ISSN 2277-9663

AGRES – An International e-Journal

Volume 1 Issue 1 January-March,2012

STUDY OF ANTAGONISTIC POTENTIALITY OF FLUORESCENT *PSEUDOMONADS* ISOLATES AGAINST *Fusarium Oxysporum* f. sp. *ricini* CAUSING WILT OF CASTOR

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ricini* is a very serious disease in castor (*Ricinus communis* L.) growing areas of the Gujarat state in general and north Gujarat in particular. The pathogen is soil-borne and application of fungicides is very expensive and also polluting the ecosystem. Several strains of fluorescent *pseudomonads* isolates have been reported to suppress soil borne diseases caused by fungal pathogens. Fifteen fluorescent *pseudomonads* isolates were isolated on King's B medium from the rhizosphere and rhizoplane of castor plant, which fluoresced under ultraviolet light and found to be gram negative, rod shaped urease positive and phosphate solubilization positive. Antagonistic activity of fluorescent *pseudomonads* isolates under *in vitro* dual culture assay reflected that isolates FP-IV and FP-X showed maximum growth suppression (93.33 %) of *F. oxysporum* f. sp. *ricini* followed by FP-I (92.22 %), FP-IX (92.22 %), FP-XV (90.00 %), FP-II (88.89 %), FP-XII (88.89 %), FP-XI (86.67 %) and FP-XIV (85.56 %), whereas minimum suppression (56.69 %) was observed with isolate FP-VI. Phosphate solubilizing reaction by formation of clear inhibition zone around the colony on Pikovskaya's medium.

KEY WORDS Castor, Wilt, Antagonistic, Fluorescent *pseudomonads*, *F. oxysporum*

INTRODUCTION

The present days need is to develop and utilize the effective low cost eco-friendly technologies in the crop production programmes. The increasing use of potentially hazardous fungicides for the management of soil borne plant diseases have created the problem of resistance in plant pathogens, besides posed a serious problem of pollution in the ecosystem. Castor wilt is serious in Gujarat state and caused heavy losses in past and also causing in present. Wilt incidence was recorded up to 80 per cent in north Gujarat (Patel *et al.,* 2003). Castor wilt is difficult to manage due to soil borne nature. The biological control is one of the methods by which damage could be minimized up to great extent. The indigenous potential isolates of fluorescent *pseudomonads* were isolated from the rhizosphere and roots of castor plants towards the development of effective and eco-friendly management of castor wilt.

MATERIALS AND METHODS

1. Collection of Soil and Plant Samples

Fifty soil and roots samples were collected from castor field plots of different locations of Patan and Banaskantha districts (Table 1), where the castor is commonly grown. Healthy plants of castor (*Ricinus communis* L.) of 60-75 days growth were carefully uprooted along with adhering soil and were carried to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teased out and used for isolation of rhizosphere bacteria. Soil particles adhering tightly to the roots were allowed to go with the roots for isolation of rhizoplane bacteria.

2. Isolation of Fluorescent *Pseudomonads* Isolates

Each soil sample of 10g of closely associated rhizosphere was added to 250 ml flask containing 90 ml sterilized distilled water. For isolation of rhizoplane bacteria, roots were cut into approximately 2-3 cm long pieces and 10g of root bits were then transferred to 90 ml sterilized distilled water. The flasks were placed on a rotary shaker for 1 hr to allow root associated bacteria to diffuse. Three replications were kept for each location and serial dilution of rhizosphere and rhizoplane samples were made up to 10^6. An aliquot of 0.1 ml from 10^6 dilution of each sample was spread plated over solidified King's medium B (Protease peptone No. 3 20.00 g, Dipotassium hydrogen phosphate 1.50 g, Magnesium sulphate $7H_2O$ 1.50 g, Agar 20.00 g, Glycerol 15.00 ml and Distilled water 1 lit.), selective medium on which preferentially fluorescent *pseudomonads* recovered under aseptic conditions. The plates were incubated at 30 ± 1^oC for 24 - 48 hrs. Colonies of different morphology were examined for their fluorescence under ultraviolet light (240 - 340 nm). The colony showing fluorescence was picked-up and was further purified by streaking on same medium plates. The purified cultures were finally transferred on to solid King's B medium and preserved at low temperature (4^o C) in refrigerator in the Department of Plant Pathology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, for further studies.

2.1 Identification

All the rhizobacterial isolates were identified with the help of morphological, cultural and biochemical characteristics as per the "Bergey's Manual of Determinative Bacteriology." The cultures were tested for characters *viz.,* colony and cell morphology, gram reaction and urease activity.

3. Antagonistic Properties of Fluorescent *Pseudomonads* Isolates against *Fusarium oxysporum* f. sp. *ricini in vitro*

Antagonistic properties of bacterial isolates were tested against *Fusarium oxysporum* f. sp. *ricini* on Potato Dextrose Agar (PDA) plates using a dual culture technique (Skidmore and Dickinson, 1976). Agar blocks of 5 mm diameter containing seven days old mycelia were placed in the centre of Petri plates containing 20 ml PDA. A loopful of 24 hours old culture of bacterial isolate was inoculated at 2 cm juxtaposed to the pathogen on each plate. The fungal pathogen inoculated centrally on PDA plate without bacterial inoculation served as control. Three replications were kept for each treatment. The plates were incubated at 30 ± 1^oC for seven days. The growth of the fungus toward the bacterial colony was suppressed and the growth inhibition zone was calculated by using the following formula (Bhatia *et al.,* 2005).

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

Where,

I = Growth inhibition (%)

C = Growth in control plate (mm)

T = Growth in treated plate (mm)

4. Evaluation of Fluorescent *Pseudomonads* Isolates for Phosphate Solubilizing Activity

The phosphate solubilizing capacity of isolates was tested *in vitro* using Pikovskaya's agar medium (Yeast extract 0.50 g, Dextrose 10.00 g, Calcium phosphate 5.00 g, Ammonium sulphate 0.50 g, Potassium chloride 0.20 g, Magnesium sulphate 0.10 g, Manganese sulphate 0.0001 g, Ferrous sulphate 0.0001 g, Agar 15.00 g and Distilled water 1 lit.). Petri plate containing only Pikovskaya's medium served as control. Three replications were kept for each treatment. The plates were incubated at 30 ± 1^{o}C for five days. Formation of a clear inhibition zone around the colony was considered as positive reaction for phosphate solubilization.

RESULTS AND DISCUSSION

1. Isolation of Fluorescent *Pseudomonads* Isolates From Rhizosphere and Rhizoplane

Fifteen fluorescent bacterial isolates were obtained on selective medium viz., King's B medium from the rhizosphere and rhizoplane of castor by dilution plating method (10^{6} cfu ml^{-1}) after incubation period of 24 - 48 hours at 30 ± 1^{o}C and examined the fluorescence under ultraviolet light (200 - 340 nm). These isolates were designated as FP-I, FP-II, FP-III, FP-IV, FP-V, FP-VI, FP-VII, FP-VIII, FP-IX, FP-X, FP-XI, FP-XII, FP-XIII, FP-XIV and FP-XV (Plate 1). Out of 20 samples collected from ten villages of patan district (Table 2), nine fluorescent *pseudomonads* isolates (FP-I to FP-IX) were obtained, whereas six isolates (FP-X to FP-XV) were gained from 30 samples from seven villages of Banaskantha district (Table 3).

ISSN 2277-9663
AGRES – An International e-Journal

Volume 1 Issue 1 January-March,2012

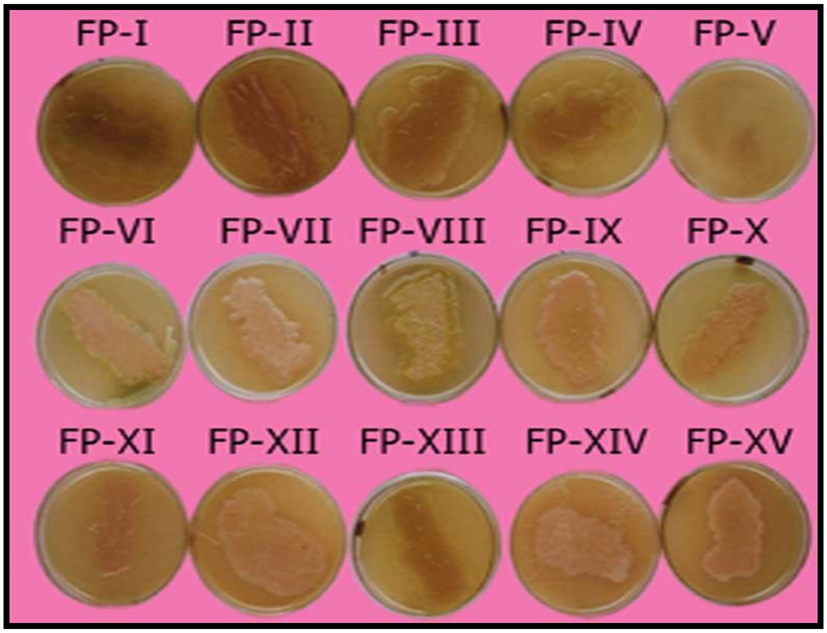

Plate 1. Different isolates of fluorescent pseudomonads

2. Characterization of fluorescent *pseudomonads* isolates

Fluorescent *pseudomonads* are characterized by their production of yellow green pigments, termed pyoverdines, which fluoresce under ultraviolet irradiation and function as siderophores (Paulitz and Loper, 1991). Hence, characterization of all the isolates was done on the basis of morphological, physiological and biochemical tests and grouped as "Fluorescent *Pseudomonads*" because all the isolates produced yellow green pigments on King's B medium, which fluoresced under ultraviolet light and were found to be gram negative, rod shaped, urease positive and phosphate solubilization positive (Table 4). *Pseudomonads* are known to be largest group of rhizosphere bacteria and are not only specific for the inner most rhizosphere but are also present at some distance from the root (Kloepper *et al.,* 1980).

3. Antagonistic Properties of Fluorescent *Pseudomonads* Isolates against *Fusarium oxysporum f. sp. ricini in vitro*

The data related to antagonistic activity of fluorescent *pseudomonads* isolates reflected that in *in vitro* dual agar culture assay, isolate FP-IV and FP-X showed maximum growth suppression (93.33 %)

ISSN 2277-9663

AGRES – An International e-Journal

Volume 1 Issue 1 January-March,2012

of *Fusarium oxysporum f. sp. ricini* followed by FP-I (92.22 %); FP-IX (92.22 %); FP-XV (90.00 %); FP-II (88.89 %); FP-XII (88.89 %); FP-XI (86.67 %) and FP-XIV (85.56 %), whereas minimum suppression (56.69 %) was observed with an isolate FP-VI. Sixty per cent isolates strongly restricted the growth of *Fusarium oxysporum f. sp. ricini* which categorized under highly antagonistic (i.e. more than 85 % growth inhibition), whereas low suppression (i.e. less than 75 % growth inhibition) categorized as low antagonistic and was noted in 26.7 per cent isolates (Table 5 and Plate 2). Fluorescent *pseudomonads* isolates FP-I, FP-IV, FP-IX, FP-X and FP-XV were at par with each other in fungal growth suppression activity.

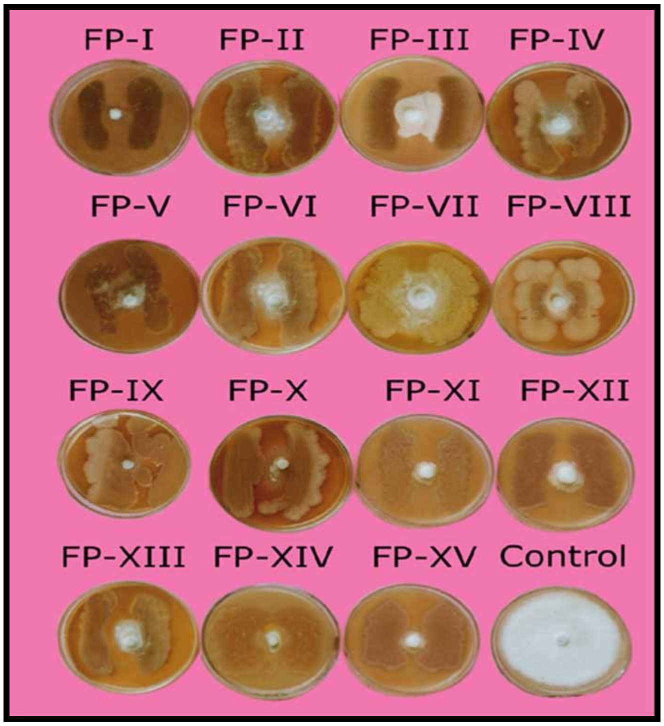

Plate 2. Antagonistic activities of isolates of fluorescent pseudomonads against Fusarium oxysporum f. sp. ricini

As far as methodology and results of antagonism activity, concurrence with Sakthivel et al. (1986); Laha *et al.* (1992); Vidhyasekaran and Muthamilan (1995), Vaidya et al. (2004) and Jha *et al.* (2005). Fluorescent *pseudomonads* antagonize the fungal pathogen by producing one or more metabolites that include antibiotics (Fravel, 1988) and siderophores (Leong, 1986).

4. Evaluation of Fluorescent *Pseudomonads* Isolates for Phosphate Solubilizing Activity

The phosphate solubilizing activity of the fluorescent *pseudomonads* isolates were tested by spot inoculation of isolate on Pikovskaya's medium. After incubation period of five days at room temperature (30 ± 1^{o}C), all the isolates displayed positive reaction by formation of clear inhibition zone around the colony (Table 4). Similar results were found by Kundu et al. (2002), Samanta and Dutta (2004), and Bhatia *et al.* (2005). Pseudomonad bacteria secrete an organic acid and lower down the pH in vicinity and bring about solubilization of insoluble phosphate in soil was described by Gaur (1990).

CONCLUSION

Fifteen fluorescent *pseudomonads* isolates were isolated on King's B medium from the rhizosphere and rhizoplane of castor plant, found to be gram negative, rod shaped urease positive and phosphate solubilization positive. Antagonistic activity of fluorescent *pseudomonads* isolates under *in vitro* dual culture assay reflected that isolates FP-IV and FP-X showed maximum growth suppression (93.33 %) of *F. oxysporum* f. sp. *ricini* followed by FP-I (92.22 %), FP-IX (92.22 %), FP-XV (90.00 %), FP-II (88.89 %), FP-XII (88.89 %), FP-XI (86.67 %) and FP-XIV (85.56 %), whereas minimum suppression (56.69 %) was observed with isolate FP-VI.

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Table -1: Details of Samples

Sr No	Name of village	Castor varieties	Sr No	Name of village	Castor varieties	Sr No	Name of village	Castor varieties
[A]	**PATAN DISTRICT**							
1.	Patan	GCH-4	19.	Vadu	GCH-2	36.	S.K.Nagar	JI-17
2.	Patan	GCH-4	20.	Vadu	GCH-4	37.	S.K.Nagar	GCH-6
3.	Anavada	GCH-2	**[B]**	**BANASKANTHA DISTRICT**		38.	S.K.Nagar	GCH-5
4.	Anavada	GCH-2	21.	S.K.Nagar	GCH-4	39.	S.K.Nagar	GCH-4
5.	Khado	GCH-4	22.	S.K.Nagar	GCH-4	40.	Vaghrol	GCH-5
6.	Anavada	GCH-4	23.	S.K.Nagar	GCH-5	41.	Vaghrol	GCH-4
7.	Rajpur	GCH-5	24.	S.K.Nagar	GCH-4	42.	Ganeshpura	GCH-4
8.	Rajpur	GCH-4	25.	S.K.Nagar	GCH-5	43.	D' Colony	GCH-5
9.	Matarvadi	GCH-4	26.	S.K.Nagar	GCH-5	44.	D' Colony	Sagar
10.	Matarvadi	GCH-4	27.	S.K.Nagar	GCH-5	45.	Chandisar	Sagar
11.	Charup	GCH-5	28.	S.K.Nagar	GCH-4	46.	Chandisar	Vijay
12.	Charup	GCH-4	29.	S.K.Nagar	GCH-4	47.	D' Colony	GCH-4
13.	Paldi	GCH-4	30.	S.K.Nagar	GCH-5	48.	Deesa	Vijay
14.	Paldi	GCH-2	31.	S.K.Nagar	GCH-4	49.	Deesa	Dantiwada seed
15.	Vagdod	GCH-5	32.	S.K.Nagar	GCH-4	50.	Rasana	Vijay
16.	Vagdod	GCH-4	33.	S.K.Nagar	GCH-4			
17.	Brahmanvada	GCH-4	34.	S.K.Nagar	GCH-5			
18.	Brahmanvada	GCH-2	35.	S.K.Nagar	GCH-6			

ISSN 2277-9663
AGRES - An International e-Journal
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Table 2 Village wise isolates obtained from the Patan district

Sr No	Village	No. of Samples Collected	No. of Isolates Obtained	Isolates Designated As
1.	Patan	02	02	FP-I, FP-II
2.	Anavada	03	01	FP-III
3.	Khado	01	01	FP-IV
4.	Rajpur	02	01	FP-V
5.	Matarvadi	02	00	-
6.	Charup	02	01	FP-VI
7.	Paldi	02	00	-
8.	Vagdod	02	01	FP-VII
9.	Brahmanvada	02	01	FP-VIII
10.	Vadu	02	01	FP-IX
Total		**20**	**09**	

Table 3 Village wise isolates obtained from the Banaskantha district

Sr No	Village	No. of Samples Collected	No. of Isolates Obtained	Isolates Designated As
1.	Sardarkrushinagar	19	04	FP-X, FP-XI, FP-XII, FP-XIII
2.	Vaghrol	02	00	-
3.	Ganeshpura	01	01	FP-XIV
4.	Dantiwada Colony	03	00	-
5.	Chandisar	02	00	-
6.	Deesa	02	01	FP-XV
7.	Rasana	01	00	-
Total		**30**	**06**	

Table 4 Characterization of fluorescent *pseudomonads* isolates

Sr. No.	Character	Isolates														
		FP-I	FP-II	FP-III	FP-IV	FP-V	FP-VI	FP-VII	FP-VIII	FP-IX	FP-X	FP-XI	FP-XII	FPXIII	FPXIV	FP-XV
	[A] Morphological															
1.	Colony colour	Yellow Brown	Light Brown	Light Brown	Yellow Brown	Cream Brown	Cream Yellow	Cream Yellow	Light Yellow	Yellow Brown	Light Brown	Cream Brown	Yellow Brown	Cream Brown	Yellow Brown	Light Brown
2.	Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
3.	Pigment colour	Light Brown	Brown	Brown	Light Brown	Brown	Light Brown	Light Yellow	Brown	Brown	Light Yellow	Light Brown	Light Brown	Light Brown	Light Brown	Brown
4.	Media colour	Brown	Brown	Brown	Brown	No change	No change	No change	No change	Brown	Brown	Brown	Light Brown	Light Brown	Brown	Brown
5.	On UV light pigment colour	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green	Yellow Green
	[B] Physiological															
6.	Growth measurement	Fast	Slow	Slow	Fast	Fast	Slow	Slow	Fast	Fast	Slow	Slow	Slow	Slow	Slow	Fast
	[C] Biochemical															
7.	Gram reaction	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve
8.	Phosphate solubilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9.	Urease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Growth ; – = No Grow

ISSN 2277-9663
AGRES – An International e-Journal

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Table 5 Antagonism of different isolates of fluorescent *pseudomonads* against *Fusarium oxysporum f. sp. ricini in vitro*

Sr. No.	Isolates	Growth of Pathogen (mm)*	Per Cent Growth Inhibition
1.	FP-I	7.0	92.22 (73.81)**
2.	FP-II	10.0	88.89 (70.53)
3.	FP-III	34.0	62.22 (52.08)
4.	FP-IV	6.0	93.33 (75.04)
5.	FP-V	17.0	81.11 (64.24)
6.	FP-VI	39.0	56.69 (48.85)
7.	FP-VII	14.0	84.44 (66.77)
8.	FP-VIII	27.0	70.00 (56.79)
9.	FP-IX	7.0	92.22 (73.81)
10.	FP-X	6.0	93.33 (75.04)
11.	FP-XI	12.0	86.67 (68.59)
12.	FP-XII	10.0	88.89 (70.53)
13.	FP-XIII	26.0	71.11 (57.49)
14.	FP-XIV	13.0	85.56 (67.67)
15.	FP-XV	9.0	90.00 (71.56)
16.	Control (Pathogen)	90.00	-
	S.Em.±	0.89	1.21
	C.D. at 5 %	2.82	3.62
	C.V.%	2.01	3.95

*** Average of three replications**

**** Figures in the parentheses are arc sin transformed values**