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ISOLATION AND EVALUATION OF FLUORESCENT *Pseudomonas* ISOLATES AGAINST *Fusarium oxysporum* f.sp. *ciceris* UNDER *INVITRO* CONDITIONS

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ABSTRACT

A total of 12 fluorescent *Pseudomonas* isolates collected from rhizosphere soils of healthy chickpea plants from six districts of Andhra Pradesh were evaluated against chickpea Fusarium wilt pathogen *i.e*., *Fusarium oxysporium* f.sp. *ciceris* by dual culture technique. Significant differences in inhibition percentage were observed among the treatments. Maximum per cent inhibition was observed with CRP-6 isolate (58.32%) followed by CRP-8 (56.90%) and minimum per cent inhibition of 4.57 was observed in CRP-10 isolate.

KEY WORDS: Chickpea, fluorescent *Pseudomonas*, dual culture, *Fusarium.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop of India, belonging to leguminasae family and commonly known as Bengal gram. India occupies world's 70 per cent of chickpea cropped area and 67 per cent production and Andhra Pradesh contributes total 7 per cent of production in the country approximately. In A.P, it is grown in an area of 3.42 lakh ha with a production of 3.91 lakh tonnes accounting for productivity of 1143 Kg ha^{-1} (Anonymous, 2017).

Among the diseases, Fusarium wilt caused by *Fusarium oxysporum* f.sp.*ciceris* (Padwick) Matuo & K.Sato (FOC) is highly destructive and worldwide in occurrence (Nene *et al*., 1989). The disease appears at almost all stages of plant growth. It survives in the soil for many years and is also seed borne in nature. Rhizosphere bacteria such as *Pseudomonas* have proved to be effective biocontrol agents against soil borne diseases of many crop plants. In this experiment, isolation of *Pseudomonas* and their efficacy was tested against the wilt pathogen *i.e*., *Fusarium oxysporum* f.sp. *ciceris.*

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MATERIAL AND METHODS

The pathogen *Fusarium oxysporum* f.sp.*ciceris* (Foc) was isolated from the wilt affected parts of chickpea plant and its pathogenecity was proved on susceptible chickpea cultivar *i.e.*, JG-62. The pathogen *Fusarium oxysporum* f.sp.*ciceris* (Foc) was isolated from the wilt affected parts of chickpea plant and its pathogenicity was proved on susceptible chickpea cultivar *i.e.*, JG-62. For isolation of *Pseudomonas,* serial dilution method proposed by Johnson and Curl (1972) was followed. For isolation, composite soil samples were collected from rhizosphere of healthy plants of chickpea. The soil was dried under shade and then used for serial dilution. For isolation of *Pseudomonas* 10^{-3} to 10^{-6} dilutions were prepared and 0.1 ml of the required dilution was spread onto sterilized Petri plates containing cooled Kings B medium (King *et al*., 1954). After solidification they were kept in an incubator at 28±2°C and observed at frequent intervals for the development of colonies. Three Petri plates were maintained for each dilution. After 48 hours of incubation from the dilution plates, transparent, smooth margined bacterial colonies were selected and exposed to UV light in UV chamber at 365 nm for few seconds and based on fluorescens they were identified as fluorescent Pseudomonads. Then these colonies of *Pseudomonas* were transferred onto fresh Nutrient agar/ Kings-B medium by streak plate method (Dilip Kumar and Dube, 1992) and pure cultures were obtained. All purified isolates were found Gram-negative and rod shaped upon Gram staining.

After confirmation of fluorescent nature of these isolates, they were tested against the pathogen by dual culture method. A 5 mm disc of pathogen (one week old culture) was placed at the centre of PDA plate. Antagonistic bacteria were streaked individually on both sides of the pathogen at 2.5 cm distance leaving 2.0 cm from periphery. Plates inoculated with pathogen alone served as control. Three replicates were maintained for each bacterial bioagent and incubated at 28 ± 2°C. Observations were recorded after one week to see the effect of antagonism on pathogen by bioagents. Per cent inhibition of mycelial growth of test pathogen over control was calculated by using the formula.

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

C = Radial growth (mm) of pathogen in control plate.

T = Radial growth (mm) of the pathogen in treatment plate.

Two potential *Pseudomonas* isolates from the above experiment was selected and qualitative HCN production was estimated by following Bakers and Schippers (1987) method.

RESULTS AND DISCUSSION

A total of 12 fluorescent *Pseudomonas* isolates were isolated from chickpea rhizosphere of healthy plants on Kings B medium. Out of suspected 20 isolates, 12 isolates were identified and confirmed as fluorescent *Pseudomonads* based on fluorescens nature and these isolates were numbered from CRP 1 to CRP 12 (Table 1, Fig.1). Gram staining of these 12 fluorescent *Pseudomonas* isolates exhibited Gram negative reaction. This is in accordance with King *et al. (*1954**)** who isolated *Pseudomonas* spp. from rhizosphere soil of chickpea plants by using King's B medium. Sharma (2012) isolated 23 *Pseudomonas* isolates from rhizosphere and rhizoplane of different crops including chickpea and identified based on their growth on *Pseudomonas* selective (fluorescein) agar medium and Gram staining.

Dual culture test results indicated that among 12 *Pseudomonas* isolates evaluated against the pathogen the isolate CRP-6 recorded highest inhibition percentage of 58.32 (Table 2) followed by the isolate CRP-8, which recorded inhibition percentage of 56.90 (on par with each other). The isolates CRP-7, CRP-11 and CRP-9 - recorded inhibition percentage of 43.21, 41.10 and 41.00 respectively and were on par with each other. The lowest percentage of inhibition was recorded with the isolate CRP-10 (4.57).

The experimental results were in agreement with Kandoliya and Vakharia (2013) who isolated ten *Pseudomonas fluorescens* isolates from chickpea rhizosphere and evaluated against Foc in dual culture and noted highest per cent inhibition of 83.5 with Pf-3. In similar experiment, Manjunatha *et al*. (2012) evaluated two already identified potential *Pseudomonas* isolates viz., Pf-4 and Pf-6 against *F. udum* and recorded more inhibition in Pf-4 than Pf-6.

Generally, the biocontrol quality of antagonistic bacteria involves either competition or production of metabolites such as HCN, siderophores, an-tibiotics or extracellular enzymes etc., that acts antagonistically towards the plant pathogens (Sang *et al.,* 2006).

In the present study clear inhibition zones were observed in dual culture of pathogen with *Pseudomonas* (CRP-6, CRP-8). Highest inhibition zone of 6 mm was observed in the isolate CRP-8 followed by CRP-6 which has an inhibition zone of 4mm. It might be due to the antifungal substances and/or cell wall degrading enzymes released by the bacteria into the culture medium (Fatima *et al*., 2009). The area of inhibition zone was taken as a measure of antagonistic potential of the isolate. *Pseudomonas fluorescens* inhibits the development of mycelium much more with volatile compounds.

These two potential isolates *i.e.* CRP-6 and CRP-8 also produced HCN which was confirmed by the change in the colour of the filter paper form yellow to reddish brown (+++). HCN effectively blocks the cytochrome oxidase pathway and also inhibits the transport of electrons thereby disrupting energy supply to the cell leading to the death of the organism (Manwar *et al*., 2011). Manjunatha *et al*. (2012) reported that two strains of *Pseudomonas* Pf-4 and Pf-6 strains produced antibiotic compounds such as phenazine, HCN and salicylic acid that showed antagonistic activity towards *F.udum.*

In the present experiment, *in vitro* studies indicated that two fluorescent *Pseudomonas* isolates *i.e.* CRP-6 and CRP-8, have shown highest inhibition activity against *Fusarium oxysporum* f.sp. *ciceris.*

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Table 1. List of fluorescent *Pseudomonas* isolates collected from soils of chickpea from different parts of Andhra Pradesh

S. No.	Isolate name	Place of collection	Mandal	District
1.	CRP-1	Soganuru	Yemmiganur	Kurnool
2.	CRP-2	Giddalur	Giddalur	Prakasam
3.	CRP-3	P.D.Palli	Dharmavaram	Anantapuramu
4.	CRP-4	Ponnalur	Ponnalur	Prakasam
5.	CRP-5	Lakshmipuram	Duttalur	Nellore
6.	CRP-6	Tullur	Tullur	Guntur
7.	CRP-7	Mandadam	Tullur	Guntur
8.	CRP-8	Karamchedu	Karamchedu	Prakasam
9.	CRP-9	Chejarla	Chejarla	Nellore
10.	CRP-10	Koppolu	Ongole	Prakasam
11.	CRP-11	Palaparru	Pedanandipadu	Guntur
12.	CRP-12	Proddutur	Proddutur	Kadapa

Table 2. *In vitro* evaluation of fluorescent *Pseudomonas* isolates *Fusarium oxysporum* f. ps. *ciceris* by dual culture technique

S. No.	Name of the isolate	Radial growth (mm)	Inhibition per cent over control
1.	CRP-1	43.00	32.17 (34.57)
2.	CRP -2	47.16	25.63 (30.35)
3.	CRP -3	40.78	37.30 (37.66)
4.	CRP -4	44.68	29.53 (32.92)
5.	CRP -5	41.50	34.54 (36.01)
6.	CRP -6	26.41	58.32 (49.82)
7.	CRP -7	36.00	43.21 (41.12)
8.	CRP -8	27.30	56.90 (48.99)
9.	CRP -9	37.50	41.00 (39.84)
10.	CRP -10	60.50	04.57 (12.35)
11.	CRP -11	37.52	41.10 (39.90)
12.	CRP -12	39.75	37.30 (37.66)
	control	63.40	0.00 (0.00)
	Sem±		0.81
	CD 5%		2.40
	CV (%)		3.62