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IN VITRO EFFICACY OF CULTURE FILTRATE OF *PSEUDOMONAS FLUORESCENS* ON THE GROWTH OF *ALTERNARIA JASMINI*

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Abstract

Seven isolates of *P. fluorescens* (BPf, TPf, VPPf, MEPf, MKPf, MOPf and SAPf) was isolated from jasmine phyllosphere were evaluated to test the antagonism against *Alternaria jasmini* under *in vitro* conditions. Of the seven antagonists tested, VPPf- isolate recorded the highest inhibition of mycelial growth (22.60 mm) of *A. jasmini* over control by recording 74.70 per cent reduction of mycelial growth over control. Biochemical tests were conducted for all the isolates. All the isolates produced similar result with regard to gram staining and KOH test showed negative, whereas fluorescent pigment test showed positive results. Generally all the isolates showed positive results in motility test. The eight bacterial isolates tested in this experiment showed variation with colony type, colour, growth type and reaction to UV light. The effect of different concentration of culture filtrate of bacterial isolates on the mycelial growth of *A. jasmini* under *in vitro* conditions revealed that the culture filtrate of the isolate- VPPf totally inhibited the mycelial growth of *A. jasmini* at 25% concentration under *in vitro* conditions followed by the isolate-SAPf.

Key words : Leaf blight, culture filtrate, bio-chemical test.

Introduction

Jasmine (*Jasminum sambac* L. Aiton) belongs to the family Oleaceae, is an important leading traditional flower crop of India, which constitute of high value commercial loose flower in Tamil Nadu. The flowers also used for the production of perfumed hair oils and attars. Jasmine essential oil has a sweet and floral aroma. It is regarded as unique, as it blends well with other floral extracts and which is highly valued throughout the world. Jasmines are native of tropical and subtropical regions and introduced in the mid sixteenth century. In India, Jasmines are cultivated throughout the country. Tamil Nadu is the leading producer of jasmine in the country with an annual production of 130070 t from the cultivated area of 12590 ha (Anonymous, 2016). In Tamil Nadu, jasmine is produced mainly in Madurai District, with an area of 1503 ha while the district produces nearly 15150 t per year (Anonymous, 2016) and is transported to Mumbai / Bombay in trade, as well as being exported to other countries. The jasmine plants suffer due to several diseases caused by the fungal, bacterial and viral pathogens and are of major constraints causing economic yield loss. Among the fungal diseases, leaf spot of jasmine is caused by *Alternaria jasmini* is becoming common disease on jasmine cause serious losses to jasmine plant. The pathogen infects the crop mainly under dry and warm conditions and it was air borne in nature. Peak incidence occurs during rainy season. *A. jasmini* affected leaves are evidenced by formation of brown, necrotic spots with concentric rings on the leaf tip of the leaves, spreading rapidly in the rainy season. The infected leaves curl and start drying from margins. In severe cases, the young shoots also dry up. The flower production is very much reduced in infected plants and may cause yield loss up to 50% (Conn and Tewari, 1990).

Control of these diseases is currently achieved through the use of chemicals but there is increasing interest in utilizing alternative approaches such as biological control agents (Belanger, 2006). Due to the increasing concern about potentially harmful effects of chemical pesticides on agricultural land, water and soil pollution as well as other health problems have demanded that agricultural scientist pursue alternative controls that are more environmentally friendly, ecologically viable, medically safe and specific for controlling plant pathogens

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(EI-Kassas and Khairy, 2009). More attention has been given to using biological control agents to manage diseases of flower crops (Amin *et al.,* 2010; Nandhini, 2016). Alternatively, antifungal agents produced by microorganisms may be used as bio-control agent (Chitarra *et al.,* 2003), as the materials based on microorganisms have properties such as: high specificity against target plant pathogens, easy degradability and low cost of mass production. The successful application of antagonistic micro organisms especially *Pseudomonas* species (Kavitha *et al.,* (2016); Venkata Siva Prasad *et al.,* (2017) for the control of *Alternaria* has been previously reported by several workers in various crops. With this background, the present study has been undertaken with the following objectives. i) isolation and identification of bacterial antagonist from phylloplane of jasmine leaf ii) in vitro efficacy of bacterial antagonist against pathogen iii) to test the efficacy of culture filtrate of bacterial antagonist against pathogen

Materials and Methods

Isolation a and Establishment of isolates

Jasmine plants showing typical symptoms of leaf blight were collected from different places *viz.,* B. mutlur, Theethampalayam, Vallampadugai, Melur, Melakadu, Morepalayam and Salur. Isolation of leaf blight pathogen *i.e.*, Alternaria jasmini was made by tissue segment method (Rangaswami, 1958). Fresh leaves showing typical symptoms were collected and edge of the lesions were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in changes of sterile distilled water thrice and then placed on Potato Dextrose Agar (PDA) medium in Petri dish. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified based on their cultural and morphological characters. Totally seven isolates were obtained and designated as I_1 to I_7. Based on the virulence study, the highly virulent isolate of I_1 was used for my entire studies.

Isolation of bacterial antagonist from jasmine phylloplane micro flora

Jasmine leaves were collected from different jasmine growing areas and cut into small bits by means of sterile scalpel. These leaf bits were suspended in ten ml of sterile distilled water and thoroughly shaken for five min and allowed to stand for five minutes. One ml of this suspension was pipette out into sterilized Petri plates using sterile Pipette. King's B medium was used for *Pseudomonas fluorescens* and Nutrient agar medium was used for other bacteria for isolation. Twenty ml of the media was poured into each of these plates, gently rotated for uniform mixing and allowed to solidify. Three replications were maintained. The plates were incubated at room temperature (28 ± 2°C). Forty eight hours after incubation, the bacterial colonies were sub cultured and subsequently purified by streak plate method (Rangaswami and Sowmini Rajagopalan, 1973). Seven isolates of *P. fluorescens* (BPf, TPf, VPPf, MEPf, MKPf, MOPf and SAPf) was isolated from jasmine phyllosphere.

Identification of phylloplane bacteria

Identification of the different cultures, if antagonistic bacteria was done as per the methods recommended in the laboratory guide for identification of bacteria published by the American Phytopathological Society (Schaad, 1992). For each test, 48 hrs old culture was used.

Efficacy of phylloplane bacterial antagonist *P. fluorescens* against *A. jasmini in vitro*

Seven isolates of *P. fluorescens* were obtained from different jasmine growing areas of Tamil Nadu and tested for their antagonistic effect on *A. jasmini* by dual culture technique (Dennis and Webster, 1971). *P. fluorescens* was multiplied on King's B medium. A 9 mm actively growing PDA culture disc of the pathogen was placed on PDA medium in sterilized Petri dish at one side 1.5 cm away from the edge of the plate, and incubated at room temperature (28 ± 2°C). Forty eight hrs later, actively growing 48 hrs old cultures of the respective test bacteria were separately streaked onto the medium at the opposite side of the plate, 1.5 cm away from the edge of the plate. The inoculated plates were incubated at room temperature (28 ± 2°C). Three replications were maintained for each antagonist. Potato dextrose agar medium with the pathogen alone serve as control. After 9 days the radial growth of the pathogen was measured. The results were expressed as per cent growth inhibition over control.

Bioassay of culture filtrates of bacterial isolates on the mycelial growth of *A. jasmini*

Preparation of the culture filtrates of bacterial isolates

The bacterial isolates were inoculated into Erlenmeyer flasks containing 50 ml of sterile King's B broth and kept on a rotary shaker at 100 rpm for 48 h. Then the cultures were filtrates thus obtained were used for the studies.

Effect of culture filtrates on the mycelial growth of *A. jasmini*

The culture filtrates of the bacterial antagonists were

separately incorporated into sterilized PDA medium at 5, 10, 15, 20 and 25 per cent by adding the calculated quantity of the culture filtrate to the medium by means of a sterile pipette. The amended media were transferred to sterile Petri plates separately @ 15 ml and allowed to solidify. Each plate was inoculated at the center with nine days old (9 mm) culture disc of *A. jasmini* grown on PDA. Three replications were maintained for each treatment. Sterile water served as control. The diameter of the mycelial growth (in mm) of *A.jasmini* was measured when the mycelial growth fully covered the control plates.

Results and Discussion

The seven bacterial isolates tested in this experiment showed variations with colony type, colour, growth type and reaction to UV light (Table 2). The isolates *viz.,* BPf, TPf, VPPf, MEPf, MKPf, MOPf and SAPf were short rod in shape and produced bright fluorescens when exposed to UV light. With regard to colony type varied from round to circular undulated margin. The colony colour varied from yellowish, cream colour and greenish yellow. Further, the growth type varied from fast to slow.

Effect of *Pseudomonas fluorescens* isolates on mycelial growth of *A.jasmini*

The results showed in table 1 revealed varying degree of antagonism by the *P. fluorescens* isolates in the dual culture technique. Among the seven isolates of *P. fluorescens* tested against *A. jasmini,* VPPf isolate recorded the highest inhibition of mycelial growth (22.60 mm) of *A. jasmini* over control by recording 74.70 per cent reduction of mycelial growth over control. Similarly, Thangeshwari (2012) reported that in our study three isolates of *P. fluorescens viz.,* Pf1, CFP1 and MFP3 were highly inhibitory to *A. alternata*. Jeeva Priscila (2014) reported that three bacterial isolates *viz.,* Pf_3, Pf_7 and Bs_2 were found more effective against *A. jasmini*. Koley *et al.,* (2015) determined the efficacy of six bio-control agents against fungus *A. solani* causing early leaf blight of tomato. *B. subtilis* showed the highest growth inhibition (52.77%) of *A. solani* over the control followed by isolate 2 and isolate 1 of *P. fluorescens* with 47.22% and 45.55% of growth inhibition, respectively. Venkata Siva Prasad *et al.,* (2017) reported that *B. subtilis* strain, *P. fluorescens* strain 1, *P. fluorescens* strain 3 and *P. fluorescens* strain 2 recorded 1.73 cm, 2.08 cm, 2.15 cm and 2.45 cm radial growth with 51.68%, 41.89%, 39.94% and 31.56% inhibition respectively.

Table 1: Effect of *Pseudomonas fluorescens* isolates on the mycelial growth of *A. jasmini.*

S. No	Treatments	Mycelial growth (mm)	Per cent inhibition over control
1.	*P. fluorescens* (BPf)	34.21[b]	61.70
2.	*P. fluorescens* (TPf)	31.67[c]	64.55
3.	*P. fluorescens* (VPPf)	22.60[g]	74.70
4.	*P. fluorescens* (MRPf)	29.23[d]	67.28
5.	*P. fluorescens* (MKPf)	34.13[b]	61.79
6.	*P. fluorescens* (MOPf)	27.22[e]	69.53
7.	*P. fluorescens* (SAPf)	24.72[f]	72.33
8.	Control	89.33[a]	-
CD (P = 0.05%)		1.626	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs

Production of siderophores and chitinases are two factors that may be involved in biological control activity. Indeed, it is known that chitinolytic activity and siderophore production are correlated with antifungal activity (Kamensky *et al.,* 2003; Quecine *et al.,* 2008). In addition, *P. fluorescens* is capable of solubilizing phosphate and producing IAA, characteristics that may enhance its potential use as an effective biological control agent to contribute to the control of *L. theobromae*. Mahesh (2007) suggested that fungal growth is mainly inhibited by HCN production and siderophore production. All these earlier results lend support to the present findings.

In addition to this, *Pseudomonas* spp. are well known for production of broad spectrum antibiotics such as phenazine by *Pseudomona*s sp. B-109 in tomato (Chin-A-Woeng *et al.,* 1998); 2, 4-diacetylphloroglucinol (2,4-DAPG) by *Pseudomonas* sp. 28r/-96 in wheat (Raajimakers and Weller, 2001); Pyoluteorin by *P.*

Table 2: Cultural characteristics of native bacterial isolates.

S.No.	Isolates	Cell shape	Colony type	Colony colour	Growth type	Reaction to UV light fluorescens emission
I. *Pseudomonas fluorescens*						
1.	BPf	Short rod	Round	Greenish yellow	Fast	Bright
2.	TPf	Short rod	Round	Yellowish	Fast	Bright
3.	VPPf	Short rod	Round	Greenish yellow	Fast	Bright
4.	MEPf	Short rod	Round	Greenish yellow	Fast	Bright
5.	MKPf	Short rod	Round	Greenish yellow	Fast	Bright
6.	MOPf	Short rod	Round	Greenish yellow	Fast	Bright
7.	SAPf	Short rod	Round	Yellowish	Fast	Bright

Table 3: Effect of culture filtrate of *Pseudomonas fluorescens* isolates on the mycelial growth of *A. jasmini* .

S.No.	I.No.	Concentration of culture filtrate (%)									
		5		10		15		20		25	
		Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control
1.	BPf	88.09^{a}	0.6	79.51^{b}	10.66	72.70^{b}	17.70	33.76^{b}	62.35	8.90^{b}	90.00
2.	TPf	85.66^{ab}	3.4	76.93^{bcd}	13.56	70.20^{bcd}	20.53	31.98^{c}	64.33	6.82^{d}	92.30
3.	VPPf	77.32^{d}	12.8	69.57^{f}	21.83	61.32^{f}	30.58	21.66^{f}	75.84	1.23^{h}	98.60
4.	MEPf	82.79^{bc}	6.6	74.55^{cde}	16.24	69.32^{cd}	21.52	29.80^{d}	66.76	5.89^{e}	93.38
5.	MKPf	88.09^{a}	0.6	77.74^{bc}	12.65	71.22^{bc}	19.37	32.40^{bc}	63.86	7.34^{c}	91.75
6.	MOPf	87.32^{a}	1.5	73.91^{de}	16.96	67.33^{de}	23.77	27.56^{e}	69.26	4.66^{f}	94.76
7.	SAPf	80.48^{cd}	9.2	72.54ef	18.49	66.39^{e}	24.84	27.04^{e}	69.84	3.20^{g}	96.4
8.	Control	88.66^{a}	-	89.00^{a}	-	88.33^{a}	-	89.66^{a}	-	89.00^{a}	-
CD (P = 0.05%)		3.754		3.474		2.903		1.672		0.470	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs

fluorescens CHAO in tobacco (Keel *et al.,* 1992); Pyrrolnitrin by *P. fluorescens* BL 915 in cotton (Ligon *et al.,* 2000) and Viscosinamide by *P. fluorescens* D1254 in sugarbeet (Nielsen *et al.,* 1998) which proved to be a major mechanism involved in their biocontrol activity. Moreover, Bakker *et al.,* (2003) reported that ability of some *Pseudomonas* spp. in producing siderophores, antibiotics and lipopolysaccharides as important factors in improving the effectiveness of the antagonist. All the above reports were in line with the present observations.

Effect of culture filtrate of *Pseudomonas fluorescens* isolates on the mycelial growth of *A. jasmini*

The results on the effect of different concentration of culture filtrate of bacterial isolates on the mycelial growth of *A. jasmini* under *in vitro* conditions revealed that the culture filtrate of the isolate- VPPf, were highly inhibited to the mycelial growth (1.23 mm; 98.60 %) of *A. jasmini* at 25% concentration under *in vitro* conditions followed by the isolate SAPf and MOPf (Table 3). From the above studies mycelial growth of *A. jasmini* was found reduced with an increase in the concentration of culture filtrate of *Pseudomonas* isolates.

In solid media, the culture filtrate of *P. fluorescens* @40 per cent concentration completely inhibited the mycelial growth of *A. solani* (Maharani, 2017). Studies on the effect of culture filtrate of *B. subtilis* on the mycelia growth of *C. gloeosporioides* revealed that the culture filtrate of the isolate BIL8 completely inhibited the mycelial growth of *C. gloeosporioides* at 15 per cent concentration under *in vitro* conditions followed by the isolate BIF11, which recorded 95.93 per cent inhibition of mycelial growth (Udhayakumar *et al.,* 2019). Chanutsa *et al*., (2014) reported 100 per cent inhibition in the growth of *S. rolfsii* with culture filtrate of *P. fluorescens*. Recently, Muthukumar and Suthinraj (2019) reported that the results on the effect of different concentration of culture filtrate of bacterial isolates *P. fluorescens* on the mycelial growth of *S. rolfsii* under *in vitro* conditions revealed that the culture filtrate of the isolate-I_7 completely inhibited the mycelial growth of *S. rolfsii* at 15% concentration under *in vitro* conditions followed by the isolate-I4.

The antifungal compounds such as pseudobactin, HCN, salicylic acid and 2-hydroxy phenazine produced by Fluorescent pseudomonads suppressed plant pathogenic fungi (Hofte and Bakker, 2007; Reddy *et al.,* 2008). Similar results were observed by Sariah (1994) and Rahman *et al.,* (2007) who reported that the fungal mycelial malformation might be due to the antibiotic metabolites produced by the bacteria which can penetrate and cause protoplasmic dissolution and disintegration. These earlier reports corroborate with the present findings.

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