Indian Phytopath. 56 (2) : 210-211 (2003)

Efficacy of *Pseudomonas fluorescens* against leaf rot in root (wilt) affected coconut palms

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Key words: Biocontrol, coconut, leaf rot, root (wilt), *Pseudomonas fluorescens*

Coconut root (wilt) disease (RWD), a systemic malady caused by phytoplasma (3), is endemically prevalent in eight southern districts of Kerala. In RWD affected palms, the leaf rot disease (LRD) is commonly superimposed and LRD a part of RWD complex (4). With incidence of root (wilt) in northern districts of Kerala and in certain districts of Tamil Nadu also, leaf rot has appeared in these regions (7). Leaf rot is due to a complex of fungi *(Colletotrichum gloeosporioides* and *Exserohilum rostratum* as the main pathogens) and dynamics of the pathogens, induced by weather factors, is also distinct (5). The emerging spindle leaf of coconut is particularly vulnerable to LRD. *In vitro* studies of biocontrol of leaf rot pathogens with *Pseudomonas fluorescens* were conducted and field trials were also initiated on biocontrol of leaf rot.

The pathogens of LRD (*C. gloeosporioides, E. rostratum, Gliocladium vermoeseni, Fusarium* sp., *Thielaviopsis paradoxa* and *Rhizoctonia solani)* were isolated from naturally affected coconut palms on potato dextrose agar medium (PDA) and purified. In the primary *in vitro* evaluation, the culture and culture filtrate of a standard bacterial antagonist, *P. fluorescens (Pfl),* obtained from Tamil Nadu Agricultural University, Coimbatore was used against all these candidate fungi. The bacterium was grown in King's B agar or King's B broth medium for experiments as per requirements. By dual culture method efficacy of the antagonist was tested against the pathogens on PDA. Appropriate controls were maintained. The antagonistic activity of P. *fluorescens* was elicited. Efficacy of the bacterium (10^9 cells/ml) was also assessed in detached leaf pieces of spindle leaves of coconut and as such in leaf lets of spindle leaves of field palms by mixing the bacterial culture with the main pathogens of the disease (*C. gloeosporioides* and *E. rostratum* - individually and in combination) before inoculation.

In controls only the pathogens were inoculated. The general impact of the bacterium on lesion development was assessed.

The field trial was conducted using a talc-based formulation of *P. fluorescens* (2.5x 10^8 cfu/g). A thrust was given to protect the young emerging spindles of affected palms by adopting the simple application technique standardized (6). About 30-year old palms with typical symptoms of LRD were marked and pre-treatment disease index for the palms were computed. The spindle leaves in individual palms were marked and palms distributed among treatment (20 palms) and control (20 palms) categories with single palm plot size. The talc-based antagonist material was suspended in clean water and poured into the axel of the spindle of treatment palms (@ 50g/l (w/v) per palm). Taking into consideration the known plant growth-promoting attribute of the bacterium, the antagonist material was also added onto the basins of the treatment palms (@100 g /palm) and incorporated into the soil. Application of the material was continued at six monthly intervals up to two stages after recording the disease status in each stage in emerged leaves and compared with control.

Growing culture of *P. fluorescens* inhibited the growth of all the fungi at different levels (44-76%) implying antagonism of the bacterium on leaf rot pathogens and with notable inhibition zone in Petri

Table 1. *In vitro* antagonism of *Pseudomonas fluorescens* on leaf rot pathogens*

Fungus	Inhibition zone (mm) after 7 days in PDA		Percent inhibition in growth after 7 days
	Culture	Culture filtrate	
Colletotrichum gloeosporioides	8.2	3.7	68
Exserohilum rostratum	12.5	5.8	72
Gliocladium vermoeseni	10.6	6.2	76
Fusarium sp.	11.2	4.8	52
Thielaviopsis paradoxa	13.4	7.2	73
Rhizoctonia solani	6.5	3.7	44
CD: (P=0.05)	2.9	2.4	14

*Mean of 3 replications

Table 2. Effect of talc-based *Pseudomonas fluorescens* formulation on leaf rot disease of coconut*

Category of palms	Pre-treatment Disease index**	Disease index in emerged leaves***	
		Stage I	Stage II
Treated	14.71	12.65	11.80
(Over control)	-	(-32.75%)	(-41.35%)
Control	15.68	18.81	20.12
(Over treatment)	-	(+48.69%)	(+70.50%)

*Mean of 20 palms, **For leaves in whole crown, ***For leaves emerged subsequent to pre-treatment

plates (6.5-13.4 mm). The cell-free culture filtrate of the bacterium also caused inhibition on these fungi (3.7-7.2 mm) indicating the movement of anti fungal substance secreted by *P. fluorescens* in the medium (Table 1). Inoculating the culture of *P. fluorescens* along with the (main) pathogens reduced the development of leaf rot lesions in detached - spindle leaf let pieces *in vitro* and in leaflets of spindles of field palms (22-35%). The leaf rot intensity (disease index) in the treated palms was less than in control palms (Table 2). While the disease intensity had risen (+ 70.50%) in control palms (over the treatment), the disease severity was reduced (- 41.35%) in the bacterial treated palms (over control).

In recent years, *P. fluorescens* has been used as an effective biocontrol agent against various plant diseases by differential application techniques based on the need and nature of disease (8,9). Talc is one of the common carriers used in biocontrol involving bacterial antagonists. *Pseudomonas* treatment has been found to significantly reduce the pathogen colonization in host plant tissues and the antagonist produces secondary metabolites inimical to fungal pathogens. Thus inhibition of leaf not development by *P. fluorescens* has been elucidafed in the study (1, 2).

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Received for publication November 20, 2002