



## Effect of rhizobacteria on *Phytophthora meadii*, *Fusarium oxysporum* f. sp. *vanillae* and *Colletotrichum vanillae* infecting vanilla

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**ABSTRACT:** Rhizobacterial isolates, *Pseudomonas fluorescens* (5 isolates), *Enterobacter agglomerans* (one isolate) and *Bacillus* spp. (14 isolates), were screened for growth promotion and against rot pathogens of vanilla such as *Phytophthora meadii* McRae, *Fusarium oxysporum* f.sp. *vanillae* and *Colletotrichum vanillae* Massae. All the rhizobacterial isolates tested except *Bacillus polymixa* (isolate IISR909) and one *Bacillus* sp. (isolate IISR915) were inhibitory to *P. meadii* to an extent of 74 percent, while *F. oxysporum* was highly inhibited (91.0%) by *Bacillus polymixa* (isolate IISR909) *in vitro*. *Bacillus* sp. (IISR153) was highly inhibitory to *C. vanillae* with an inhibition of 77.8%. The maximum growth promotion in terms of shoot length (27cm) in vanilla was observed in plants treated with *P. fluorescens* (isolate IISR13). Different combination of isolates found promising as growth promoting such as *P. fluorescens* isolates (IISR6, IISR853) *B. lentus* (IISR906) *B. polymixa* (IISR909) *E. agglomerans* (IISR912), *Bacillus* spp. (isolates IISR910, IISR913, IISR914, IISR915 and IISR149) as well as suppressing the rot pathogens, viz., *P. fluorescens* (isolates IISR6, IISR51, IISR853), *Bacillus* spp. (isolates IISR147, IISR148 and IISR152), were tested against root rot of vanilla caused by *F. oxysporum* f. sp. *vanillae*. The consortia of rhizobacterial isolates, viz., 1)- *P. fluorescens* isolates (IISR13, IISR51), *Bacillus* sp. (IISR152) and *B. polymixa* (IISR909); 2)- *P. fluorescens* isolates (IISR13, IISR51), *Bacillus* sp. isolates (IISR148, IISR149, IISR152, IISR 907), *B. polymixa* (IISR909) and *B. lentus* (IISR 906); 3) *P. fluorescens* isolates (IISR6, IISR13, IISR51), *Bacillus* sp. isolates (IISR147, IISR151, IISR152, IISR153) and *B. polymixa* (IISR909); and 4) *P. fluorescens* isolates (IISR6, IISR51, IISR147, IISR148, IISR149 and IISR907) and *B. lentus* (IISR906), gave significant disease reduction (88.22- 92.85%) when compared to control. However, among the four rhizobacterial consortia, 3 showed the maximum disease reduction of 92.9%.

**KEY WORDS:** *Colletotrichum vanillae*, consortium, *Fusarium oxysporum*, PGPR, *Phytophthora meadii*, rhizobacteria, *Vanilla planifolia*

### INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews syn. *V. fragrans*), a native to the humid tropical rain forests, is now widely cultivated in India for its aromatic vanillin. The area under vanilla in India during 2002-

2003 was 2545 hectares with a production of 92 metric tones. The initial attractive market of vanilla tempted the farmers to extend the area of cultivation. As the extent of cultivation was increased, the crop became susceptible to a number of fungal and viral pathogens, which seriously affected the

production. The major fungal pathogens reported to be associated with vanilla are *Fusarium oxysporum* f. sp. *vanillae* (Tucker) Gordon (Alconero, 1968) causing stem and root rot, *Colletotrichum vanillae* Massae causing bean shedding and stem rot, and *Phytophthora meadii* McRae causing bean rot, (Suseela Bhai and Joseph Thomas, 2000). Among the diseases, root rot caused by *F.oxysporum* f.sp. *vanillae* is the most serious disease in most of the vanilla gardens. Stem rot is caused by *F. oxysporum* f.sp. *vanillae* (Tombe *et al.*, 1992). Epidemiology of this disease has been worked out by Hadisutrismo *et al.* (1976) in Indonesia and the mode of dissemination of pathogen has been described (Sitepu and Purakusumah, 1985). Host range studies with *F.oxysporum* isolated from stem rot of vanilla revealed that the pathogen was specific to vanilla and it failed to cause infection in alternative crops such as tomato, potato, groundnut, cucumber, ginger and cotton (Nurawan, 1990). The characteristic symptom of the disease is browning and death of roots. Infected roots may become soft and watery or dry depending on the moisture conditions. Although Tsao and Mu (1987) reported *Phytophthora* species in causation of root rot, *F. oxysporum* was recovered frequently in India (Suseela Bhai, unpublished). At present, only carbendazim is being used for the control of this disease (Tombe and Sitepu, 1986). As the extraction of vanillin is from the cured beans, the quality of the beans is greatly affected by this chemical application. Hence, there is a very high demand for the organically produced crop. Moreover, the presence of chemical residues beyond the permissible limit is being rejected in most cases. Thus, it is thought of exploiting the potential of plant growth promoting and bioprotecting rhizobacteria in overcoming these problems. The concept of PGPR is intended to cover both PBPR (Plant Bioprotection Promoting Rhizobacteria), which are rhizobacteria that promote the protection against major plant pathogens and PGPR (Kloepper and Schroth, 1978) which are rhizobacteria that promote beneficial effects on plant growth through control of deleterious microorganisms (Willmar, 2001). Plant growth promoting rhizobacteria (PGPR)

are commonly used as inoculants for improving the growth and yield of agricultural crops. They can as well be used for disease suppressiveness. However, screening for the selection of effective PGPR strains is very critical. Therefore, an attempt was made to exploit the antagonistic potential of rhizobacteria for combating these pathogens with the objectives 1) to study the efficacy of rhizobacteria on growth promotion, 2) to test the potential of rhizobacterial strains against *P. meadii*, *C. vanillae* and *F. oxysporum* f. sp. *vanillae* *in vitro* and 3) to develop a consortium of rhizobacteria for the management of root rot disease of vanilla caused by *F. oxysporum* f. sp. *vanillae* (*Fov*).

## MATERIALS AND METHODS

### Test organisms – Rhizobacteria

The rhizobacterial isolates maintained in the biocontrol repository of IISR, Calicut, were used for the experiment, which are furnished in Table 1. The cultures were initiated by streaking the bacteria in nutrient agar plates and incubated at 24°C for 24h. Single colonies were selected and transferred to 10ml sterile distilled water and distributed thoroughly by shaking. An aliquot of 100µl from this was inoculated to 50ml of nutrient broth and incubated at 24-25°C for 48h in a shake culture. The population level was estimated by the MPN method. Here a ten-fold dilution series was made with water sample of each rhizobacterial isolate and one ml of each dilution inoculated into a separate tube containing nutrient broth and incubated at 24-25°C for 48h. After incubation, the tubes were observed for the presence or absence of growth. The dilution after which no growth was observed was taken for the calculation of CFU (Oblinger and Koburger, 1975; John Lindquist, 2000). The resultant broth culture had a population of approximately  $2 \times 10^9$  cells ml<sup>-1</sup>. The isolates found promising as growth promotive (such as *P. fluorescens* (isolates IISR 6, IISR 853), *B. lentus* (IISR 906), *B. polymixa* (IISR 909), *Bacillus* spp. (IISR 910, 913, 914, 915 and 149) and *E. agglomerans* (IISR 912) as well as pathogen suppressive isolates such as *P. fluorescens*

(isolates IISR6, 51, 853), *Bacillus* spp. (IISR 147, 148 and 152) were made into different combinations and tested against the root rot pathogen, *F. oxysporum* f. sp. *vanillae*.

### Fungal pathogens

Pure cultures of *P. meadii*, *F. oxysporum* f. sp. *vanillae* and *C. vanillae* were isolated from diseased vanilla vines. For *P. meadii* isolation, rot affected beans were collected from the infected field, surface sterilized in 0.1% mercuric chloride solution and washed in several changes of water, blot dried using a sterile filter paper and placed in PVPH media (Tsao and Guy, 1977) and incubated at 24-25°C for 72h-120h. The *Phytophthora* growth obtained was subcultured into carrot agar (CA) slants and transferred into carrot agar plates as and when required.

*F. oxysporum* f. sp. *vanillae* was isolated from root rot infected roots of vanilla collected from infected field. Roots were washed as above and plated in potato dextrose agar (PDA) and incubated at room temperature for 72h-120h and the pure culture obtained was maintained in PDA slants and transferred on to PDA plates as and when required.

*C. vanillae* was isolated from premature yellowing and rot affected beans collected from the infected field. The infected beans were surface sterilized and plated as above on PDA and pure cultures were transferred to PDA. The fungal isolates were maintained in potato dextrose agar (PDA) slants and were further subcultured onto PDA for *in vitro* evaluations. *F. oxysporum* f. sp. *vanillae* for artificial inoculation was prepared in sand-sorghum medium (1: 1)

### Test plants

Three node cuttings of *Vanilla planifolia* were used for greenhouse studies.

### *In vitro* studies on suppression of fungal pathogens

The rhizobacterial isolates were tested against *P. meadii*, *F. oxysporum* and *C. vanillae* for their efficacy *in vitro* following dual culture method (Jin

and Hee, 1989). For this, the test pathogens, viz., *P. meadii*, *F. oxysporum* f. sp. *vanillae* and *C. vanillae*, were grown in PDA for 72h. From this, 3mm discs were cut from the periphery and placed on the centre of the Petri dish already poured with 15ml of PDA. The rhizobacterial isolates were streaked on either side of the pathogen at a distance of 3cm apart. The dual cultured plates were incubated at 28 ± 2°C. The linear growth of the pathogen towards the bacterial growth was measured after 72h and the per cent inhibition was compared with control (pathogen alone) using the formula  $I = [(C-T)/C] \times 100$ , where I is the per cent inhibition, C and T are the radial growth of the pathogen in control and treatment, respectively.

### Greenhouse studies

#### Test for induction of systemic resistance

To study the induction of systemic resistance (if any) due to these rhizobacterial isolates, vanilla shoots of 10cm size were cut off from the individual rhizobacteria treated plants and inoculated with the pathogen under green house conditions. Internodal region and leaves of these detached cuttings were inoculated with 5mm diameter culture discs of *P. meadii* and incubated for 72-120h under humid conditions maintaining a temperature of 22-24° and RH >90%. There were five replications /treatment. Observations on leaf area (A) infected as well as length of the internode infection was taken after 72 h of inoculation and the percent area infected was calculated using the formula  $A = -62.246 + 3.376L = 13.294W$  where L = length of the leaf, W = width of the leaf (Krishnakumar *et al.*, 1997).

#### Effect of individual rhizobacteria on growth promotion

Potting mixture consisting of soil, sand, and farmyard manure and coir compost in 1:1:1:1 proportion was filled in polybags of size 22x14cm. These bags were planted with three node shoots of vanilla @ one shoot/bag. The experiment was designed in CRD. Twenty plants were used for each treatment. The broth culture of the effective isolates viz., *P. fluorescens* (IISR 6, IISR 51, IISR 853),

*B. lentus* (IISR 906), *B. polymixa* (IISR 909), *Bacillus* spp. (IISR 910, IISR 913, IISR 914, IISR 915, IISR 149, IISR 147, IISR 148 and IISR 152) and *E. agglomerans* (IISR 912), having CFUs of approximately  $2 \times 10^9$  ml<sup>-1</sup> was diluted to one liter and added @ 50ml vine<sup>-1</sup> soon after planting and repeated at monthly interval for three months. Observation on growth with respect to shoot length was recorded after three months (Table 1).

#### Effect of mixture of rhizobacteria on growth promotion

The experiment was designed in CRD with five treatments. Each treatment consisted of seven plants (Table 4). The potting mixture was prepared as described above and filled in bags of size 22x14cm and planted with three node shoots of vanilla @ one shoot/bag. Consortia of rhizobacteria, viz, 1) *P. fluorescens* (isolates IISR 13, IISR 51), *Bacillus* sp. (IISR 152) and *B. polymixa* (IISR 909); 2) *P. fluorescens* isolates IISR 13, IISR 51), *Bacillus* sp. isolates (IISR 148, IISR 149, IISR 152, IISR 907), *B. polymixa* (IISR 909) and *B. lentus* (IISR 906); 3) *P. fluorescens* isolates IISR 6, IISR 13, IISR 51), *Bacillus* sp. isolates (IISR 147, IISR 151, IISR 152, IISR 153) and *B. polymixa* (isolate IISR 909); 4) *P. fluorescens* isolates (IISR 6, IISR 51, IISR 147, IISR 148, IISR 149 and IISR 907) and *B. lentus* (IISR 906) were prepared and diluted to one liter and added to the plants @ 50ml vine<sup>-1</sup> soon after planting. The CFUs of the culture were adjusted to  $2 \times 10^8$  ml<sup>-1</sup> by the Most Probable Number (MPN) method as described elsewhere. The application of consortia was repeated at monthly interval for three months and the plants were grown in poly bags for three months and the growth was recorded.

#### Effect of mixture of rhizobacteria on *Fusarium* infection

The three-month-old plants grown in polybags were transplanted into earthen pots containing 10kg potting mixture in the ratio 1:1:1 consisting of sand, soil and FYM and mulched with sterile coir compost @ 250g pot<sup>-1</sup>. The consortium was applied at the time of transplanting and repeated thrice at two months interval. Six months

after establishment, the plants were inoculated with  $4 \times 10^8$  spores g<sup>-1</sup> of *F. oxysporum* f. sp. *vanillae*. The plants were observed for four months for disease incidence and the data were analyzed statistically.

## RESULTS AND DISCUSSION

The rhizobacterial isolates, viz. *P. fluorescens* (isolates IISR 6, IISR 13, IISR 51, IISR 853, IISR 859), *Bacillus* sp. (IISR 147, IISR 148, IISR 149, IISR 150, IISR 151, IISR 152, IISR 153, IISR 907, IISR 910, IISR 913, IISR 914, IISR 915), *B. lentus*, (IISR 906), *B. polymixa* (IISR 909) and *E. agglomerans* (IISR 912) when screened against *P. meadii*, *F. oxysporum* f. sp. *vanillae* and *C. vanillae in vitro*, showed varying levels of inhibition (Table 1). Isolate IISR 859 of *P. fluorescens* inhibited *P. meadii* to an extent of 73.8% followed by *B. lentus* (71%). *B. polymixa* (isolate IISR 909) exhibited highest inhibition (91.7%) against *F. oxysporum* f. sp. *vanillae*. *P. fluorescens* isolates, namely, IISR 6, IISR 51, IISR 853 and *Bacillus* sp. isolates IISR 147, IISR 148 and IISR 152 showed 52-58% inhibition against *Fov*. Other isolates showed below 50% inhibition. In contrast unknown *Bacillus* sp. (IISR 153) caused 77.8% inhibition of *C. vanilla in vitro* followed by *P. fluorescens* isolate IISR 13 and *Bacillus* isolate IISR915, showing 58% and 50% inhibition respectively. Interestingly, *P. fluorescens* (IISR 6, IISR 13), *Bacillus* isolates (IISR 153) and *E. agglomerans* (IISR 912) showed multiple antagonistic potential against all the three pathogens. High degree of inhibition of *in vitro* growth of fungal pathogens by fluorescent pseudomonas and their efficiency variation have been observed in earlier studies (Sivakumar and Narayanaswamy 1999; Rangeshwaran and Prasad, 2000; Bhai *et al.*, 2005), which is in agreement with the present results. In the present study also, varying degrees of *in vitro* inhibition were expressed by different rhizobacterial isolates against each pathogen.

Rhizobacterial isolates were tested against *P. meadii* under greenhouse conditions to study the induction of systemic resistance (if any). The

lesions produced within 72h of inoculation showed the susceptibility of plants to pathogen whereas zero infection indicated the resistance acquired due to rhizobacterial inoculation (Table 1). Plants treated with rhizobacterial isolates of *P. fluorescens* (isolate IISR 51) and *Bacillus* (IISR 907, IISR 147 and IISR 148) resisted infection whereas plants treated with other isolates showed varying levels of

infection both on the leaves and stem (Fig. 1). The results obtained are in agreement with those of Raupach and Kloepper (1998) who observed suppression of symptoms of *C. orbiculare* and *P. syringae* pv. *lachrymans* in plants grown from seeds that had been treated with rhizobacteria. The rhizobacterial isolates mentioned in this study were already evaluated against black pepper and ginger

**Table 1. Effect of individual rhizobacteria on growth promotion and pathogen inhibition**

| Rhizobacterial isolate          | Shoot length (cm)** | <i>In vitro</i> Inhibition (%)* |  |                    |
|---------------------------------|---------------------|---------------------------------|--|--------------------|
|                                 |                     | <i>P. meadii</i>                | <i>F. oxysporum</i> f. sp. <i>vanillae</i> | <i>C. vanillae</i> |
| <i>Pseudomonas fluorescens</i>  |                     |                                 |  |                    |
| IISR 06                         | 20.1 <sup>a-d</sup> | 59.6 <sup>ab</sup>              | 58.3 <sup>ab</sup>                         | 44.4 <sup>ab</sup> |
| IISR 13                         | 27.0 <sup>a</sup>   | 64.9 <sup>ab</sup>              | 44.4 <sup>ab</sup>                         | 58.3 <sup>ab</sup> |
| IISR 51                         | 16.2 <sup>b-c</sup> | 59.6 <sup>ab</sup>              | 55.5 <sup>ab</sup>                         | 27.8 <sup>ab</sup> |
| IISR 853                        | 22.9 <sup>a-c</sup> | 60.5 <sup>ab</sup>              | 55.5 <sup>ab</sup>                         | 16.7 <sup>b</sup>  |
| IISR 859                        | 17.9 <sup>b-e</sup> | 73.8 <sup>a</sup>               | 0.0 <sup>b</sup>                           | 16.7 <sup>b</sup>  |
| <i>Enterobacter agglomerans</i> |                     |                                 |  |                    |
| IISR 912                        | 18.5 <sup>ae</sup>  | 64.0 <sup>ab</sup>              | 44.4 <sup>ab</sup>                         | 50.0 <sup>ab</sup> |
| <i>Bacillus lentus</i>          |                     |                                 |  |                    |
| IISR 906                        | 23.9 <sup>ab</sup>  | 71.1 <sup>ab</sup>              | 44.4 <sup>ab</sup>                         | 13.9 <sup>b</sup>  |
| <i>B. polymixa</i>              |                     |                                 |  |                    |
| IISR 909                        | 23.2 <sup>ac</sup>  | 21.1 <sup>bc</sup>              | 91.7 <sup>a</sup>                          | 38.9 <sup>ab</sup> |
| <i>Bacillus</i> sp.             |                     |                                 |  |                    |
| IISR 907                        | 14.8 <sup>ce</sup>  | 63.1 <sup>ab</sup>              | 30.6 <sup>ab</sup>                         | 11.1 <sup>b</sup>  |
| IISR 910                        | 19.8 <sup>ad</sup>  | 63.2 <sup>ab</sup>              | 23.7 <sup>b</sup>                          | 22.2 <sup>b</sup>  |
| IISR 913                        | 20.0 <sup>a-d</sup> | 59.6 <sup>ab</sup>              | 2.8 <sup>b</sup>                           | 30.7 <sup>ab</sup> |
| IISR 914                        | 23.2 <sup>a-c</sup> | 49.1 <sup>ab</sup>              | 18.1 <sup>b</sup>                          | 16.7 <sup>b</sup>  |
| IISR 915                        | 22.7 <sup>a-c</sup> | 0.0 <sup>c</sup>                | 16.7 <sup>b</sup>                          | 50.0 <sup>ab</sup> |
| IISR 147                        | 14.8 <sup>a-c</sup> | 56.1 <sup>ab</sup>              | 51.4 <sup>ab</sup>                         | 16.7 <sup>b</sup>  |
| IISR 148                        | 14.2 <sup>c-e</sup> | 56.1 <sup>ab</sup>              | 52.8 <sup>ab</sup>                         | 27.8 <sup>ab</sup> |
| IISR 149                        | 18.6 <sup>a-e</sup> | 65.9 <sup>ab</sup>              | 38.9 <sup>ab</sup>                         | 16.7 <sup>b</sup>  |
| IISR 150                        | 13.1 <sup>d-e</sup> | 64.1 <sup>ab</sup>              | 44.4 <sup>ab</sup>                         | 33.3 <sup>ab</sup> |
| IISR 151                        | 10.5 <sup>e</sup>   | 65.9 <sup>ab</sup>              | 30.6 <sup>ab</sup>                         | 16.7 <sup>b</sup>  |
| IISR 152                        | 17.8 <sup>b-e</sup> | 67.2 <sup>ab</sup>              | 58.3 <sup>ab</sup>                         | 27.8 <sup>ab</sup> |
| IISR 153                        | 16.7 <sup>b-e</sup> | 64.9 <sup>ab</sup>              | 47.2 <sup>ab</sup>                         | 77.8 <sup>a</sup>  |
| Control                         | 18.2 <sup>b-e</sup> | -                               | -  | -                  |

\* Values within a column followed by the same letter(s) do not differ significantly ( $P = 0.01$ ) according to Duncan's multiple range test; \*\* Mean of ten replications

pathogens in similar studies conducted at Indian Institute of Spices Research, Calicut (Diby *et al.*, 2001; Bhai *et al.*, 2005).

The results of the present study are also supported by the experiments done on black pepper, where five bacterial strains which had been proved efficient in suppressing *P. capsici* were made into consortia in different combinations and their effect in suppression and growth promotion was evaluated (Jisha *et al.*, 2002)

Treatment of vanilla cuttings with different isolates of *P. pseudomonas*, *B. lentus*, *B. polymixa*, *Bacillus sp.*, and *E. agglomerans* showed varying

bioconsortia, consortium 3 containing *P. fluorescens* isolates IISR13, IISR 51, IISR 6, *Bacillus sp.* isolates IISR 152, IISR 147, IISR 151, IISR 153 and *B. polymixa* isolate IISR909 recorded the maximum shoot length when compared to control (Table 2).

The mixture of rhizobacteria in various combinations was very promising in controlling the infection caused by *F. oxysporum* f. sp. *vanillae*. Though the plants initially took up infection, there was no further spread of the disease in any of the treated plants, whereas, in the control plants, the infection extended upwards from the root and the whole plant collapsed within a period of one month. This clearly showed the protection offered by the

**Table 2. Effect of mixture of rhizobacteria on *Fusarium* infection & growth promotion**

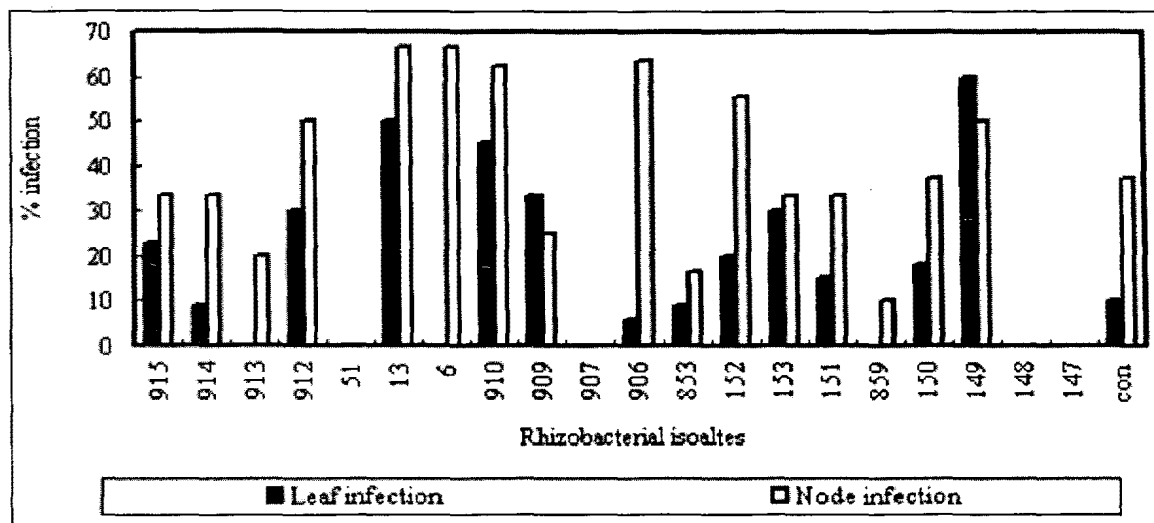
| Sl. No.        | Treatment  | <i>Fusarium</i> infection (%) | Shoot length (cm) |
|----------------|--|-------------------------------|-------------------|
| T <sub>1</sub> | <i>P. fluorescens</i> (IISR 13, 51), <i>Bacillus sp.</i> (IISR 52) and <i>B. polymixa</i> (IISR 909) | 8.6 (88.3)                    | 83.6              |
| T <sub>2</sub> | T1 + <i>Bacillus sp.</i> (IISR 148, 149, 907) and <i>B. lentus</i> (IISR 906)                        | 8.1 (88.9)                    | 71.9              |
| T <sub>3</sub> | T1+ <i>P. fluorescens</i> (IISR 6), <i>Bacillus sp.</i> (IISR 147, 151 and 153)                      | 5.2 (92.9)                    | 84.3              |
| T <sub>4</sub> | <i>P. fluorescens</i> (IISR 6, 51, 147, 148, 149 and 907), <i>B. lentus</i> (IISR 906)               | 7.9 (89.2)                    | 60.6              |
| T <sub>5</sub> | Control – (only pathogen)  | 73.3 (0.0)                    | 77.0              |
|                | LSD  | 24.3                          | NS                |

Values in parenthesis are percentage disease reduction

levels of growth. The maximum growth promotion with respect to shoot length (27cm) in vanilla was observed in plants treated with *P. fluorescens* isolate IISR13 when compared to control (18.2cm). *P. fluorescens* isolates (IISR 6, IISR 853), *B. lentus* (isolate IISR 906), *B. polymixa* (isolate IISR909), *E. agglomerans* (isolate IISR 912) and *Bacillus sp.* isolates (IISR 910, IISR 913, IISR 914, IISR 915 and IISR149) also increased the growth of vanilla compared to the control (Table 1).

Though no significant difference in growth was observed in plants treated with any of the

mixture of rhizobacteria in controlling *Fusarium* infection. All the four mixtures showed minimum disease incidence in all the treatments and were significantly superior to control giving more than 88.2 - 92.9% disease reduction (Table 2). The results emphasized the potential of the rhizobacterial mixture in the suppression of *Fusarium* infection in vanilla, though the effect varied with the isolates used. However, among the four rhizobacterial consortia, the consortium containing *P. fluorescens* isolate IISR 6, IISR 13, IISR 51, *Bacillus sp.* isolate (IISR 152, IISR 147, IISR 151 and IISR 153) and *B. polymixa* (IISR 909), *i.e.* treatment T<sub>3</sub>, showed the



**Fig 1. Induction of systemic resistance by rhizobacterial isolates against *P. meadii***

maximum disease reduction of 92.9%. It may be due to the fact that rhizobacterium activates different defense mechanisms such as physical (lignification) and chemical (quinines) barriers within the induced resistance pathway resulting in differential induction in symptoms (Silva *et al.*, 2004).

Earlier also, attempts were made to control stem rot disease of vanilla caused by *F. oxysporum* through biological means (Tombe, *et al.*, 1992) by using a bacterium isolated from the rhizosphere of *Allium* sp. There are also reports that non-pathogenic isolates and mutants of some *Fusarium* sp. were effective in suppressing stem rot disease of vanilla under *in planta* conditions (Tombe, *et al.*, 1994). The experiment proved that there is synergistic effect when the consortium was made into combinations (Anandaraj and Sarma, 2003). Earlier workers also supported the consortium approach. Duffy and Weller (1995) stated that the possible approach to improve biological control is the application of combinations of biocontrol agents. Failure of the establishment of antagonistic microorganisms may be due to environmental factors resulting in inadequate distribution, insufficient establishment of rhizobacterial strains, or poor expression of their antagonistic activity. Increasing the biodiversity of biological control systems through the use of mixtures of

microorganisms may result in treatments that persist longer in the rhizosphere and utilize a wide array of biocontrol mechanisms such as induction of systemic resistance, production of antibiotics and competition for nutrients under a broader range of environmental conditions (Pearson and Weller, 1994). By combining microorganisms, multiple antifungal traits can be combined and at least one biocontrol mechanism will be functional under the conditions faced by the released biocontrol agents. Combinations of biocontrol strains are expected to result in a higher level of protection (Dunne *et al.*, 1998; Jetiyanon and Kloepper, 2002). *Fusarium* wilts can be suppressed through the activity of fluorescent *Pseudomonas* spp. strains. The disease-suppressive mechanisms of these biocontrol agents include siderophore-mediated competition for iron, competition for substrates, and induction of systemic resistance and production of antibiotics (de Boer, 2003). Enhanced disease suppression may involve not only different disease-suppressive mechanisms, but can also result from interactions between the introduced strains that positively influence growth, root colonization or activity of the strains (de Boer *et al.*, 1999, 2003). The results of the present experiment are also in accordance with the work done by Albuquerque *et al.* (2000) wherein they reported the effect of PGPR on *Fusarium* wilt in micro-propagated banana plantlets

against Panama wilt. Similarly, bacterial consortia have been developed to improve banana cultivation (Jaizme Vega *et al.*, 2004). They demonstrated for the first time that a *Bacillus* sp. consortium was able to improve banana development and increase levels of foliar mineral contents. Thus, the present study indicated that *Fusarium* infection in vanilla can be managed using consortia of rhizobacterial strains, viz. *P. fluorescens* isolates (IISR 6, IISR13, IISR 51), *Bacillus* sp. isolates (IISR147, IISR151, IISR 152, IISR 153) and *B. polymixa* (IISR 909).

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