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Research Note

In vitro and *in planta* assays for biological control of *Fusarium* root rot disease of vanilla

R. SUSEELA BHAI, B. REMYA, JITHYA DANESH and S. J. EAPEN
Division of Crop Protection, Indian Institute of Spices Research, Marikunnu PO,
Calicut 673012, Kerala, India.
E-mail: suseela@spices.res.in

ABSTRACT: Rhizosphere and phyllosphere organisms isolated from vanilla consisted of predominant colonizers such as *Fusarium* sp. (24 isolates) and *Colletotrichum* sp. (20 isolates). Other organisms were *Rhizoctonia* sp. (two isolates), *Trichoderma* spp. (seven isolates), *Paecilomyces* sp. (one isolate), *Mucor* sp. (three isolates), non-sporulating fungal species (10 isolates) and *Pseudomonas fluorescens* (three isolates). When tested *in vitro*, six isolates showed more than 50% inhibition of *Fusarium oxysporum* f. sp. *vanillae*. These were two isolates of *Trichoderma* sp. (53.30-70.58), one isolate of *Paecilomyces* sp. (65.00%) and two isolates of *P. fluorescens* (59.15-62.50%) that were antagonistic to the pathogen. None of the non-pathogenic *Fusarium* species tested showed promising inhibitory effect on *F. oxysporum* f. sp. *vanillae*. The five promising isolates were tested *in planta* by challenge inoculation. *Paecilomyces* sp. provided 100 per cent protection against root rot. *T. harzianum* and *P. fluorescens* provided 40% protection. Thus the present study indicated the possibility of using *Paecilomyces* sp. as a potential antagonist for *F. oxysporum* f. sp. *vanillae*.

KEY WORDS: Biological control, *Colletotrichum* sp., *Fusarium oxysporum* f. sp. *vanillae, in vitro* screening, root rot, *Vanilla planifolia*.

Vanilla planifolia Andrews is a tropical herbaceous perennial vine, climbing on trees or other supports to a height of 10-15m by means of its adventitious roots and thrives best under moist conditions. It requires a warm and humid climate for its proper growth and economic production. The major fungal diseases reported on vanilla are root rot, stem rot, stem blight, and shoot tip rot, fruit rot and immature bean shedding. Species of *Phytophthora*, *Fusarium, Colletotrichum* and *Sclerotium* are main diseases (Purseglove, 1988). Among these root rot caused by *F. oxysporum* f. sp. *vanillae* (FOV) is devastating in several vanilla plantations. As the root system is almost completely destroyed due to the above disease, the entire vine wilts off. At present only chemicals are used for the control of this disease, but the indiscriminate use of chemicals is not advisable as this will leave residues in the produce. There are very few reports on the use of bioagents in controlling diseases of vanilla. Tombe *et al*. (1992) reported that pre-treatments of the plants with a non-pathogenic isolate of *Fusarium* sp. suppressed stem rot disease. Similarly, a number of mutants of *Fusarium oxysporum* have also been reported as biocontrol agents against pathogenic forms of *Fusarium* sp. (Tombe *et al*., 1994). But none of them has been recommended for the biological control of either root rot or stem rot disease of vanilla. Hence, in the present study, an attempt was made to isolate and test more biocontrol organisms against *F. oxysporum* and the results are presented here.

In vitro screening of organisms against pathogens

Source of pathogen

Pathogenic isolate of *F. oxysporum* f. sp. *vanillae* (Isolate no. IISR V39), maintained in the repository of Indian Institute of Spices Research (IISR), Calicut, was used for screening. The isolate was sub-cultured on PDA and used in the inoculation studies.

In vitro screening of bacterial isolates

Dual culture method was followed for *in vitro* studies. The pathogen was grown on PDA for 72h and a 5mm disc from this was placed at the centre of a Petri dish with PDA. Cultures of selected bacteria (24h old) were streaked 3cm apart on either side of the pathogen disc. The control plate was kept without bacteria to compare the growth of the fungus. The inoculated plates were incubated at room temperature (24-25°C). In the case of fungi, 5mm discs were cut from the edge of the fungal culture grown on PDA for 72h and placed 3cm apart from the target pathogen.

The radial growth of the pathogen on either side towards the bacteria or the test fungi was measured at 72h and 120h and compared with the control. The per cent inhibition was calculated using the formula I = (C - T) / C x 100, where I is the percentage inhibition of the pathogen, C and T are radial growth of the pathogen in control and treatment, respectively.

In planta* testing of short-listed fungal and bacterial isolates against pathogenic *Fusarium

Test plants

Potting mixture was prepared using soil, sand and farm yard manure in a 1: 1: 1 proportion and filled in poly bags of size 22 x 14cm. These bags were planted with three node cuttings of vanilla @ 1 cutting/bag.

***In planta* screening**

Vanilla cuttings raised in polybags were used for the *in planta* assay. The experiment was conducted in CRD with 10 plants /treatment. There were seven treatments, *viz,* two isolates of *Trichoderma harzianum*, two isolates of *P. fluorescens,* one isolate of *Paecilomyces* sp. and two sets of control, where one set was with the pathogen and another set (absolute control) was without the pathogen. The plants were maintained under 20% moisture level. The plants in polybags (one month after planting) were initially applied with the antagonistic cultures of *Paecilomyces* sp., *T. harzianum* and *P. fluorescens* as described above. After 4 days, these plants were inoculated with the pathogenic *F. oxysporum* f.sp. *vanillae* isolate (IISR V39). Observations were recorded on mortality and root infection after seven days.

Isolation of organisms from plant material and Soil

***In vitro* screening of isolated organisms**

Fusarium oxysporum f. sp. *vanillae* (IISR V39) was used in *in vitro* screening. Among the 30 isolates subjected to *in vitro* testing only six isolates showed more than 50% inhibition against *F. oxysporum* f.sp. *vanillae*. The antagonistic isolates were *T. harzianum* (IISR V184, IISR V 193, IISR V194), *Paecilomyces* sp, (IISR V202) and *P. fluorescens* (IISR V203 and IISR V 204) (Table 1).

***In planta* testing of short-listed fungal and bacterial isolates against root rot of vanilla**

Fusarium infection was manifested as water soaked areas followed by yellowing and rotting of the basal portion of the vine along with root rot. The *Fusarium* treated plants showed yellowing and basal root rot as described by Deqvaire (1976). The symptoms appeared on the 13thday of inoculation of the pathogen. Rotting gradually extended upwards leading to the death of the vine. Varying degrees of rotting was expressed in each treatment. Pathogen treated plants exhibited wilting, yellowing and root rot as mentioned above and showed mortality on 15th day and all the plants died in 60 days. *T. harzianum* (IISR V194) treated plants showed no infection till the 15th day and later the roots got infection and 60% of the plants died in 60 days. *P. fluorescens* isolates IISRV 203 and IISRV204 also showed initial protection and later all the plants got infection. *Paecilomyces* sp. (IISRV202) isolated from the soil gave an inhibition of 65% *in vitro* and 100% *in vivo*, showing its potential as a promising biocontrol candidate. *Trichoderma* isolates showed an inhibition of 53.30% and

Table 1. Effect of short-listed antagonistic isolates against major pathogens *in vitro*

Organism	Plant part	Inhibition (%)**		
		F. oxysporum f. sp. *vanillae*	*Colletotrichum vanillae*	*Phytophthora meadii*
T. harzianum (IISRV184)	Aerial root	53.30^{a-f}	54.00^{m-p}	Nd*
T. harzianum (IISRV193)	Aerial root	70.58^{a}	72.50^{op}	Nd
T. harzianum (IISRV194)	Aerial root	70.58^{a}	57.50^{op}	Nd
Paecilomyces sp. (ISRV202)	Soil	65.00^{ab}	62.50^{p}	67.50
P. fluorescens (IISRV203)	Soil	59.15^{a-d}	62.50^{p}	Nd
P. fluorescens (IISRV204)	Soil	62.50^{a-c}	62.50^{p}	Nd

*Nd- Not done; ** Values followed by the same alphabets in a column do not differ significantly by DMRT

70.58%, respectively *in vitro*, but failed to give satisfactory protection when tested *in vivo* (Table 2). Compared to *Paecilomyces* sp., it gave only 40% inhibition. Similarly, two *Pseudomonas* isolates (Isolate No. IISR V203 & IISR V204) also gave *in vitro* inhibition of 59.15-62.50 % but were found non-protective *in planta*. The plants were found absolutely healthy without any aerial or ground root infection. In absolute control, where no treatments were given, the plants showed 60% infection (Fig 1) (This may be due to the spore splash from the adjacent pathogen alone treated polybags). The results revealed the potential of *Paecilomyces* sp. as a promising biocontrol agent for the management of root rot of vanilla.

Table 2. Incidence of root rot (expressed as % mortality)

Organism and Repository No	Mortality due to *F. oxysporum* f. sp. *vanillae* in %
T. harzianum IISRV194	60.00
Paecilomyces sp. IISRV202	0.00
P. fluorescens IISRV203	60.00
P. fluorescens IISRV204	100.0
Fusarium oxysporum f. sp. *vanillae*	100.00
Control	60.00

Amongst several strains of *Trichoderma* isolated from both phyllosphere and rhizosphere, three isolates (IISR V184, IISR V193, and IISR V194) showed satisfactory inhibition against both the pathogens *in vitro*. *Mucor* sp. was isolated from leaf, stem and bean samples but found pathogenic on vanilla (Bhai, unreported). *P. fluorescens* and *Paecilomyces* sp. were isolated from the rhizosphere soil. Of the total 69 fungal and bacterial isolates, only 30 isolates qualified for antagonistic evaluation. In the present study, none of the non-pathogenic *Fusarium* isolates was found inhibitory to *F. oxysporum* f.sp. *vanillae*. But there are reports where pre-treatments of the plants with non-pathogenic isolate of *Fusarium* sp. suppressed stem

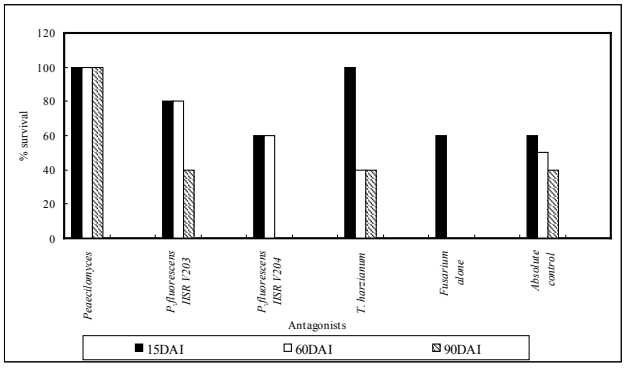

Fig. 1. Screening short listed antagonists against *F. oxysporum* f. sp. *vanillae* ***invivo***

rot disease (Tombe *et al*., 1992). Similarly, a number of mutants of *F. oxysporum* have also been reported as biocontrol agents against pathogenic forms of *Fusarium* sp. (Tombe *et al*., 1994).

Thus, from the present study it is found that *Paecilomyces* sp. (IISR V202) is a potential bioagent for the control of root rot disease of vanilla. The findings of the present study are supported by the works done on other crops. Fang *et al.* (2005, 2006) studied the antagonistic effect of *P. lilacinus* and its mechanism against *F. oxysporum* and found that the secondary metabolite produced by the fungus suppressed the colony growth, sporulation and germination of *F. oxysporum*. The antagonistic mechanism is related to nutrient competition and beta-glucosidases activity that caused cell wall degradation and antibiotic production such as toxic protein and amylase and suggested that *P. lilacinus* can be used as a biocontrol agent against *F. oxysporum*. Shahnaz and Ghaffar (2003) studied the effect of inorganic fertilizer on the efficacy of *P. lilacinus* in the control of soil borne root infecting fungi on mung bean. They observed that when seeds were sown 10 days after treatment, a complete control of *Fusarium* infection was possible in soil amended with *P. lilacinus* and di-ammonium phosphate. Biological control of *Meloidogyne incognita* and *F. solani* disease complex in papaya using *P. lilacinus* and *T. harzianum* was reported by Khan *et al*. (1997). Praveen and Ghaffar (1998) reported the use of *P. lilacinus* in the control of *F. oxysporum* root rot and *M. javanica* root knot infection on tomato. Moreover, there were also reports on its effectiveness as a biocontrol agent against *M. incognita* on black pepper (Sosamma and Koshy, 1997).

From the present study, it is found that *Paecilomyces* inhibited *Colletotrichum vanillae* (Table 1) which is another major pathogen on vanilla causing premature bean shedding (Bhai and Thomas 2000, Bhai, *et.al*. 2006) and also *Phytophthora meadii* (67.5%) casing stem and bean rot of vanilla. Attempts were also made to multiply the fungus in various easily available and economically cheap carrier media. It was found that the fungus can be grown profusely in wheat bran, or sorghum grains or wheat rava or in potato dextrose or Czapek broth (Bhai, unreported). Thus, the result of the present study is highlighting the efficiency of *Paecilomyces* isolate *as a* potential biocontrol agent in suppressing root rot of vanilla caused by *F. oxysporum* f. sp. *vanillae*.

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