

## 8.2.3 ISOLATION OF BACTERIAL ANTAGONISTS FROM RHIZOSPHERE AND THEIR *IN VITRO* EVALUATION AGAINST PATHOGENS OF COCONUT LEAF ROT DISEASE

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### INTRODUCTION

Leaf rot disease (LRD) of coconut is widely prevalent in association with root (wilt) disease (RWD) in Kerala State. An average of 65% of RWD affected coconut palms is infected with LRD. RWD is caused by Phytoplasma<sup>[1]</sup> and LRD is due to a fungal complex. Among the fungi associated with LRD, *Colletotrichum gloeosporioides* and *Exserohilum rostratum* are considered as the main pathogens<sup>[2]</sup>.

Control of LRD is very important in view of its destructive potential. As complete control of LRD is not achieved by fungicidal sprayings<sup>[3]</sup>, it becomes necessary to seek alternative methods like biological control, which can be adopted as such or as part of the measures for integrated disease management. Hence, efforts were made to isolate the native bacterial antagonists from coconut rhizosphere and to evaluate their potential as biocontrol agents against the main pathogens of LRD.

### MATERIALS AND METHODS

**Fungal Culture** *Colletotrichum gloeosporioides* and *Exserohilum rostratum* were isolated on potato dextrose agar (PDA) medium from naturally leaf rot affected coconut palms.

**Isolation of rhizosphere bacteria** It was done from the soil samples collected from the rhizosphere of coconut palms (from basins at a depth of 0–25 cm) by serial dilution technique. Both selective and non-selective media were used. For each medium, representative colonies were isolated and repeatedly cultured till pure culture was obtained.

***In vitro* antagonism** Bacterial isolates were tested for antagonism against the main pathogens of LRD on PDA by dual culture method.

**Antagonism by culture filtrate** The bacterial culture filtrate was filled in a central well in PDA plate, on either side of which agar blocks with mycelial growth of leaf rot fungi were placed.

**Identification of selected bacterial isolates** The bacterial isolates were identified upto generic level with the help of morphological, cultural and biochemical characteristics as per the "Bergey's Manual of Systematic Bacteriology" (Vol. I and II).

### RESULTS AND DISCUSSION

**Isolation of rhizosphere bacteria and screening for antagonistic activity** 21 isolates from rhizosphere were obtained and purified. Antagonistic activity of these isolates against *C. gloeosporioides* and *E. rostratum* was qualitatively recorded. Growing cultures of 9 out of 21 rhizosphere isolates inhibited the mycelial growth of both the fungi. 4 isolates showed weak antagonism where eight showed no reaction.

***In vitro* biocontrol potential of rhizosphere isolates** The isolate 9 was found to be effectively inhibiting the mycelial growth

of both *C. gloeosporioides* and *E. rostratum* followed by isolate 8. The cell-free culture filtrates of all the nine bacterial isolates also inhibited the mycelial growth of *C. gloeosporioides* and *E. rostratum*, thereby indicating the secretion of antifungal substance into the growth medium.

Tab. 1 Antagonistic potential of fluorescent *Pseudomonas* from rhizosphere (mean of 6 replications)

Isolate	Inhibition zone (mm) after 7 days in PDA	
	<i>C. gloeosporioides</i>	<i>E. rostratum</i>
1	3.0	9.0
2	3.0	12.0
3	2.5	6.5
4	4.0	9.0
5	2.0	4.0
6	1.5	5.0
7	2.5	9.0
8	5.5	10.0
9	7.0	12.0

Tab. 2 Growth of *C. gloeosporioides* and *E. rostratum* (radius in mm) as affected by fluorescent *Pseudomonas* spp. (mean of 6 replications)

Fungus	Per cent inhibition (after days of incubation)				
	1	2	3	5	7
<i>C. gloeosporioides</i>					
Isolate 8	0.0	8.7	38	45	55
Isolate 9	0.0	12.5	30	45	61
<i>E. rostratum</i>					
Isolate 8	0.0	12.0	34	60	60
Isolate 9	0.0	25.0	40	68	68

**Identification of selected bacterial isolates** The bacterial isolate numbers 8 and 9, which showed maximum antagonism against both the main fungal pathogens of LRD, were identified as fluorescent *Pseudomonas* spp.

As the fluorescent *Pseudomonas* spp. are naturally occurring antagonists and have the ability to grow fast, they may have an edge over the other competitors, and establish themselves to an effective population levels in competition with the existing ones. In addition, they produce a variety of secondary metabolites including siderophores, which play a very important role in the plant disease suppression.

### REFERENCES

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