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In vitro biocontrol of coconut leaf rot pathogens with *Pseudomonas fluorescens*

Key words: Coconut, leaf rot disease, root (wilt) disease, *Colletotrichum gloeosporioides, Exserohilum rostratum, Pseudomonas fluorescens*, bio-control.

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Leaf rot disease (LRD) is prevalent in areas of Kerala State where root (wilt) disease (RWD) is endemic. On an average, 65% of RWD affected palms also have superimposed LRD (Srinivasan, 1991). The occurrence of LRD has increased over the years with increased intensity of RWD. Recent studies on LRD have established a complex fungal etiology. *Colletotrichum gloeosporioides* and *Exserohilum rostratum* are the main pathogens and *Gliocladium vermoeseni, Fusarium solani, Thielaviopsis paradoxa* and *Rhizoctonia solani* are other associated fungi involved in the disease (Srinivasan and Gunasekaran, 1996a, b). A series of attempts have been made in the past to control the disease with contact and systemic fungicides (Menon and Nair, 1952;Nair and Radha, 1959; Samraj *et al.*, 1966; Srinivasan and Gunasekaran, 1996c). Prevention or control of the disease had only limited success. Biological control of fungal diseases of different crop plants using microorganisms has been reported by many workers (e.g., Blakeman and Fokkema, 1982; Bolland, 1990; Arya and Parashar, 1997; Nallathambi, *et al.*, 1997a). Lily *et al.* (1952, 1955) reported that a strain of *Bacillus subtilis* inhibited the growth of a leaf root pathogen, *Helminthosporium halodes* on agar medium. The culture filtrate of the bacterium was also found to inhibit the fungus. Such environment friendly biocontrol agents might therefore, be suitable for controlling LRD. In recent years, *Pseudomonas fluorescens* has been utilized by various workers for the control of fungal diseases (Gnanamanickam and Krishnamurthy, 1998; Vidhyasekaran, 1998). Hence, an attempt has been made to evaluate the efficacy of *P. fluorescens* (Pf) against the LRD pathogens. The results are presented in this paper.

Fungal Cultures: LRD fungi, *viz. Colletotrichum gloeosporioides, Exserohilum rostratum, Gliocladium vermoeseni, Fusarium solani, Thielaviopsis paradoxa and Rhizoctonia solani* were isolated from naturally infected palms and the pure cultures were used for the studies. For inoculation, 7-day-old cultures grown on PDA were used as inoculum.

An isolate of *P. fluorescens* obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore was maintained in King's B (KB) agar medium. For evaluation of bacterial antagonism *in vitro* the fungi were grown on PDA. A culture of *P. fluorescens* grown in KB broth was used to prepare the culture filtrate.

***In vitro* assay of antagonism by dual culture:** Sterilized PDA (10 ml) was poured into culture dishes (90 mm dia.) and allowed to cool. Mycelial blocks (5 mm dia. cut from 7 day old cultures of the respective fungus and the aqueous cell suspension of *P. fluorescens* in sterile water (from 3 day old culture) were placed on the medium at equal distance (25mm) from the centre of the dish; the bacterial inoculum was placed as a streak parallel to the median line of the dish. The cultures were incubated at 30°C and the radial mycelial growth towards the centre and the inhibition zone were recorded up to 5 days at regular intervals.

Sterile culture filtrate of P. *fluorescens* was prepared from 3-day-old liquid culture (KB.broth) by passing through Seitz filters (0.22 m size). A well of 5 mm dia. was made at the centre in the medium with

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ork borer and filled with 0.1 ml of culture filtrate under eptic conditions. The plates were inoculated with test ngus on both sides of the well at equal distance. The lates were then incubated at 30°C and the inhibitory fect of culture filtrate was recorded qualitatively (- and for 5 days at regular intervals.

hibitory effect on LRD pathogens: The biocontrol otential of *P. fluorescens* against LRD was also tested leaflet pieces. Young, emerging spindle leaves from WD affected palms (free from LRD) were collected. nder leaflets were excised and cut into pieces (70 mm ngth). Pieces from the middle of the leaflet with uniform dth were selected for testing.

The biocontrol potential of *P. fluorescens* was essed against two main pathogens of LRD, *viz.*, *C. oeosporioides* and *E. rostratum,* in leaf rot velopment. Aqueous cell suspension of *P. fluorescens* sterile distilled water was prepared, incorporated to fungal inoculum to give a final concentration of proximately 10^6 bacterial cells/ml. The fungi, *C. oeosporioides* and *E. rostratum* individually and in mbination, were co-inoculated (with Pf) on leaflet ces. The diameter of leaf rot lesion was recorded at ular intervals for 5 days. Appropriate controls, for all ve experiments, were maintained for comparison. All eriments were repeated thrice.

vitro* antagonism of *P. fluorescens: Antagonistic cts of *P. fluorescens* against LRD fungi, *viz, C. osporioides, E. rostratum, G. vermoeseni, F. solani, aradoxa* and *R. solani* were assessed *in vitro*. The wing bacterial culture was found to be inhibitory to mycelial growth of all the above fungi to different nt (Table 1). Maximum inhibition zone of 15.5 mm recorded for *T. paradoxa* followed by *E. rostratum* 4 mm). Filter sterilized culture filtrate of Pf was found hibit the mycelial growth of LRD fungi in varying ees. The growth of *T. paradoxa* was completely pressed by the culture filtrate (Table 2).

1. Antagonistic effect of *P. fluorescens* on LRD pathogens (after 5 days)*

	Inhibition zone (mm)	Max. inhibition of mycelial growth (mm)	% inhibition (over control)
oeosporioides	8.2	23.2	31.0
ratum	13.4	28.4	38.9
moeseni	11.7	26.7	35.6
ni	12.0	27.0	36.0
doxa	15.5	30.5	40.7
ni	11.9	26.9	35.9

of 5 samples

Table. 2. Effect of culture filtrate of *P. fluorescens* on LRD pathogens *

Fungus	Comparative inhibition index 2nd day	5th day
C. gloeosporioides	+	+
E. rostratum	+	+
G. vermoeseni	-	+
F. solani	-	+
T. paradoxa	+	+
R. solani	+	+

- = No response
+ = Positive reaction
* = Mean of 5 samples

Inhibition of LRD development: Incorporation of Pf with two LRD pathogens while inoculating detached leaflet pieces resulted in substantial reduction in leaf rot lesion size and also reduced the initial onset of the disease in general (26 - 33%). Inhibition of lesion size and reduction in LRD development was noticed under both categories of inoculation, *i.e., C. gloeosporioides* and *E. rostratum* inoculated individually and in combination (Table 3).

The results indicate that *P. fluorescens* is inhibitory to *C. gloeosporioides* and *E. rostratum,* the main pathogens of LRD as well as the other associated fungi, *Gliocladium vermoeseni, Fusarium solani, Thielaviopsis paradoxa* and *Rhizoctonia solani*. The culture filtrate of Pf was also inhibitory to the mycelial growth of LRD fungi. In terms of mode of action of an antagonist it should be recognized that greater the number and diversity of methods used by an antagonist to inhibit a pathogen, the more successful it will be in biocontrol of a fungal complex. *P. fluorescens*, which is acting in more than one ways, is effectively used as biocontrol agent of many plant diseases (Arya and Parashar, 1997; Gnanamanickam and Krishnamurthy, 1998; Kumar and Ranganathan, 1997; Nallathambi *et al.*, 1997 a;b; Vidhyasekaran, 1998). Hence, there is scope for *Pf* in the biocontrol of LRD pathogens. Further evaluation of its potential under field condition is being done for effective incorporation of this agent as a means of disease control in the LRD management.

Table 3. Co-inoculation of *P. fluorescens* and main pathogens of LRD and leaf rot development

Fungus inoculated (with *P. fluorescens*)	Inhibition in leaf rot development (%) 3rd day	5th day	Mean
C. gloeosporioides	40.3	26.7	33.5
E. rostratum	18.3	25.5	21.9
C. gloeosporioides and *E. rostratum*	28.1	33.2	30.7

Mean of 20 samples
Values in parentheses represent Standard Deviation

References

Blakeman, J. P. and Fokkema, N. J. 1982. Potential for biological control of plant diseases on the phylloplane. *Ann. Rev. Phytopathol.* **20** : 162-192.

Lily, V. G., Nair, U. K., Pandalai, K. M. and Menon, K. P. V. 1952. Observations on the inhibitory activity of a bacterium on some fungi parasitic on the coconut palm. *Indian Coconut J.* **5** (4) 162-170.

Lily, V. G., Radha, K. and Menon, K. P. V. 1955. Observations on the inhibitory activity of a bacterium, Activity of the fungal substance produced by the bacterium, *Bacillus subtilis*. *Indian Coconut J.* **8** (3) : 137-140.

Menon, K. P. V. and Nair, U. K. 1952. Scheme for the investigation of the root and leaf diseases of the coconut palm in South India. *Indian Coconut J.* **5** (2) : 81-100, 113.

Samraj, J. Paily, P. V. and Cheeran, A. 1966. Observations on the aerial application of oil based fungicides against leaf disease of coconut palm. *Agrl. Res. J. Kerala* **4**: 67-70.

Srinivasan, N. 1991. Occurrence of coconut leaf rot in relation to root (wilt) disease. *Indian Coconut J.* **21** : 14-18.

Srinivasan, N and Gunasekaran, M. 1996a. Pathogenicity of preponderant fungi associated with leaf rot disease of coconut palm. *Indian Coconut J.* **27** (3) : 2-4.

Srinivasan, N and Gunasekaran, M. 1996b. Incidence of fungal species associated with leaf rot disease of coconut palm in relation to weather and stage of lesion development. *Ann. appl. Biol.* **129** : 433-449.

Srinivasan, N and Gunasekaran, M. 1996c. Field control of leaf rot disease of coconut with fungicides. *CORD* **12** : 34-42.

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