} = \frac{\text{No of seed germinated}}{\text{Total no of seed sown}} \text{x100}$$

$$\text{Per cent disease incidence} = \frac{\text{Total infected plants}}{\text{Total number of plants observed}} \text{ x 100}$$

Result and Discussion

Compatibility of potential isolates of *Pseudomonas* spp. (PF2) with fungicides

Present investigations were carried to test the compatibility of *Pseudomonas* spp. with fungicide in order to integrate *P. fluorescens* with fungicide for management of wilt of chickpea.

Effective fungicides against pathogen *viz*., Carbendazim, Tebuconazole, Carboxin + Thirum, Tebuconazole+ Trifloxystrobin and Carbendazim + Mancozeb tested at various concentrations *in vitro* by inhibition zone technique. Inhibition zone indicated the degree of compatibility of *P. fluorescens* with test fungicides. The result revealed that, all five fungicides were showed significant difference in inhibition zone (mm) recorded at 48 and 72 hrs. of incubation (PLATE I, Fig. 1a).

At 48 hrs. of incubation, amount of inhibition zone produced with test fungicides ranged from 00.00 to 16.25 mm whereas, at 72 hrs. of incubation, amount of inhibition zone produced ranged from 00.00 to 18.12 mm, with average inhibition zone ranged from 00.00 to 17.18 mm (Table 2).

The fungicides Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and Tebuconazole 50% + Trifloxystrobin 25% WG was most compatible with *P. fluorescens*, as they didn't showed any zone of inhibition (00.00 mm), both after 48 and 72 hrs. Whereas, Tebuconazole 25.9% EC (8.75 to 10.12 mm) and Carboxin 37.5% + Thirum 37.5% WP (16.25 to 18.12 mm) were found non compatible with *P. fluorescens*, they showed zone of inhibition, at both 48 and 72 hrs respectively. Thus, among tested fungicides Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and Tebuconazole 50% + Trifloxystrobin 25% WG were found highly compatible with *P. fluorescens* and Tebuconazole 25.9% EC and Carboxin 37.5% + Thirum 37.5% WP were found incompatible with *P. fluorescens*.

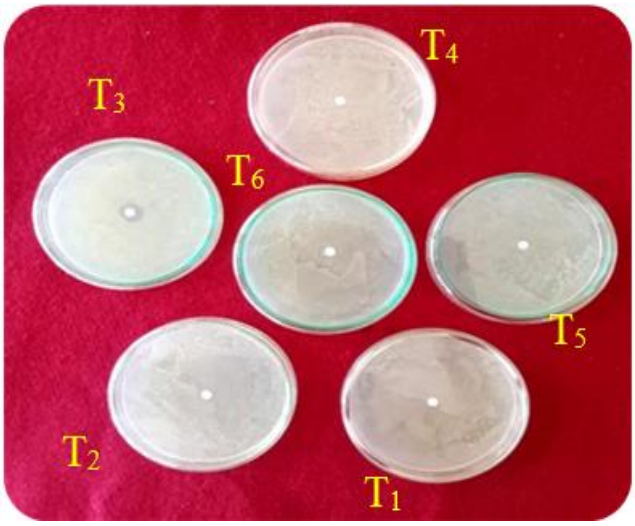


Plate I: *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens* (PF2)

Table 2. *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens* (PF2)

Tr. No.	Inhibition Zone (mm) at 48 hrs.	Inhibition Zone (mm) at 72 hrs.	Average
T_1	0.00	0.00	0.00
T_2	8.75	10.12	9.43
T_3	16.25	18.12	17.18
T_4	0.00	0.00	0.00
T_5	0.00	0.00	0.00
T_6	0.00	0.00	0.00
SE±	0.35	0.32	
CD at 1%	1.01	0.94	

Inhibition zone = Average of four replications

Compatibility of potential isolates *Pseudomonas* spp. (PF5) with fungicides

Five fungicides *viz*., Carbendazim, Tebuconazole, Carboxin + Thirum, Tebuconazole + Trifloxystrobin and Carbendazim + Mancozeb tested various concentrations *in vitro* by inhibition zone technique which exhibited inhibition zone indicated the degree of compatibility of *P. fluorescens* with test fungicides. The result revealed that, all five fungicides shown significance difference in inhibition zone (mm) recorded at 48 and 72 hrs. of incubation (PLATE II, Fig. 1b).

At 48 hrs. of incubation, amount of inhibition zone produced with test fungicides ranged from 00.00 to 16.87 mm whereas, at 72 hrs. of incubation, amount of inhibition zone produced ranged from 00.00 to 18.12 mm, with average inhibition zone ranged from 00.00 to 17.49 mm (Table 3).

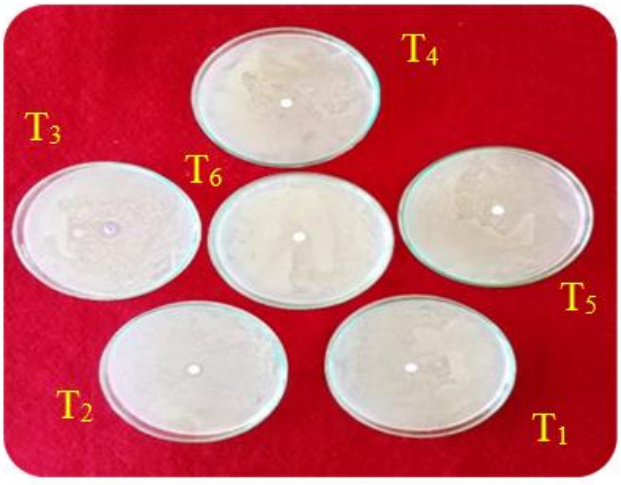


Plate 2: *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens* (PF5)

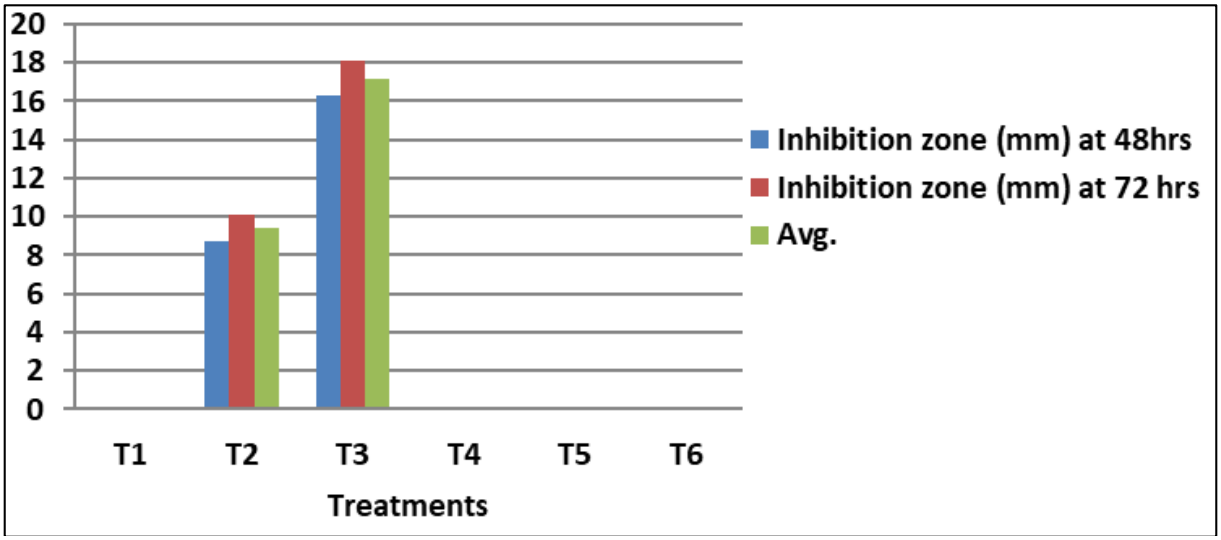
The fungicides Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and Tebuconazole 50% + Trifloxystrobin 25% WG found highly compatible with *P. fluorescens*, as they didn't showed any zone of inhibition (00.00 mm), both 48 and 72 hrs. Whereas, Tebuconazole 25.9% EC (9.25 to 9.93 mm) and Carboxin 37.5% + Thirum 37.5% WP (16.87 to 18.12 mm) were found non compatible with *P. fluorescens*, they showed zone of inhibition, at both 48 and 72 hrs respectively. Thus, among tested fungicides Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and Tebuconazole 50% + Trifloxystrobin 25% WG were found highly compatible with *P. fluorescens* and Tebuconazole 25.9% EC and Carboxin 37.5% + Thirum 37.5% WP were found incompatible with *P. fluorescens*.

Table 3: *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens* (PF5)

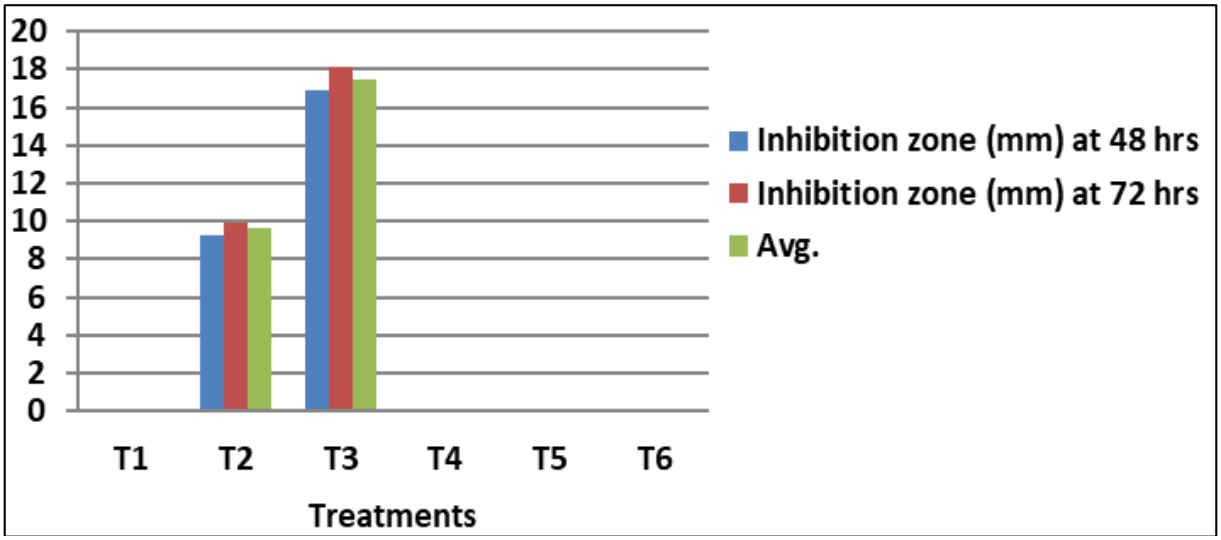
Tr. No.	Inhibition Zone (mm) at 48 hrs.	Inhibition Zone (mm) at 72 hrs.	Average
T_1	0.00	0.00	0.00
T_2	9.25	9.93	9.59
T_3	16.87	18.12	17.49
T_4	0.00	0.00	0.00
T_5	0.00	0.00	0.00
T_6	0.00	0.00	0.00
SE±	0.30	0.34	
CD at 1%	0.88	0.98	

Inhibition zone = Average of four replications

The results of present investigation have resembled with earlier records of scietists *viz*., Chennakesavulu *et al*. (2013) [3] reported that, Carbendazim was high compatible with *P. fluorescens* followed by Mancozeb. Geethu and Gautam (2015) [5] reported the compatibility of three neem formulations, nine fungicides and seven insecticides with *P. fluorescens*. Among fungicides *viz*., Carbendazim, Isoprothiolane, Kitazin, Hexaconazole, Mancozeb, Carbendazim + Mancozeb and Bordeaux mixture are highly compatible with *P. fluorescens* and Copper oxychloride and Wettable sulphur incompatible with *P. fluorescens*. Priya *et al*. (2019) [10] reported that, Carbendazim, Tebuconazole + Trifloxystrobin and Propiconazole were compatible at 500, 1000 and 1500 ppm concentration. Basha *et al*. (2018) [2] tested compatibility of *P. fluorescens* with agrochemicals and reported that, Carbendazim, Hexaconazole, Propiconazole, Hisulphur and Tricyclazole compatible with *P. fluorescens*. The other scientists like Khan and Gangopadhyay (2008) [6], Telangre *et al*. (2013) [12], Loius *et al*. (2016) [7] and Praful Kumar and Mane (2017) [9] had also found the compatibility of *P. fluorescens* with fungicide.



a) *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens* (PF2)



b) *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens* (PF5)

Fig 1: *In vitro* compatibility of fungicides with potential isolates of *Pseudomonas fluorescens*

Evaluation of compatible *Pseudomonas* isolates and effective fungicides against *Fusarium oxysporum* f. sp. *ciceri* (pot culture)

The pot culture experiment conducted during *Rabi* season 2019-20 to evaluate efficacy of two *Pseudomonas* isolates, two fungicides and their combinations by applying seed treatment in pot culture using susceptible variety JG-62 against *F. oxysporum* f. sp. *ciceri* (PLATE III, Fig. 2). Result (Table 4) revealed that, all the treatments were found effective against the test pathogen and significantly enhance the seed germination and reduced the per cent disease incidence in chickpea over untreated control.

Per cent germination

All treatments increased germination percentage in the ranged 81.11 to 100 per cent compare to control (71.11%). Among the all treatments the Carbendazim 50% WP + PF2, Carbendazim 50% WP + PF5 and Carbendazim 25% + Mancozeb 50% WP + PF5 were given 100 per cent seed germination. This was followed by Carbendazim 25% + Mancozeb 50% WP + PF2 (97.77%), Carbendazim 50% WP (92.22%), Carbendazim 25% + Mancozeb 50% WP (91.11%) and PF5 (84.44%). The least germination percentage (81.11%) was observed with seed treatment of PF2.

Per cent wilt incidence

All treatments influenced significantly the disease incidence which was recorded against untreated control. The wilt incidence recorded ranged from 13.33 per cent (Carbendazim 50% WP + PF5) to 42.44 per cent (PF2), and 69.21 per cent in untreated control. The lowest wilt incidence recorded with Carbendazim 50% WP + PF5 (13.33%) followed by Carbendazim 50% WP + PF2 (14.44%) and both treatments were found at par with each other. Carbendazim 25% + Mancozeb 50% WP + PF5, Carbendazim 25% + Mancozeb 50% WP + PF2, Carbendazim 50% WP, Carbendazim 25% + Mancozeb 50% WP and PF5 recorded of 18.88%, 21.60%, 22.88%, 25.61% and 36.82% wilt incidence, respectively. The highest wilt incidence observed in PF2 (42.44%) and which was found least effective.

Per cent disease control

All treatments significantly improved the disease control percentage as compared untreated control. The disease control percentage recorded ranged from 38.67 to 80.73 per cent over untreated control. The highest disease control percentage recorded with Carbendazim 50% WP + PF5 (80.73%). This was followed by seed treatments with Carbendazim 50% WP + PF2 (79.13%), Carbendazim 25% + Mancozeb 50% WP + PF5 (72.72%), Carbendazim 25% + Mancozeb 50% WP + PF2 (68.79%), Carbendazim 50% WP (66.94%), Carbendazim 25% + Mancozeb 50% WP (62.99%) and PF5 (46.79%). The lowest disease control percentage was observed in PF2 (38.67%).

The results of present investigation have resembled the finding of earlier records of scientist *viz*., Chennakesavulu *et al*. (2013**)** [3] studied the different seed treatment and soil application of bioagents against *F. udum* inciting pigeon pea wilt and reported that, seed treatment of Carbendazim @ 20 ml/kg + soil application of effective *P. fluorescens* @ 2g/kg gave least PDI of 11.1 per cent as compared to inoculated control (64.9%). Patil *et al*. (2015) [8] conducted experiment of seed treatment with bioagents and fungicides against wilt of chickpea and reported that among tested bioagents, *Trichoderma viride* recorded less PDI (6.67%) followed by Carbendazim (7.66%) and *P. fluorescens* (10.66%) PDI.

Plate 3: Evaluation of compatible *Pseudomonas* isolates and effective fungicides against *Fusarium oxysporum* f. sp. *ciceri* (pot culture)

Table 4: Evaluation of effective *Pseudomonas* isolates and fungicides against *Fusarium oxysporum* f. sp. *ciceri* (Pot culture)

Treat no	Fungicides/ Isolates *Pseudomonas*	Per cent germination	Per cent Wilt	Disease inhibition per cent over control
T_1	PF2	81.11 (64.23)	42.44 (40.65)	38.67
T_2	PF5	84.44 (66.76)	36.82 (37.35)	46.79
T_3	Carbendazim 50% WP	92.22 (73.80)	22.88 (28.57)	66.94
T_4	Carbendazim 25% + Mancozeb 50% WP	91.11 (72.65)	25.61 (30.40)	62.99
T_5	Carbendazim 50% WP + PF2	100 (90)	14.44 (22.33)	79.13
T_6	Carbendazim 50% WP + PF5	100 (90)	13.33 (21.41)	80.73
T_7	Carbendazim 25% + Mancozeb 50% WP + PF2	97.77 (81.41)	21.60 (27.69)	68.79
T_8	Carbendazim 25% + Mancozeb 50% WP + PF5	100 (90)	18.88 (25.75)	72.72
T_9	Control	71.11 (57.48)	69.21 (56.29)	-
SE±		1.28	1.65	
CD at 1%		3.48	4.48	

Observation = Average of three replications and figures in parenthesis are arcsine transformation value

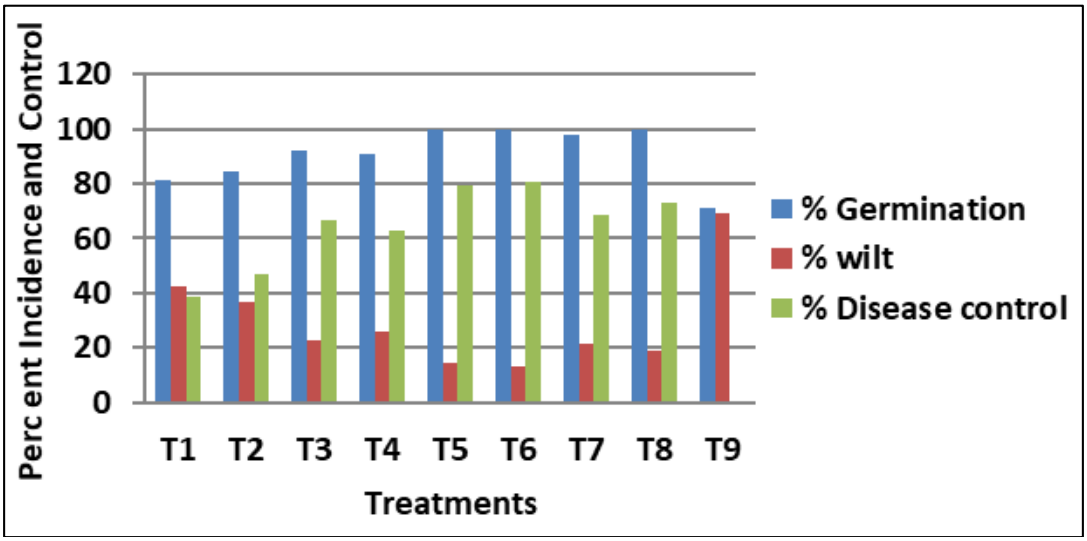


Fig 2: Evaluation of effective *Pseudomonas* isolates and fungicides against *Fusarium oxysporum* f. sp. *ciceri* (pot culture)

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