**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. Jasmimes are native of tropical and subtropical regions and introduced in the mid sixteenth century. In India, Jasmimes 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.
(El-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 I₁ to I₇. Based on the virulence
study, the highly virulent isolate of I₁ 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, CPF1 and MFP3 were highly inhibitory to *A. alternata*. Jeeva Priscila (2014) reported that three bacterial isolates viz., Pf1, Pf2, and B8, 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.

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 phenzone by *Pseudomonas* 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. fluorescens* isolates on the mycelial growth of *A. jasmini*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatments</th>
<th>Mycelial growth (mm)</th>
<th>Per cent inhibition over control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. fluorescens</em> (BPf)</td>
<td>34.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.70</td>
</tr>
<tr>
<td>2</td>
<td><em>P. fluorescens</em> (TPf)</td>
<td>31.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.55</td>
</tr>
<tr>
<td>3</td>
<td><em>P. fluorescens</em> (VPPf)</td>
<td>22.60&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>74.70</td>
</tr>
<tr>
<td>4</td>
<td><em>P. fluorescens</em> (MPf)</td>
<td>29.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>67.28</td>
</tr>
<tr>
<td>5</td>
<td><em>P. fluorescens</em> (MKPf)</td>
<td>34.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>61.79</td>
</tr>
<tr>
<td>6</td>
<td><em>P. fluorescens</em> (MOpf)</td>
<td>27.22&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>69.53</td>
</tr>
<tr>
<td>7</td>
<td><em>P. fluorescens</em> (SAPf)</td>
<td>24.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.33</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>89.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

*CD (P = 0.05%)* 1626

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

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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Isolates</th>
<th>Cell shape</th>
<th>Colony type</th>
<th>Colony colour</th>
<th>Growth type</th>
<th>Reaction to UV light fluorescences emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>2.</td>
<td>TPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Yellowish</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>3.</td>
<td>VPPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>4.</td>
<td>MEPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>5.</td>
<td>MKPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>6.</td>
<td>MOPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>7.</td>
<td>SAPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Yellowish</td>
<td>Fast</td>
<td>Bright</td>
</tr>
</tbody>
</table>

**Table 2: Cultural characteristics of native bacterial isolates.**

1. *Pseudomonas fluorescens*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Isolates</th>
<th>Cell shape</th>
<th>Colony type</th>
<th>Colony colour</th>
<th>Growth type</th>
<th>Reaction to UV light fluorescences emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>2.</td>
<td>TPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Yellowish</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>3.</td>
<td>VPPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>4.</td>
<td>MEPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>5.</td>
<td>MKPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>6.</td>
<td>MOPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Greenish yellow</td>
<td>Fast</td>
<td>Bright</td>
</tr>
<tr>
<td>7.</td>
<td>SAPf</td>
<td>Short rod</td>
<td>Round</td>
<td>Yellowish</td>
<td>Fast</td>
<td>Bright</td>
</tr>
</tbody>
</table>
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 DI254 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, 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.

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>I.No.</th>
<th>Concentration of culture filtrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycelial growth (mm)</td>
</tr>
<tr>
<td>1.</td>
<td>BPF</td>
<td>88.09a</td>
</tr>
<tr>
<td>2.</td>
<td>TPf</td>
<td>85.66b</td>
</tr>
<tr>
<td>3.</td>
<td>VPPf</td>
<td>77.32d</td>
</tr>
<tr>
<td>4.</td>
<td>MEPf</td>
<td>82.79ce</td>
</tr>
<tr>
<td>5.</td>
<td>MKPf</td>
<td>80.89e</td>
</tr>
<tr>
<td>6.</td>
<td>SAPf</td>
<td>87.32e</td>
</tr>
<tr>
<td>7.</td>
<td>Control</td>
<td>80.48c</td>
</tr>
<tr>
<td>8.</td>
<td>Control</td>
<td>88.66e</td>
</tr>
</tbody>
</table>

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.

References


Bacillus subtilis isolates from sugarbeet (Ellis and Martin) YM10-20 inhibits and potential for bio-control.


