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Efficacy of *Pseudomonas* spp. against *Fusarium oxysporum* f. sp. *ciceri*

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Abstract
Efficacy of five isolates of *Pseudomonas fluorescens* were evaluated *in vitro* and using pot culture study against *Fusarium oxysporum* f. sp. *ciceri*. All isolates inhibited mycelial growth of test pathogen, but none of *Pseudomonas fluorescens* isolates showed complete inhibition of pathogen. PF5 was found most effective in inhibition of mycelial growth (77.92%) followed by isolates PF2 (68.61%). The PF4 and PF3 isolates recorded 64.58% and 55.27% inhibition of mycelial growth, respectively. The PF1 was found least effective in arresting the mycelial growth with 40.42 per cent mycelial inhibition of test pathogen. All isolates of *Pseudomonas fluorescens* significantly improved the percentage seed germination as compared to control (69.16%) with minimizing the seedling mortality both at pre emergence and post emergence stage. Highest seed germination was recorded in PF5 (87.49%) followed by the PF2 (84.16%), PF4 (80.83%) and PF3 (79.16%). PF1 gave least seed germination (77.49%) of chickpea (JG-62). The least pre and post emergence seedling mortality was found with PF5 followed by seed treatments of PF2, PF4 and PF3.

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

Introduction
Chickpea (*Cicer arietinum* L.) is world's third most important pulse crop after dry bean (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) and occupies a prominent place as preferred food legume. Chickpea is affected by several diseases, however among various diseases, major economic losses ranging from 10-40% worldwide were reported due to chickpea wilt (Nene *et al*., 1984) [8] which is one of the most important and destructive vascular disease of chickpea (Dileep kumar, 1999) [1]. 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]. Plant Growth Promoting Rhizobacteria (PGPR), such as *Pseudomonas* and *Bacillus* strains which are the major root colonizers (Manikandan *et al*., 2010) [6] can be utilized in management of soil borne diseases. Exploitation of local isolates of *Pseudomonas* can be utilized as safer and effective ecofriendly tool alternative to fungicide in management of chickpea wilt, hence investigation on study on efficacy of *Pseudomonas* spp. against *Fusarium oxysporum* f. sp. *ciceri* was undertaken.

Material and Methods
Present research work entitled "study on *in vitro* efficacy of *Pseudomonas* spp. against *Fusarium oxysporum* f. sp. *ciceri*" was conducted at the Department of Plant Pathology College of Agriculture, Latur during the year 2019-20.

In vitro efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri*
Seven days old culture of five isolates of *Pseudomonas fluorescens* and pathogen *i.e. Fusarium oxysporum* f. sp. *ciceri* were used for the study. Efficacy of *Pseudomonas fluorescens* was tested using Dual Culture Technique, where a loopful of bacterial culture was taken and streaked longitudinally in a zigzag fashion at one end of the petri plate, simultaneously 5 mm disc of mycelial growth of *F. oxysporum* f. sp. *ciceri* was placed on opposite side of the streak and incubated at 28 ± 2 ^{0}C for 5 days (Narasimha Rao *et al*., 2004) [7]. Four replications were maintained for each isolate with suitable control without antagonist. Growth of pathogen was measured 7 days after recording full growth of the test pathogen in control plate. Per cent inhibition of mycelial growth of test pathogen was calculated by using the formula.

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

where,
I = Per cent reduction
C = Radial growth of test pathogen (mm) in control
T = Radial growth of test pathogen (mm) in treatment

***In vitro* efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in pot culture**

Steam sterilized potting media of soil: sand: farm yard manure in 3:1:1 was filled in pot sterilized with five per cent sodium hypochlorite solution. Each pot is inoculated with *Fusarium oxysporum* f. sp. *ciceri* cultures multiplied on sand maize medium. These pots were kept slightly moist and incubated for seven days under glass house conditions. Surface sterilized seeds of chickpea Cv. JG 62 with one per cent sodium hypochlorite were given treatment with *Pseudomonas* spp. and sown in each of the pots. Four replications were maintained in each treatment. A control was maintained without seed treatment. Pots were watered regularly so as to maintain 50 per cent water holding capacity of the soil. Observations were recorded regularly for number of wilted plant.

Result and discussion

In vitro* efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri

All five isolates of *Pseudomonas fluorescens viz.*, PF1, PF2, PF3, PF4 and PF5 isolated from chickpea rhizosphere were tested *in vitro* against *Fusarium oxysporum* f. sp. *ciceri.* All isolates inhibited mycelial growth of test pathogen, but none of *Pseudomonas fluorescens* isolates showed complete inhibition of pathogen (PLATE I).

Among five isolates tested (Table 1 and Fig.1), PF5 was found most effective in inhibition of mycelial growth (77.92%) with least mycelial growth (19.87 mm). The second best isolates PF2 recorded 28.25 mm mycelial growth with per cent inhibition of mycelial growth up to 68.61%. The PF4 and PF3 isolates recorded 64.58% and 55.27% inhibition of mycelial growth, respectively. The PF1 was found least effective in arresting the mycelial growth and recorded 40.42 per cent mycelial inhibition of test pathogen.

Plate I: *Fusarium oxysporum* f. sp. *ciceri*

Table 1: *In vitro* efficacy of *P. fluorescens* against *Fusarium oxysporum* f. sp. *Cicero*

Tr. No.	Treatments	Colony diameter of test pathogen (mm)	Per cent inhibition
T_1	PF1	53.62	40.42
T_2	PF2	28.25	68.61
T_3	PF3	40.25	55.27
T_4	PF4	31.87	64.58
T_5	PF5	19.87	77.92
T_6	Control	90.00	
SE±		0.64	
CD at 1%		1.85	

Colony diameter of test pathogen = Average of four replications

Results obtained are in similar line with previous findings of anatgonastic nature of *Pseudomonas fluorescens*, Pandey and Sobitasimon (2015) [9] reported antagonistic activity of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri in vitro* and showed that all six isolates *viz*., PF18, PF4, PF20, PF19, PF13 and PF14 were found effective in maximum inhibition of pathogen in dual culture over control. Shahzaman *et al*. (2016) [12] recorded the effectiveness of thirty isolates isolated from chickpea rhizosphere against wilt pathogen in dual culture over control. Sankar *et al*. (2018) [10] evaluated ten isolates of *Pseudomonas* sp. against *Fusarium oxysporum* f. sp. *ciceri* and recorded maximum inhibition of pathogen with CPs3 (54.07%) followed by Pf1 (51.47%). Other workers like Karimi *et al*. (2012) [2] and Kandoliya and Vakhariya (2013) [4] also found similar results in inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* with anatagonistic action of *Pseudomonas* sp.

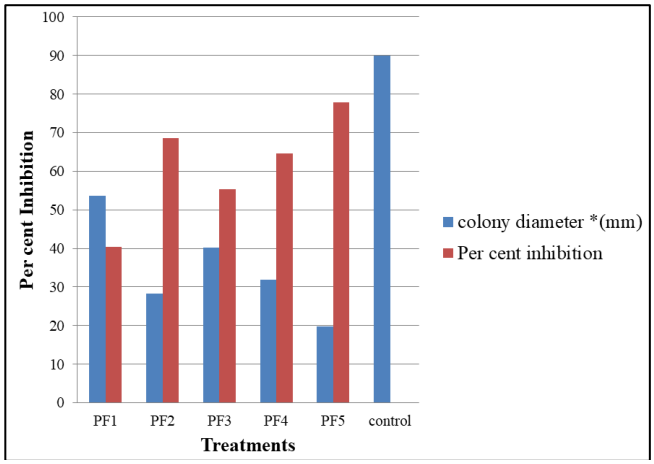

Fig 1: *In vitro* efficacy of *P. fluorescens* against *Fusarium oxysporum* f. sp. *ciceri*

Management of *Fusarium oxysporum* f. sp. *ciceri* with *Pseudomonas fluorescens* (Pot culture)

The pot culture experiment was conducted during *Rabi* season 2019-20 to evaluate efficacy of different isolates *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* using susceptible variety JG-62 (Table 2, PLATE II and Fig. 2).

Seed germination

Results revealed that seed treatment with all the isolates of *Pseudomonas fluorescens* significantly improved the percentage seed germination and it was observed in the range of 77.49 to 87.49 per cent as compared to control (69.16%). Highest seed germination was recorded in PF5 (87.49%) followed by the PF2 (84.16%), PF4 (80.83%) and PF3

(79.16%). PF1 gave least seed germination (77.49%) of chickpea cultivar JG-62.

Pre emergence seedling mortality

Results revealed that, all the isolates *Pseudomonas fluorescens* tested significantly minimized the seedling mortality both at pre emergence and post emergence stage and mortality of seedling recorded in the range of 12.50 to 22.50 per cent at pre emergence stage as compared to control (30.83%). Least pre emergence seedling mortality of 12.50 per cent was observed with PF5. This was followed by the other isolates *viz*., PF2 (15.83%), PF4 (19.16%) and PF3 (20.83%). PF1 was found least effective with highest pre emergence seedling mortality (22.50%) of chickpea cultivar JG-62.

Post emergence seedling mortality

Post emergence seedling mortality was observed from 16.20 to 30.11 per cent in all treatments as against, 38.56 per cent in control. The least post emergence seedling mortality was found in PF5 (16.20%). This was followed treatments *viz*., PF2 (18.84%), PF4 (22.66%) and PF3 (25.27%). The seed treated with PF1 was found least effective with highest percentage of post emergence seedling mortality (30.11%).

Plate II: Management of *Fusarium oxysporum* f. sp. *ciceri* with *Pseudomonas fluorescens* (Pot culture)

Table 2: Efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* (Pot culture)

Treatment no	Per cent Germination	Pre emergence mortality (%)	Post emergence mortality (%)
T_1	77.49 (61.67)	22.50 (28.31)	30.11 (34.27)
T_2	84.16 (66.54)	15.83 (23.44)	18.84 (25.72)
T_3	79.16 (62.83)	20.83 (27.15)	25.27 (30.17)
T_4	80.83 (64.03)	19.16 (25.95)	22.66 (28.42)
T_5	87.49 (69.28)	12.50 (20.70)	16.20 (23.73)
T_6	69.16 (56.26)	30.83 (33.72)	38.56 (38.38)
SE±	1.17	1.17	1.14
CD at 1%	3.38	3.38	3.28

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

The results of present investigation resembles with the finding of earlier records of scientist. Kala *et al*. (2014) [3] reported that seed treatment with *Pseudomonas fluorescens*, *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *ciceri* and *Pseudomonas fluorescens* were effective in controlling disease. Mahmood *et al*. (2015) [5] gave seed treatment to three different varieties of chickpea with six bioagents for *Fusarium oxysporum* f. sp. *ciceri* in glass house assay and observed that *Pseudomonas fluorescens* was most effective on all three varieties in reducing disease as compared to inoculated control. Pandey and Sobitasimon (2015) [9] gave soil application of six isolates of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* and observed the effectiveness of isolate PF18 (41.75%) in reducing the percentage of wilted plant followed by other isolates.

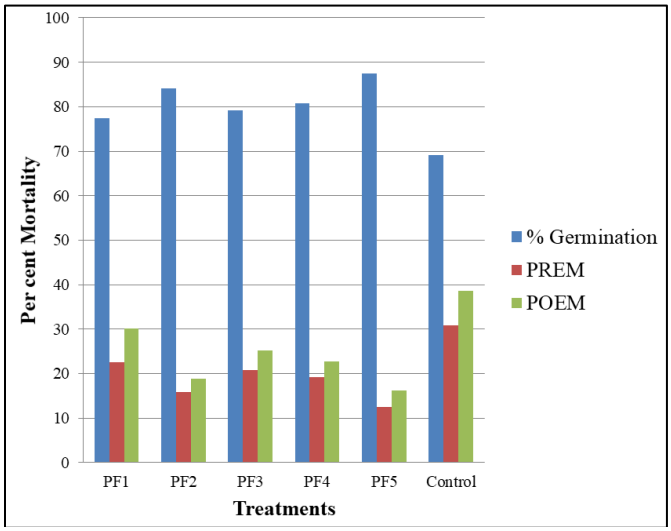

Fig 2: *In vitro* efficacy of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* (pot culture)

Conclusions

Thus from the results obtained on various aspects during present investigation on "study on efficacy of *Pseudomonas* spp. against *Fusarium oxysporum* f. sp. *ciceri*" it could be concluded that the isolates PF5 and PF2 were found to be more effective antagonists against *Fusarium oxysporum* f. sp. *ciceri* due to their high percentage of inhibition in dual culture technique and also they were effective in obtaining maximum germination with least post emergence mortality of seedling in pot culture study.

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