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Biosourcing of Rhizosphere isolates of *Pseudomonas Fluorescens* against Chick Pea wilt

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The present experiment was conducted during year 2018-2019 at JNKVV, Jabalpur.The bacterial bioagents were isolated from different rhizosphere soils and These isolates were used aganist *F. oxysporum f. sp. ciceri in vitro* and pot cultivation.The Observations were recorded antagonist effect of *Pseudomonas flurorescens* towards *F. oxysporum f.sp. ciceri* and phenotypic characters like Germination (%), Vigour index (%), Pre-emergence mortality (%), Post-emergence mortality (%), Root and Shoot length of chickpea variety's – JG 11 and JG 14 in vitro and pot cultivtion . A mong *Pseudomonas flurorescens* isolates of Pf-2 is effective in vitro and Pot culture

Key words : Bioagent, chickpea, phenotypic characters, rhizosphere,

INTRODUCTION

Chickpea (*Cicer arietinum* L) is cultivated in almost all parts of the world covering Asia, Africa, Europe, Australia, North America and South America continent. It is the largest produced food legume in South Asia and the third largest produced food legume globally, after common bean (*Phaseolus vulgaris* L.) and ûeld pea (*Pisum sativum* L.).It is also an important source of protein for millions of people in the developing countries, particularly in South Asia, who are largely vegetarian either by choice or because of economic reasons. In addition to having high protein content (20-22%), rich in ûber, minerals (phosphorus, calcium, magnesium, iron and zinc) and â-carotene. Chickpea is affected by several soil, seed and air borne diseases, among of all diseases Fusarium wilt is epidemics can be devastating to individual crops and cause up to 100% loss under favorable conditions. *F. oxysporum* f.sp*. ciceris* infects chickpea at seedling as well as at flowering and pod forming stage (Grewal, 1969), with more incidence at flowering and podding stage if the crop is subjected to sudden temperature rise and water stress (Chaudhry *et al*., 2007).

Research on plant growth promoting rhizobacteria (PGPR) over the last three decades has reveal their efficacy in improving the growth of plant by increasing seed emergence, plant weight, plant height and ultimately crop yield (Kloepper *et al*., 1981). Among PGPRs, most common are *Azotobacter, Acenitobacter, Pseudomonas fluorescens*, *Bacillus* and *Rhizobium* spp., etc. During the last decade much of the research has focused on an organisms belonging to *Bacillus* and *Pseudomonas* species. *Pseudomonas fluorescens* is a gram-negative, rod-shaped, and non pathogenic bacterium belong to the genus *Pseudomonas*. that is known to inhabit primarily the soil, plants, and water (Peix et al., 2009). *Pseudomonas* are the most extensively studied biocontrol agents for management of soil borne and seed borne pathogen. It posses plant growth promoting rhizobacteria especially *Pseudomonas fluorescens* assist in plant colonization and suppress various plant diseases caused by soil borne pathogenic microorganisms (Handelsman

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and Stabb, 1996). The mechanisms of plant diseases control by soil *Pseudomonas* includes production of antibiotics, siderophores, volatile compounds like HCN, ammonia, and induction of systemic resistant and competition for nutrients (O'Sallivan and O'Gara, 1992). Bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *Pseudomonas fluorescens* have been successfully used for biological control of several plant pathogens (Ramamoorthy *et al*., 2001). Earlier studies indicated that disease could be controlled by application of fluorescent Pseudomonads as seed, soil or root treatment and induced systemic resistance and thus protect the leaves. The synthesis of yellow-green, fluorescent, Pigmentation under UV light is a characteristic property of some *Pseudomonas* spp. (Stanier *et al*., 1966).

MATERIAL AND METHODS

Collection of soil samples

Composite soil samples was collected from different rhizosphere of healthy plants of Brinjal, Chickpea, Chilli, Paddy, Red gram, Tomato, Soybean, Sesamum at Maharajpur Horticulture Field Breeding Field, Sesamum and Niger Field at JNKVV, Jabalpur.

Isolation of Rhizospheric native Pseudomonas sp. bacteria

Antagonistic bacteria were isolated by following serial dilution technique (Johnson and Curl, 1977). Composite soil sample was collected from rhizosphere of healthy plants. The soil was dried under shade and then used for serial dilution. To get 10^{-1} dilution, ten gram of this soil was dissolved in 90 ml of sterile distilled water. From this one ml of soil suspension was taken and added to nine ml of sterile distilled water to get 10^{-2} dilution. This was repeated until a final dilution of 1-8 for bacteria. One milliliter of each 10^{-7} or 10^{-8} dilution was pipetted into sterile petri-dishes containing prepared selective medium *Pseudomonas* agar (fluorescein). These petriplates were incubated in incubator at 28±1°C for 24 hours. On the basis of visual observation, it is identified as Pseudomonas sp. Then by using loop streak method, it was streaked in zig-zag manner over the solidified agar plate, care was taken not to break the medium surface. The developing colonies were discrete and then transferred to fresh medium for getting pure culture of *Pseudomonas spp.*

Table 1: List of isolates *Pseudomonas fluorescens* isolated from rhizosphere used in the study

Different rhizosphere Isolates	Name of the crop	Area
Pf 1	Brinjal	Maharajpur Horticulture Field
Pf 2	Chickpea	Breeding Field
Pf 3	Chilli	Maharajpur Horticulture Field
Pf 4	Paddy	Breeding Field
Pf 5	Redgram	Breeding Field
Pf 6	Tomato	Maharajpur Horticulture Field
Pf 7	Soyabean	Breeding Field
Pf 8	Seasmum	Sesamum and Niger Field

Collection of disease sample

Chickpea plants showing the symptoms of wilt at seedling, flowering and pod formation stage like drooping and pale-coloured leaves and Discoloration of the pith and xylem in roots were collected from sick plots at Department of Plant Breeding and Genetics, JNKVV, Jabalpur.

Dual culture test

The efficacy of biocontrol agents was evaluated in vitro against *F. oxysporum f. sp. ciceri*, by dual culture method (Dhingra and Sinclair, 1985) and the seven days old culture grown on PDA media was used. Inoculum disc of 5 mm pathogen was slotted with cork borer and were picked up with sterile needle. The autoclaved and cooled PDA medium was poured in sterilized glass petri plates (90 mm dia.), allowed to solidify and 5 mm disc of the test pathogen was picked up with sterile needle and inoculated at one end and the *P fluorescens* was streaked at one end at the same time. They were incubated for 7 days at temperature 28 + 2ºC and the observation on colony diameter of the target pathogen were noted vertically and horizontally and their mean was noted as average colony diameter. The percentage inhibition over

control was calculated by following formula (Vincent, 1947)

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

Where,

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

Seed Source

Four varieties JG11, JG14, JG62 and JG315 of chickpea were taken from Chickpea Breeding Unit, JNKVV, Jabalpur.

Mass multiplication and Pathogenicity test by Soil infestation

Soil infestation method was followed to prove the pathogenicity of the fungus. The *Fusarium oxysporum f. sp. ciceris* was mass multiplied on sand maize meal medium (90 g of sand, 10 g of maize meal and 40 ml of water) in 250 ml conical flasks.The medium was inoculated with two discs of 10 mm diameter mycelial growth of ten days old culture of *Fusarium oxysporum* grown on PDA. The flasks were incubated at 28 ± 2°C. for 3 weeks. The three weeks of old inoculum was added to soil in pots @ 100 g kg-1. The seeds of chickpea were sown simultaneously with pathogen inoculation @ 15 seeds per pot and an un-inoculated control was maintained. The plants were observed for wilt symptoms. Each treatment replicated thrice (Padmodaya and Reddy,1998).

Effect of Pseudomonas fluorescens on growth parameters

Data on germination percentage was recorded after 10 days and at the time of maturity Plant height (cm), was Calculated the Vigor index percentage as follows (Abdul Baki and Anderson 1973).

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Vigour index (%) = Germination% × Seedling length on the day of final count

Pre and post-emergence mortality (%)

Pre-emergence mortality was recorded immediate after complete emergence of the plants and post emergence mortality was recorded at 90 days after sowing. The pre- and post-emergence mortality was calculated using the following formula;

$$\text{Pre-emergence mortality (\%)} = \frac{\text{No. of diseased un-germinated seed}}{\text{Number of sown seed}} \times 100$$

$$\text{Pre-emergence mortality (\%)} = \frac{\text{No. of seedling collapsed}}{\text{Total no. seedling emerged}} \times 100$$

$$\text{Total Mortality (\%)} = \text{Pre-emergence mortality (\%)} + \text{Post-emergence mortality(\%)}$$

Root and Shoot length

The healthy plants were carefully uprooted to measure root and shoot length using scale after 50 days. The experiment of designed in Completely Randomized Design with three replications.

RESULTS AND DISCUSSION

Table 1: Screening of eight different *Pseudomonas fluorescens*(PF) isolates against *Fusarium oxysporum f.sp. ciceris* (FOC) under dual culture on PDA

Out of all isolates *Pf* -2isolate was recorded Maximum percent inhibition (50.21%) followed by *Pf*-3(47.92%), *Pf*-1(44.02%), *Pf*-4(41.27%), *Pf*-6(.37.82%) and *Pf*-7(35.30%) on PDA whereas there was less inhibition recorded in isolate *Pf*-5(32.82%), *Pf*-8(26.35%) and there was no inhibition in control. Results explained by Akter *et al*. (2014) reported that the bacterial isolate KMB25 showed maximum inhibition (68.44%) fallowed by *Pseudomonas fluorescens* isolates TMB33 (60.89%), PMB38 (60.22%), UMB20 (50.00%) and BMB42 (48.22%)

Table 2: Effect of different isolates of *Pseudomonas fluorescens* on fusarium wilt (Fusarium oxysporum f.sp ciceris) disease incidence on JG 11 variety of chickpea in Pots

Germination percentage

A mong the treatments maximum germination percent was observed in T_2 (93.33%) followed by T_3(91.10%) ,T_1(88.88%),T_4(86.66%), T_6(84.44%), T_7(82.22%), T_8(80.00%), T_5(77.77%), T_{10}(73.33%) and lowest germination per cent was observed in T_9 (70.00%).

Pre-emergence mortality (%)

Maximum pre emergence mortality was observed T_9(28.91%) fallowed by T_{10}(26.67%), T_5(22.22%), T_8(20.00%), T_7(17.78%), T_6(15.56%), T_4(13.34%), T_1(11.11%), T_3(8.89%) and lowest pre emergence mortality was observed in T_2(6.67%).

Post-emergence mortality (%)

A mong all treatments maximum post emergence mortality was T_9(31.21%) fallowed by T_{10}(18.18%), T_5(16.83%), T_8(16.16%), T_7(16.06%), T_6(15.80%), T_4(15.38%), T_1(15.01%), T_3(14.64%) and lowest post emergence mortality was observed in T_2(14.28%).

Total mortality (%)

Maximum Total mortality was observed T_9(51.00%) fallowed by T_{10}(40.00%), T_5(35.55%), T_8(33.33%), T_7(32.59%), T_6(31.85%), T_4(31.11%), T_1(27.65%), T_3(24.19%) and lowest total mortality was observed in T_2(20.74%)

Table 3: Combined influence of different isolates of PF and FOC on phenotypic characters of chickpea – JG 11 variety

Root length

A mong the treatments maximum root length was observed in T_2(18.50cm) followed by T_3(17.00cm), T_1(15.30cm), T_4(15.50cm), T_6(15.23cm), T_7(14.22cm), T_8(13.46cm), T_5(14.53), T_{10}(14.00cm) and lowest root length was observed in T_9(10.33cm).

Shoot length

Maximum shoot length was observed in T_2(39.40cm) followed by T_3 (36.20cm), T_1(34.76cm), T_4(35.16cm), T_6(35.33cm), T_7(34.90cm), T_8(31.46cm), T_5(33.70cm),

T_{10}(32.00cm)and lowest root length was observed in T_9(27.16cm).

Vigor index (%)

Among the treatments maximum vigor index observed in T_2(5403.80) followed by T_3(484-6.52),T_1(4449.33),T_4(4390.19), T_6(4269.28), T_7(4038.64),T_8(3593.60), T_5(3750.84), T_{10}(3373.18) and lowest vigor index was observed in T_9 (2664.778).

Table 4: Effect of different isolates of *Pseudomonas fluorescens* on fusarium wilt (Fusarium oxysporum f.sp ciceris) disease incidence on JG14 variety of chickpea

Germination percentage (%)

A mong the treatments maximum germination percent was observed in T_2 (88.88%) followed by T_3(86.66%), T_1(84.44%), T_4(82.22%),T_6(80.00%), T_7(77.77%), T_8(73.33%), T_5(75.55%), T_{10}(71.10%) and lowest germination per cent was observed in T_9 (68.88%).

Pre-emergence mortality (%)

Maximum pre emergence mortality was observed T_9(31.11%) fallowed by T_{10}(28.89%), T_5(24.44%), T_8(26.67%), T_7(22.22%), T_6(20.00%), T_4(17.78%), T_1(15.56%), T_3(13.34%) and lowest pre emergence mortality was observed in T_2(11.11%).

Post-emergence mortality (%)

Maximum post emergence mortality wasT_9(35.45%) fallowed by T_{10}(24.84%), T_5(20.53%), T_8(21.21%), T_7(22.72%), T_6(19.44%), T_4(24.35%), T_1(18.37%), T_3(17.94%) and lowest post emergence mortality was observed in T_2(17.57%).

Total mortality (%)

Maximum Total mortality was observed T_9(55.55%) fallowed by T_{10}(46.66%), T_5(39.99%), T_8(42.22%), T_7(40.00%), T_6(35.55%), T_4(37.77%), T_1(31.10%), T_3(28.88%) and lowest total mortality was observed in T_2(26.66%).

Table 5: Combined influence of different isolates of PF and FOC on phenotypic characters of chickpea – JG 14 variety

Root length

A mong the treatments maximum root length was observed in T_2(19.26cm) followed by T_3(17.53cm),

T_1(16.30cm), T_4(16.00cm), T_6(15.80cm), T_7(15.73cm) ,T_8(14.80cm), T_5(14.53cm), T_{10}(15.33cm) and lowest root length was observed in T_9 (10.50cm).

Table 1: Screening of eight different *Pseudomonas fluorescens* isolates against *Fusarium oxysporum* f.sp. *ciceris* under dual culture on PDA

Treatment	*Pf* Isolates	Fungal radial growth (mm) on PDA	Growth inhibition(%)
T_1	*Pf*-1	40.66	44.02
T_2	*Pf*-2	36.16	50.21
T_3	*Pf*-3	37.83	47.92
T_4	*Pf*-4	42.66	41.27
T_5	*Pf*-5	49.00	32.54
T_6	*Pf*-6	45.16	37.82
T_7	*Pf*-7	47.00	35.30
T_8	*Pf*-8	53.50	26.30
T_9 (control)		72.65	00.00
SEm±		1.01	
CD at 5%		3.04	

Shoot length

A mong the treatments maximum shoot length was observed in T_2(40.90cm) followed by T_3(39.30cm), T_1(35.76cm), T_4(37.16cm), T_6(36.23cm), T_7(35.90cm), T_8(35.50cm), T_5(35.03cm), T_{10}(32.00cm) and lowest root length was observed in T_9 (30.06cm).

Vigor index (%)

A mong the treatments maximum vigor index observed in T_2(5347.02) followed by T_3 (4924.88), T_1(4395x.94), T_4(4370.81), T_6(4162.40), T_7(40-15.26), T_8(3688.49), T_5(3744.25), T_{10}(3365.16) and lowest vigor index was observed in T_9 (2835.10). Usharani *etal.*(2009) reported that treatment with *Pseudomonas fluorescens* had increased the plant stand (92.33%), root length(17.25cm), shoot length(36.32cm), vigor index(4982.94), reduced disease incidence (19.43%) as compared with control which had the plant stand(81.33%), root length(11.57cm), shoot length(21.12cm), vigor index(2747.64) and disease incidence (38.25%). Saravanan *et al.*(2013) reported that Inoculation with fluorescent Pseudomonas induced a significant increase in root and shoot length (123 and 96%, respectively) over the uninoculated control. In contrast, pathogen treatment caused a reduction in root and shoot length (4 to 24% and 13 to 23%, respectively).

Table 2: Effect of different isolates of *Pseudomonas fluorescens* on fusarium wilt (*Fusarium oxysporum* f.sp *ciceris*) disease incidence on JG 11 variety of chickpea in Pots

Isolates	Treatments	Germination (%)	Pre emergence mortality (%)	Post emergence mortality (%)	Total mortality (%)
Pf-1	T_1	88.88	11.11	15.01	27.65
Pf-2	T_2	93.33	6.67	14.28	20.74
Pf-3	T_3	91.10	8.89	14.64	24.19
Pf-4	T_4	86.66	13.34	15.38	31.11
Pf-5	T_5	77.77	22.22	16.83	35.55
Pf-6	T_6	84.44	15.56	15.80	31.85
Pf-7	T_7	82.22	17.78	16.06	32.59
Pf-8	T_8	80.00	20.00	16.16	33.33
T C	T_9	71.08	28.91	31.21	51.10
Control	T_{10}	73.33	26.67	18.18	40.00
SEm±		1.72	1.72	0.91	1.86
CD		5.12	5.12	2.70	5.54

CONCLUSION

The above experiment concluded that Pf-2, Pf-3 isolates were shown increasing in germination Percentage, Root, Shoot, length, vigour index, low Pre and Post emergence mortality

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Table 3: Combined influence of different isolates of PF and FOC on phenotypic characters of chickpea – JG 11 variety

Isolates	Treatments	Germination (%)	Pre emergence mortality (%)	Post emergence mortality (%)	Total mortality (%)
Pf-1	T_1	88.88	11.11	15.01	27.65
Pf-2	T_2	93.33	6.67	14.28	20.74
Pf-3	T_3	91.10	8.89	14.64	24.19
Pf-4	T_4	86.66	13.34	15.38	31.11
Pf-5	T_5	77.77	22.22	16.83	35.55
Pf-6	T_6	84.44	15.56	15.80	31.85
Pf-7	T_7	82.22	17.78	16.06	32.59
Pf-8	T_8	80.00	20.00	16.16	33.33
T C	T_9	71.08	28.91	31.21	51.10
Control	T_{10}	73.33	26.67	18.18	40.00
SEm±		1.72	1.72	0.91	1.86
CD		5.12	5.12	2.70	5.54

Table 4: Effect of different isolates of *Pseudomonas fluorescens* on fusarium wilt (*Fusarium oxysporum* f.sp *ciceris*) disease incidence on JG14 variety of chickpea

Isolates	Treatments	Germination (%)	Pre emergence mortality (%)	Post emergence mortality (%)	Total mortality (%)
Pf-1	T_1	84.44	15.56	18.37	31.10
Pf-2	T_2	88.88	11.11	17.57	26.66
Pf-3	T_3	86.66	13.34	17.94	28.88
Pf-4	T_4	82.22	17.78	24.35	37.77
Pf-5	T_5	75.55	24.44	20.53	39.99
Pf-6	T_6	80.00	20.00	19.44	35.55
Pf-7	T_7	77.77	22.22	22.72	40.00
Pf-8	T_8	73.33	26.67	21.21	42.22
T C	T_9	68.88	31.11	35.45	55.55
Control	T_{10}	71.10	28.89	24.84	46.66
SEm±		1.85	1.85	2.61	2.43
CD		5.52	5.52	7.77	7.23

Table 5: Combined influence of different isolates of PF and FOC on phenotypic characters of chickpea – JG 14 variety

Isolates	Treatments	Root length (cm)	Shoot length (cm)	Vigor index (%)
Pf-1	T_1	16.30	35.76	4395.94
Pf-2	T_2	19.26	40.90	5347.02
Pf-3	T_3	17.53	39.30	4924.88
Pf-4	T_4	16.00	37.16	4370.81
Pf-5	T_5	14.53	35.03	3744.25
Pf-6	T_6	15.80	36.23	4162.40
Pf-7	T_7	15.73	35.90	4015.26
Pf-8	T_8	14.80	35.50	3688.49
T C	T_9	10.50	30.66	2835.10
Control	T_{10}	15.33	32.00	3365.16
SEm±		0.31	0.29	
CD		0.93	0.87	

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