CHAPTER 6

In vivo Evaluation of
Pseudomonas putida Strains
ASO1 and AS04 as Biocontrol
Agents Against Wilt in Brinjal
IN VIVO EVALUATION OF Pseudomonas putida STRAINS AS01 AND AS04 AS BIOCONTROL AGENTS AGAINST WILT IN BRINJAL

6.1. Introduction

The eggplant or brinjal (Solanum melongena L.), also called aubergine, is one of the most widely grown vegetable crop all over the world. Although brinjal is cultivated in all the continents, it is grown extensively in tropical Asia and Mediterranean countries (Sunseri et al., 2003). Brinjal is a major commercial vegetable crop and is grown all over India except at high altitude (Wesley, 1956). Brinjal is of high nutritive value and has been a staple vegetable in our diet since ancient times. Brinjal fruits are considered especially useful for those who have to maintain low calorie diets. It is very useful as a dietary element of old and sick people due to the unparallel taste as well as the presence of a number of phytochemical compounds in its flesh, which protects against cancer and atheromatosis (Adamicki, 1995; Esteban et al., 1992). As it contains large amount of mineral salts, brinjal fruits are effective in strengthening the heart activity and lowering the level of cholesterol in blood (Adamicki, 1995).

The market demand of brinjal is increasing rapidly leading to an increase in the cultivation of the crop into new areas. West Bengal is the largest producer of brinjal in India sharing 24.13 % of the total yield (http://agriexchange.apeda.gov.in/India_Productions.aspx?hscode=07093000). It is a major vegetable crop and is cultivated in large areas in sub-Himalayan West Bengal. Brinjal is subjected to attack by many fungi, bacteria, viruses, nematodes and insect pests. Fungal diseases are considered to be a major factor limiting the successful cultivation of the crop. Some common fungal diseases of brinjal are damping-off (caused by Pythium sp., Fusarium sp., Rhizoctonia solani etc.), phomopsis blight (caused by Phomopsis vexans), anthracnose (caused by Colletotrichum gloeosporioides), fruit rot (caused by Pythium spp.), southern blight (caused by Sclerotium rolfsii), early blight (caused by Alternaria solani) etc. (Pandey,
Wilt and root rot caused by several species of *Fusarium* leads to massive loss of the crop (Chakraborty and Chatterjee, 2007, 2008; Joseph et al., 2008; Akhtar et al., 2010). Because of the increasing restriction in the use of chemical fungicides due to concern for the environment and human health, microbial inoculants have been experimented extensively during the last decade to control wilt and other plant diseases (Siddiqui and Shakeel, 2006; Chakraborty and Chatterjee, 2008; Akhtar et al., 2010).

Fluorescent *Pseudomonas* spp. have emerged as the largest and potentially most promising group of microbes involved in the biocontrol of plant diseases (Weller et al., 2007; Couillerot et al., 2009). This comprises of the species that produce the water-soluble yellow-green siderophore pyoverdine, a feature commonly used to distinguish them from other pseudomonads. The fluorescent pseudomonads include *P. aeruginosa*, *P. syringae*, *P. putida* and *P. fluorescens* (Bossis et al., 2000; Palleroni, 2008). Besides the fluorescent pseudomonads, there are some other species that are also reported as biocontrol agents. Table 1 lists some selected strains of pseudomonads which have been used successfully to control diseases in plants. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents. These include the ability to (i) grow rapidly in vitro and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances); (v) compete aggressively with other microorganisms; and (vi) adapt to environmental stresses (Weller, 2007). In addition, pseudomonads are responsible for the natural suppressiveness of some soils to soilborne pathogens (Weller et al., 2002).

*Pseudomonas putida* strains are known to be excellent root colonizers and can protect plants by the mechanism of competition for nutrients and niches (Scher and Baker, 1982; Gotz et al., 2006; Validov et al., 2009). The
aim of this work was to evaluate the in vivo biocontrol potential of the most promising antagonistic strains isolated during the present study. As brinjal wilt is a major disease in India, therefore the effect of two *Pseudomonas putida* strains AS01 and AS04 in suppressing wilt incidence in brinjal seedlings caused by *F. solani* were studied under green house conditions.

**6.2. MATERIALS AND METHODS**

**6.2.1. Host plant**

The experiment for in-vivo evaluation of antagonism showed by the selected bacterial isolates was conducted on brinjal, which is a widely cultivated plant in sub-Himalayan West Bengal.

**6.2.1.1. Selection of brinjal varieties**

Two varieties of brinjal plants were selected for the present study. Selection was done taking into account, the suitability of growing conditions of each variety and also emphasis was given on the choice of the farmers. All the selected varieties were cultivated at the experimental garden of Department of Botany, University of North Bengal.

**6.2.1.2. Collection of selected varieties**

Seeds of different brinjal varieties were collected from different places. Seeds of Pusa Purple Long (PPL) variety were collected from 'Indo-Japan Hybrid', Siliguri, District- Darjeeling, India. Seeds of Lalita variety and another locally cultivated variety were collected from local market of Matigara, located in the Darjeeling district of West Bengal.

**6.2.1.3. Cultivation of selected varieties**

Brinjal cultivation needs a warm sheltered sun-drenched position for optimum growth. It also requires plenty of moisture, well drained soil and the optimum pH value ranges from 5.5 to 6.8. A steady temperature of 25-
30°C is required in the growing season for best yield. All these conditions were maintained during cultivation of the varieties.

6.2.1.4. Raising of seedlings

Seeds of both brinjal varieties were sown six weeks before the plants were set in the garden. Seed sowing was done at 1 cm depth in seedbed prepared in 60 cm x 30 cm x 7 cm aluminium trays filled with sterile soil. Soil moisture was maintained by periodic spraying of water and the temperature was maintained in between 25-30°C. Soil was not allowed to dry out during the period of germination. After germination, seedbeds were watered.

6.2.1.5. Transplantation

Brinjal plant requires more care than many other plants during the time of transplantation. Before transplantation, plants need to be hardened off for 10 days. During this period, exposure to sunlight was increased and frequency of watering was reduced for slowing down growth rate. Proper care was taken to avoid wilting. After hardening, the plants were transplanted in the earthen pots (size 20 cm diameter x 15 cm height) filled with sterile soil. For biocontrol experiments, transplantation was done according to experimental requirements.

6.2.2. Pathogenicity test of *Fusarium solani* and verification of Koch's postulates

*F. solani* was selected as test pathogen because of its agronomic importance. The fungus is a severe pathogen causing wilt and major crop loss in brinjal. The present culture was purchased from IARI, New Delhi and used for biocontrol experiments after verification of Koch’s postulates. For this, six-week old potted brinjal plants of three different varieties (PPL, Lalita and a locally cultivated variety) were collected from the experimental garden. Five potted plants of each variety were used for the test and five plants were reserved as uninoculated control. Fifteen day old culture of *F. solani* (grown in twenty-five 250 ml flasks with 100ml PDB in each) was harvested and the mycelial mat along with spores was collected by filtration
through sterile muslin cloth. It was mixed with sterile distilled water (50 g of fresh mycelia in 200 ml water). The mixture was blended in a waring blender to fragmentize the mycelial mat. Soil up to 2 inches deep surrounding the stem of the plants were removed carefully and the whole mixture with spore and fragmented mycelial mat were poured in the pots. Removed soils were replaced and the pots were kept in the experimental screen house under normal conditions of light and temperature. The pots were observed regularly for 14 days and watered with sterile water as necessary. The diseased PPL plants were uprooted and damaged regions of roots were cut into 5-10 mm long pieces. The pieces were first washed with SDW and then surface sterilized with 0.1% mercuric chloride (HgCl₂) for 1-3 min and rewashed with SDW. The pieces were finally transferred aseptically to sterile PDA slants. Isolates were examined after 15 days of inoculation and the identity was confirmed after comparing them with the stock culture.

6.2.3. Disease evaluation process

Evaluation of wilt incidence was done after 8 and 16 days following the method of Chen et al. (1995). The plants were uprooted carefully and symptom severity was graded into five disease classes, as follows: 0 = no disease; 1 = 0-25% of the leaves withered; 2 = 26-50% of the leaves withered; 3 = 61-75% of the leaves withered; 4 = 76-100% of the leaves withered. Based on the classes, the disease index was calculated using the following formula: Disease index = Σ [(P x DC) x 100] / (T x 4); Where P = plants per class, DC = disease class and T = total number of plants. After calculating disease index, the percent efficacy of disease control (PEDC) was calculated using the formula: PEDC= [(Disease index in untreated control – Disease index in treated plants)/Disease index in untreated control] x100 (Purkayastha et al., 2010).
6.2.4. *In-vitro* studies on growth and antagonism of the antagonistic *Pseudomonas putida* strains AS01 and AS04

The *P. putida* strains AS01 and AS04 were selected for green house experiments as they showed maximum antifungal activity *in vitro*. Before proceeding for plant studies, it was considered worthwhile to study the growth kinetics of these bacteria and assess their *in vitro* antagonistic potential with respect to time in liquid dual cultures.

### 6.2.4.1. Growth kinetics study

Nutrient broth media (5 ml) in sterilized test tubes were inoculated aseptically with 200μl of 24 hour old culture of the bacterial isolates. The tubes were then incubated at 30°C on an orbital shaker and bacterial growth was recorded at a regular interval of one hour by measuring the absorbance at 600nm till stationary phase was reached.

### 6.2.4.2. Study of *in vitro* antagonism in liquid culture

A study of *in vitro* antagonism was carried out against the fungal pathogen *F. solani* in liquid cultures. For this, PDB media was prepared, dispensed in 250ml conical flasks and sterilized at 121°C for 15 min. A fungal mycelial disc (4mm diameter) was co-inoculated separately with each of the bacterial isolates, AS01 and AS04 to the PDB medium and incubated at 30°C. A control flask was inoculated with fungal pathogen only. Mycelial dry weight was taken at 24 h intervals up to a period of 6 days by straining the media through muslin cloth and then removing the excess media by blotting dry. The mycelia were then dried in hot air oven and dry weight was measured.

6.2.5. *In vivo* studies for management of *Fusarium* wilt in brinjal

### 6.2.5.1. Preparation of bacterial inoculum

The bacterial isolates AS01 and AS04 were grown separately in nutrient broth at 30°C for 30 h at 120 rpm in a rotary shaking incubator. The culture thus obtained was centrifuged at 7,000 rpm for 10 min. Cell pellets of bacterial culture were suspended in 0.1 M phosphate buffer (pH 7.0) to
obtain a final concentration of $10^8$ CFU per ml and 1% carboxy methyl cellulose was added to it which acted as a binder (Nandakumar et al., 2001).

6.2.5.2. Preparation of fungal inoculum

Fungal inoculum for biocontrol experiments was prepared on wheat seeds. The seeds were rinsed and soaked in distilled water for 10-12 h in 500 ml Erlenmeyer flasks. Subsequently, excess water was drained off. The flasks were filled with the imbibed wheat seeds in one-third of its volume, were autoclaved twice in two successive days. Each sterilized flask was inoculated with five mycelial disks taken from 7-day-old PDA cultures of *F. solani* (Leslie et al., 2006). The inoculated flasks were incubated at 28°C for 20 days and shaken every three days to avoid lump formation and allow uniform growth of the fungus. For soil inoculation, 10 g of the infected seeds were mixed per kg soil in the experimental pots.

6.2.5.3. In vivo studies for management of *Fusarium* wilt in brinjal

Six-week-old brinjal seedlings were transplanted to earthen pots (20 cm diameter) containing sterilized garden soil and organic manure (1:1), which was pre-treated with fungal inoculum. The bacterial culture (25 ml) was poured carefully at the root (Nandakumar et al., 2001). Severity of disease was recorded by visual observation with reference to the untreated control plants, where no biocontrol bacterium but only the pathogen was applied. For each treatment 10 healthy plants were selected. All experimental plants were kept in the experimental greenhouse under normal light and temperature conditions and watered with sterile water at regular intervals. The other set of treatment was performed by replacing sterilized garden soil with unsterilized garden soil. In this case, unsterile tap water was used for watering the seedlings. All other experimental conditions were kept similar to that of the sterilized set.
6.2.5.4. Statistical analysis

The experiment was replicated thrice and all data from three independent experiments were pooled and average was calculated. Standard error was calculated using the statistical software SPSS version 11.0.

6.3. RESULTS

6.3.1. Verification of Koch's postulates

All inoculated plants of PPL variety showed typical symptoms of wilt after 7 to 12 days which grew severe by 14th day. The locally cultivated variety showed moderate wilt after 14 days but Lalita was almost resistant. The isolated fungal culture was identified as *F. solani*. This reisolation of *F. solani* from infected plants confirmed it as a pathogen of brinjal and thereby the Koch's postulations were verified.

6.3.2. *In vitro* studies

The growth curve of the bacteria is depicted in Fig 31a,b. Both bacteria showed steady increase in OD values upto 15 h and reached a plateau by 16-17 h. There was no significant difference in the growth time and pattern among the two tested strains. In dual cultures, severe restriction of growth of the fungus was noted in flasks inoculated by both the bacteria (Table 19a,b, Fig 31c). The percentage reduction of biomass of *F. solani* after 5 days was found to be 75.72% in AS01 co-inoculated cultures and 71.67% in cultures co-inoculated with AS04. The control flask on the other hand recorded progressive fungal growth upto 5 days after which the growth slowed down. The extent of inhibition in mycelial growth inflicted by the two strains AS01 and AS04 showed little variation when compared to each other.
Table 19a: Study of *in vitro* antagonism by *P. putida* strains AS01 against *F. solani* in PDB dual culture assay

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Mycelial dry weight of <em>Fusarium solani</em> (g)</th>
<th>% reduction in biomass of <em>F. solani</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inoculated with AS01</td>
</tr>
<tr>
<td>1</td>
<td>1.09±0.03</td>
<td>0.21±0.04</td>
</tr>
<tr>
<td>2</td>
<td>1.94±0.04</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>3</td>
<td>2.83±0.05</td>
<td>0.59±0.04</td>
</tr>
<tr>
<td>4</td>
<td>4.22±0.04</td>
<td>1.02±0.06</td>
</tr>
<tr>
<td>5</td>
<td>5.19±0.07</td>
<td>1.26±0.03</td>
</tr>
<tr>
<td>6</td>
<td>5.26±0.05</td>
<td>1.64±0.04</td>
</tr>
</tbody>
</table>

Table 19b: Study of *in vitro* antagonism by *P. putida* strains AS04 against *F. solani* in PDB dual culture assay

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Mycelial dry weight of <em>Fusarium solani</em> (g)</th>
<th>% reduction in biomass of <em>F. solani</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inoculated with AS04</td>
</tr>
<tr>
<td>1</td>
<td>1.09±0.03</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>2</td>
<td>1.94±0.04</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>3</td>
<td>2.83±0.05</td>
<td>0.63±0.04</td>
</tr>
<tr>
<td>4</td>
<td>4.22±0.04</td>
<td>1.16±0.06</td>
</tr>
<tr>
<td>5</td>
<td>5.19±0.07</td>
<td>1.47±0.04</td>
</tr>
<tr>
<td>6</td>
<td>5.26±0.05</td>
<td>1.78±0.04</td>
</tr>
</tbody>
</table>
Fig. 31: Study of growth kinetics by turbidometry of *Pseudomonas putida* strains (a) AS01 and (b) AS04; (c) Study of *in vitro* antagonism by *P. putida* strains AS01 and AS04 against *F. solani* in PDB dual culture assay.
6.3.3. **In vivo evaluation of biocontrol potential of the antagonistic bacterial isolates AS01 and AS04 against *Fusarium* wilt in brinjal**

Table 20 summarizes the results of the *in vivo* evaluation of the strains AS01 and AS04 for their ability to protect brinjal plants against wilt caused by *Fusarium solani*. The strain AS04 showed significant reduction of disease incidence (Fig. 32c) as compared to untreated control (Fig. 32a) within 8 days of treatment, regardless of the soil being sterilized or unsterilized. The disease index in plants treated with bacteria reduced to 25.0 from 82.5, which was recorded in untreated sets. However, in case of sterilized soil, the disease control efficiency was slightly higher (69.6 %) than in unsterilized soil (63.6%) as evident from a lower disease index and higher PEDC value obtained under sterilized soil conditions. There was also a marginal lowering of disease index after 16 days of treatment (Table20, Fig. 32f). The result of treatment with the strain AS01 was also satisfactory as it showed considerable amount of disease inhibition (Fig. 32b,e) as compared to control (Fig. 32a,d). However, the disease control efficacy exhibited by this strain was lower than that showed by AS04. Sterilized soil condition was again found be better in controlling the wilt disease and the disease control efficiency was again recorded to be higher after 16 days of treatment than that of 8 days as evident from the disease index and PEDC values.
Table 20: Inhibition of wilt disease caused by *F. solani* in brinjal seedlings by *Pseudomonas putida* strains AS01 and AS04

<table>
<thead>
<tr>
<th>Treatments</th>
<th>8 days after inoculation</th>
<th>16 days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease Index</td>
<td>PEDC*</td>
</tr>
<tr>
<td>AS01 (sterilized soil)</td>
<td>35.0±0.46</td>
<td>57.5±0.35</td>
</tr>
<tr>
<td>AS01 (unsterilized soil)</td>
<td>42.5±0.29</td>
<td>48.5±0.50</td>
</tr>
<tr>
<td>AS04 (sterilized soil)</td>
<td>25.0±0.85</td>
<td>69.6±0.79</td>
</tr>
<tr>
<td>AS04 (unsterilized soil)</td>
<td>30.0±0.66</td>
<td>63.6±0.23</td>
</tr>
<tr>
<td>Untreated control (sterilized soil)</td>
<td>82.5±0.57</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Untreated control (unsterilized soil)</td>
<td>82.5±0.29</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>0.594</td>
<td>0.725</td>
</tr>
</tbody>
</table>

*PEDC: Percent efficacy of disease control. PEDC = [(Disease index in untreated control – Disease index in treated plants)/Disease index in untreated control] x100. Data represent the means ± standard error.

6.4. DISCUSSION

*Pseudomonas* spp. are ubiquitous in agricultural soils, and are well adapted to growing in the rhizosphere. Favourable characters such as widespread distribution in soil, ability to colonize the rhizospheres of host plants, and ability to produce a range of compounds antagonistic to a number of serious plant pathogens have made these bacteria a subject of intense research as biocontrol agents at the genetic and biochemical level (Anjaiah et al., 1998; De Souza et al., 2003; Pujol et al., 2006). Biocontrol strains have been observed markedly at the root surface, (i.e. the rhizoplane) where they form microcolonies or discontinued biofilms in between the epidermal cells (Couillerot et al., 2009). A large number of these strains have been utilized as inoculums in plant health management practices to control or inhibit plant pathogens and stimulate plant growth (Huang and Wong,
Fig. 32: Suppression of wilt disease in brinjal seedlings by the isolated *Pseudomonas putida* strains under sterile soil conditions:

(a) Considerable wilt in untreated control set after 8 days of inoculation; Disease suppression after 8 days of inoculation by *Pseudomonas putida* strains (b) AS01 and (c) AS04; (d) Severe wilt in untreated control set after 16 days of inoculation; Disease suppression after 16 days of inoculation by *Pseudomonas putida* strains (e) AS01 and (f) AS04.
Fig. 33: Disease Index (DI) of wilt in brinjal seedlings inoculated by *F. solani* and treated by isolated antagonistic *Pseudomonas putida* strains AS01 and AS04.

Fig. 34: Percent Efficacy of Disease Control (PEDC) of wilt in brinjal seedlings by the isolated antagonistic *Pseudomonas putida* strains AS01 and AS04 under sterile and unsterile conditions.
The present study has shown that *Fusarium* wilt of eggplant can be efficiently controlled by *Pseudomonas putida* strains AS01 and AS04 recovered from the rhizosphere. During the green-house studies, biocontrol bacteria and fungal pathogen were co-inoculated to assess the efficiency of the isolated bacteria in reducing wilt disease in eggplant. Method of co-inoculation was successfully used for controlling phytopathogens *Macrophomina* and *Aspergillus* infecting chickpea by *Pseudomonas* M1P3 (Saraf et al., 2008). *Pseudomonas stutzeri* YPL-1 co-inoculated with the pathogen *F. solani* was also found to suppress the root-rot disease in kidney bean (*Phaseolus vulgaris* L.) to a desirable extent (Lim and Kim, 1995). Method of application is another aspect that contributes significantly towards achieving a good biocontrol efficiency and plant growth promotion (Xue et al., 2009). We have used soil application method of bacterial inoculation as this method has been shown to produce better levels of colonization and biocontrol efficiency than other methods like root dipping (Xue et al., 2009) or seed inoculation (Gotz et al., 2006).

At the onset of this study, the pathogenicity of the *F. solani* strain was confirmed through the verification of Koch's postulates. Next, the growth kinetics of the two *P. putida* isolates was studied. The growth rate was moderate for both strains as they attained stationary phase stage after 16-17 hours. Severe restriction of growth in terms of a low mycelial dry weight was observed in dual cultures in broth. In the *in vivo* study, about 73.5% reduction in disease incidence was demonstrated in the eggplant variety PPL on direct soil application of *P. putida* isolates. The strain AS04 appeared to be more competent than AS01 in controlling pathogen infection. However, the *in vitro* experiments of dual culture in liquid medium did not show any significant difference between these strains in inhibiting the growth of *F. solani*.

A large number of workers previously reported the suppression of root diseases by *Pseudomonas* spp. both in greenhouse and field conditions (Kim...
et al., 1994; Remadi et al., 2006; Nihorimbere et al., 2009; Akhtar et al., 2010). Scher and Baker (1982) observed that addition of *P. putida* strain A12 to the soil reduced the incidence of *Fusarium* wilt caused by *Fusarium oxysporum* f. *Sp. lini*. Wilt incidence in flax after 30 days was 42.5% in the control and 10% in the presence of strain A12. Significant disease control was achieved by adding A12 against *Fusarium* wilt in cucumber (lowered to 40% of that in the control) and radish (lowered to 61% of that in the control). Further experimental evidences suggested that competition for iron was responsible for the suppressiveness since iron appeared to be necessary for germ tube elongation of the *F. oxysporum* conidia. During our experiment, although a higher suppression of disease was achieved but the involvement of siderophores could not be ascertained. Several other authors have also suggested that siderophores are major contributors in disease inhibition by fluorescent pseudomonads. For instance, *Pseudomonas fluorescens* EPS62e which was found to be highly efficient in controlling infections by *Erwinia amylovora*, the causal agent of fire blight disease in pear trees did not produce antimicrobial compounds described in *P. fluorescens* species and only developed antagonism in King’s B medium, where it produced siderophores (Cabrefiga et al., 2007). The maximum growth rate and affinity for nutrients in immature fruit extract were higher in EPS62e than in *E. amylovora*, but the cell yield was similar. In preventive inoculations of EPS62e, subsequent growth of *E. amylovora* was significantly inhibited. It was concluded that cell-to-cell interference as well as differences in growth potential and the spectrum and efficiency of nutrient use are mechanisms of antagonism of EPS62e against *E. amylovora* (Cabrefiga et al., 2007). Gupta et al. (2002) observed that bacterization of peanut seeds with the siderophore producing fluorescent *Pseudomonas* strain GRC2 resulted in increased seed germination, early seedling growth, fresh nodule weight, grain yield and reduced charcoal rot disease of peanut in *M. phaseolina*-infested soil as compared with control.

Several studies have been conducted to understand the role of siderophores produced by antagonistic bacteria in disease suppression in plants. Experimental evidences accumulated during the last three decades suggest
that competition for iron is responsible for disease suppression by several fluorescent *Pseudomonas* strains. Scher and Baker (1982) studied the effect of *P. putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt. Results revealed that *P. putida* mediated disease suppressiveness in soil may be induced by managing iron availability in the environment. Duijff et al. (1994) found that *P. putida* strain WCS358 alone significantly reduced carnation wilt caused by *F. oxysporum* and showed that siderophore mediated competition for iron was the principal mechanism involved in disease suppression. Siderophore production and inhibition of *F. oxysporum* by the biocontrol strain decreased with increasing iron availability *in vitro* supporting the more effective disease suppression at low iron availability. Biocontrol studies with a Tn5 mutant defective in siderophore biosynthesis showed that the mutant strain did not reduce disease incidence. De Boer et al. (2003) observed that *Fusarium* wilt in radish caused by *F. oxysporum* can be controlled in a more effective way by using multiple *P. putida* strains. The authors used a mixture of the strains *P. putida* WCS358 (defective in siderophore production) and *P. putida* RE8 to study the suppression of wilt in comparison to single strain treatments. They found an enhanced suppression by the combination of the strains than single applications. Leeman et al. (1996) found that iron availability affects induction of resistance to *Fusarium* wilt of radish by the antagonistic strain *P. fluorescens* WCS374. They suggested that iron chelating salicylic acid and pseudobactin siderophore produced by the strain is involved in the induction of systemic resistance to the disease. Buysens et al. (1996) showed that the plant growth-promoting rhizobacterium *Pseudomonas aeruginosa* 7NSK2 produced multiple siderophores, pyoverdin, the salicylate derivative pyochelin, and salicylic acid and was an efficient antagonist of *Pythium*-induced damping-off. Studies with mutant strains impaired in siderophore production and subsequent complementation tests revealed that siderophore-mediated iron competition could explain the observed antagonism. On the other hand, Ongena et al. (1999) reported that experimental evidences on protection of cucumber by *P. putida* strain BTP1
and its siderophore negative mutant M13 indicate that induction of resistance is the principal mechanism of disease suppression rather than involvement of siderophores.

The present study reveals that the antagonistic isolate *P. putida* AS04 which was recorded as a strong siderophore producer and possess multiple plant growth promoting traits was also capable of reducing incidence of brinjal wilt induced by *Fusarium solani*. Considering all the characters possessed by this strain it may be concluded that *P. putida* AS04 has excellent potential and may be used in experimental trials for managing crop diseases in the field.