

## Evaluation of fungicides and antagonistic organism against major pathogens of leaf rot disease of coconut and their eco-friendly management

N. Srinivasan<sup>1</sup>\*, S. Jyothi Rahna<sup>1</sup>, V.K. Anishkumar<sup>1</sup>

### Abstract

Leaf rot is an integral part of coconut root (wilt) disease complex. In the management of root (wilt), leaf rot control is of vital importance. Leaf rot phase is due to a complex of fungal pathogens - *Colletotrichum gloeosporioides*, *Exserohilum rostratum*, *Fusarium solani* etc. Investigations on effect of fungicides confirmed strong inhibition of all major pathogens *in vitro* by Contaf (Hexaconazole), Indofil M- 45 ((Mancozeb), Tilt (Propiconazole) and Bavistin (Carbendazim). Antracol (Propineb), Tag cop 50 (Copper oxychloride) and Calixin (Tridemorph) inhibited *F. solani* to a lesser extent than others. *Bacillus subtilis* was found relatively tolerant to fungicides (Contaf, Indofil M- 45) as compared to *Pseudomonas fluorescens*. *B. subtilis* was able to tolerate lower concentrations of Tilt or Bavistin in the medium while *P. fluorescens* did not. *B. subtilis*, *P. fluorescens* and *Trichoderma harzianum* were tolerant to Antracol in lower concentrations. As such, integrated use of bio agents with fungicides (at minimal concentrations) can be employed in disease management. Amelioration of leaf rot affected palms in the field was consistently effected by a talc-based preparation of *P. fluorescens*. Antagonistic potential of *B. subtilis* and *P. fluorescens* against the pathogens alone and in combination eliciting synergism in inhibition of pathogens have also been established. *T. harzianum* was found to inhibit *C. gloeosporioides* and *E. rostratum*. However, a reverse effect of *F. solani* on the fungal antagonist was seen. Combined use of fungicide (Contaf as a test case) and bacterial antagonist(s) when tested *in vitro* against the pathogens, synergism in antagonism against *C. gloeosporioides* and *E. rostratum* was evident. Prevalence of antagonistic bacteria (*Bacillus* sp.) and fungi (*Aspergillus* sp.) in the phylloplane of coconut was also brought out. Knowledge thus gained on various aspects are important in identifying effective/superior isolates of antagonists, formulation of effective microbial consortia and appropriate integration of bio agents and fungicides in the integrated management of the disease complex.

**Key words:** Coconut, root (wilt), leaf rot, fungal pathogens, fungicides, antagonists, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, Contaf, Indofil M-45, Tilt, Bavistin, Antracol, Tag cop 50, Calixin, integrated disease management.

<sup>1</sup> Central Plantation Crops Research Institute, Regional Station, Kayangulam, Krishnapuram – 690 533, Alappuzha District, Kerala, India.

\* (Corresponding author: Telephone: + 91– 0479 –2442160; Fax: + 91–0479–2445733; E-Mail: srinivasannarayanamy@yahoo.com / cpccrirskgm@yahoo.com)

## Introduction

In coconut, leaf rot is an important disease and it is a part of root (wilt) disease complex widely prevalent in southern districts of Kerala state, India. In recent years, its incidence in northern districts of Kerala and in certain districts of Tamil Nadu bordering Kerala has been also brought out (Solomon *et al.*, 1999; Srinivasan and Sasikala, 2001). Leaf rot is significant as its superimposition in root (wilt) affected palms leads to a sharp decline in the condition of the palms, thus drastically affecting their yield and quality parameters. Therefore, leaf rot control has been recognized as an important and integral part in the root (wilt) management strategy (Srinivasan, 2004a, 2005; Srinivasan and Rohini Iyer, 2004). The leaf rot phase is due to a complex of fungal pathogens and among them *Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Fusarium solani* are the major pathogens (Srinivasan, 2004b; Srinivasan and Gunasekaran, 1996a, 2000a). Earlier recommendation in control of leaf rot has been based on utilization of fungicides particularly by spraying of infected palms (Menon and Nair, 1951; Srinivasan and Gunasekaran, 1999). Inhibitory effect of fungicides on pathogens of leaf rot based on *in vitro* studies was shown (Srinivasan and Gunasekaran, 1998). Srinivasan and Gunasekaran (1996b) evolved a simple technique of applying fungicide on to well of the spindle leaf of coconut crown for amelioration of leaf rot syndrome and such system of disease control by protecting the emerging spindle leaves (Srinivasan and Gunasekaran, 2003) has received a wide attention in the disease endemic region. The possibility of using such a technique for other factors in the integrated disease management has since been forth coming. The necessity of eco-friendly management of the disease complex is increasingly felt with due consideration for environment in the disease endemic region. Hence rationale use of fungicides, antagonistic organisms, botanicals etc. effective against the pathogens have been outlined (Lily *et al.*, 1952, 1955; Alka Gupta *et al.*, 2000; Gunasekaran *et al.*, 2003; Joseph *et al.*, 2003; Srinivasan, 2003, 2005; Anishkumar *et al.*,

2004) for disease management in an environment friendly manner. In the given nature of root (wilt) complex more understanding on eco-friendly measures of disease control become necessary. Therefore, information by evaluation of certain fungicides on major pathogens of leaf rot as well as their antagonists, individual and combined effect of antagonists on the pathogens, compatibility of fungicide – antagonist *vis-à-vis* their combined effect on pathogens, consistency of field performance of antagonist on the disease and *in vivo* status of bio agents prevalent in coconut phylloplane against the pathogens were generated.

## Material and methods

The fungicides and details of their concentrations tested in the study against the pathogens and their antagonists are given in concerned Table. Cultures of *C. gloeosporioides*, *E. rostratum* and *F. solani* isolated afresh from leaf rot affected palms and grown in potato dextrose agar (PDA) medium, standard cultures of two bacterial antagonists (*Bacillus subtilis* and *Pseudomonas fluorescens*) and a fungal antagonist (*Trichoderma harzianum*) were utilized in the study. Individual and combined effect of bacterial antagonists on the pathogens, besides combined effect of antagonist and fungicide were experimented *in vitro*. A field trial was conducted with bacterial antagonist as a test case. Efficacy of certain isolates of bacteria and fungi isolated from coconut phylloplane were also evaluated *in vitro* against the pathogens. Experimental details of various aspects are as follows.

### Effect of fungicides on pathogens

Two experiments were conducted to evaluate efficacy of certain fungicides against the pathogens by employing poisoned food technique (Srinivasan and Gunasekaran, 1998). In the first experiment, seven fungicides (Table 1) were tested to study their effect on the pathogens. Fungicide amended medium with

specific concentration was poured aseptically into sterile petri dishes (15 ml/petri dish). Mycelial discs (5 mm diameter) - cut from the periphery of five-day-old cultures of each fungus was inoculated onto the centre of petri dish. Three replications were maintained for each treatment. Controls inoculated with fungus but without fungicide, were maintained for comparison. These were incubated at  $30 \pm 2^{\circ}\text{C}$ . The radial growth of the fungal colony in each petri dish was recorded on 5<sup>th</sup> day of incubation. For this the average diameter of the colony by measuring the diameters in two directions at right angles was taken. The fungal growth in fungicide treated petri dish was compared with control and percent inhibition of growth of fungi was arrived at by the following formula:

$$I = \frac{C - T}{C} \times 100$$

(where I = Percent inhibition of growth, C = diameter of fungus in control and T = diameter of fungus in treatment). Mean inhibition percent was calculated.

In the second experiment five fungicides were used (Table 2). The fungicides at different concentrations were tested for inhibitory effect on leaf rot pathogens by the method as described earlier. The fungal growth in fungicide treated petri dishes was compared on 5<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> day after incubation. Mean inhibition effect of the concentrations of fungicides for different days of incubation was analyzed after computation of percent inhibition of growth of the fungi.

#### Effect of fungicides on antagonists

The effect of these five fungicides on growth of antagonistic bacteria and fungus was also studied (Table 3). In the case of bacterial antagonists, nutrient agar (NA) was used as basal medium. *B. subtilis* and *P. fluorescens* were individually streaked onto medium previously mixed with fungicides. Three replications were maintained for each treatment and control. The inoculated petri dishes were incubated at  $30 \pm 2^{\circ}\text{C}$ . Observations on growth of bacteria upto 5<sup>th</sup> day was qualitatively recorded in comparison

with control and grouped as (-): No growth, (+): Low growth, (++) : Moderate growth and (+++): Good growth. For the antagonistic fungus, *T. harzianum*, PDA was used as basal medium. The five-day-old circular - mycelial discs of *T. harzianum* (5mm diameter) were inoculated onto centre of petri dishes containing the medium previously mixed with fungicides in different concentrations (three replications). The inoculated petri dishes and control were incubated and percent inhibition of the fungus computed as described earlier.

#### Field efficacy of *Pseudomonas fluorescens* on leaf rot

With the evidence of *P. fluorescens* inhibiting the pathogens of leaf rot *in vitro* (Srinivasan, 2003; Gunasekaran *et al.*, 2003) a field control trial was conducted with talc-based formulation of the antagonist. About 30 year old - leaf rot affected palms were selected. The experiment was designed based on single palm plot size and spindle leaves of the palms were marked at the start of the experiment. The pre-treatment disease index for the palms was quantified as described (Srinivasan and Gunasekaran, 1996b). There were 20 palms under treatment category and another 20 palms under control for comparison. As the preliminary finding of the treatment effect (Srinivasan, 2003) gave encouraging results more data were collected. The talc-based material of *P. fluorescens* ( $2.5 \times 10^8$  cfu/g) was suspended in water (@50g/l per palm) and poured into the axel of the spindle of palms (Srinivasan and Gunasekaran, 1996b, 2003). The *P. fluorescens* material was also added onto the palm basins of treatment category (@ 100g/palm) and the material incorporated in the basins of palms. This was repeated twice a year (May and September). The initial round of treatment was given in April/May 2001. Subsequently, five rounds of treatments (through September, 2003) were given to the experimental palms during which the disease index for subsequently emerged leaves of palms at each round calculated in a cumulative manner. By the end of the experiment all the leaves in the crown were replaced with newly

emerged leaves since start of the trial. The data of treated palms were compared with control for assessing the effect of *P. fluorescens* on leaf rot.

#### **Effect of antagonists, their compatibility and fungicide-antagonist interaction on pathogens**

Cultures of bacterial antagonists, *B. subtilis* and *P. fluorescens* and the fungal antagonist, *T. harzianum* were tested and compared for their antagonistic activity against the pathogens of leaf rot. The bacteria were swab inoculated individually on the petri dishes containing PDA medium using cotton swabs. Mycelial disc of each pathogenic fungus (5 mm size) was placed at the centre of the each swab culture in petri dish and incubated at  $30 \pm 2^{\circ}\text{C}$ . The bacterial combinations were also similarly studied by appropriately mixing them. The colony diameter of the fungi was measured after 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of incubation. The extent of inhibition in growth of the pathogens particularly after 5<sup>th</sup> day of incubation was computed in percent. Culture of *T. harzianum* was also tested against the pathogens by dual culture method. The mycelial discs of the pathogens were individually inoculated at the centre of petri dishes containing PDA medium and in each dish four mycelial discs (5 mm diameter) of *T. harzianum* were placed at four corners at equal distance from the disc of the pathogen. The inoculated petri dishes (three replications) were incubated, colony diameter measured at different intervals in comparison with control and percent inhibition particularly after 5<sup>th</sup> day of incubation computed.

Following the encouraging results obtained with bacterial antagonists singly and in combination, they were combined with one systemic fungicide – Contaf (Hexaconazole) in different concentrations (0.025%, 0.05%, 0.1% and 0.2%) and tested subsequently. Contaf was already identified as a potential systemic fungicide against leaf rot pathogens (Srinivasan and Gunasekaran, 1998) and use of the fungicide has since been *in vogue* in management of the disease in disease endemic region (Srinivasan and Gunasekaran, 2003). The leaf rot fungi (5mm diameter) were individually inoculated onto centre of petri dishes containing PDA medium

amended with *B. subtilis* alone, *P. fluorescens* alone, Contaf alone (in various concentrations), *B. subtilis* + Contaf (in various concentrations) and *P. fluorescens* + Contaf (in various concentrations). Suitable controls were maintained. The method of inoculation, incubation, recording of observations etc. is essentially as described previously (three replications). Percent inhibition of pathogens on 5<sup>th</sup> day of incubation was computed in comparison with the control. Qualitative and quantitative effects of various treatments on the pathogens were recorded.

#### **Assessment of bioagents from coconut phylloplane on pathogens**

Bacteria and fungi isolated from phylloplane of coconut leaves were assessed for their ability of antagonism, if any, on leaf rot pathogens. For this study, leaf samples collected were individually suspended in sterile distilled water (each one gm of leaf bit in 10 ml of water) and shaken well for 10 minutes. Serial dilutions upto  $10^{-6}$  were plated on NA and PDA for bacteria and fungi respectively. Petri dishes were incubated at  $30 \pm 2^{\circ}\text{C}$  and observed at 24 hr interval for 5 days. From this, bacterial and fungal isolates were made in pure cultures. These were screened against the pathogens of leaf rot and the interactions studied. Subsequently, isolates of bacteria (*Bacillus* sp.) and fungi (*Aspergillus* sp. and *Penicillium* sp.) showing inhibitory effect on all the three major pathogens were selected and subjected to detailed study.

A swab or streak method (as described earlier) was employed to test the inhibition of pathogens by the phylloplane bacteria. The plates were incubated for 5 days and inhibition status recorded. The inhibition zone on either side of the streak, when appeared was recorded. Fungal isolates from coconut phylloplane were also evaluated in the laboratory against the pathogens of leaf rot by adopting the dual culture as described earlier. The petri dishes were incubated for 5 days, colony diameters measured and percent inhibition calculated.

Inhibition zone, when developed, was also measured.

## Results and discussion

### Effect of fungicides on pathogens

Results on the effect of fungicides on leaf rot pathogens in the first experiment are given in Table 1. Selective inhibition of pathogens by fungicides has been observed. Among the fungicides, Indofil M-45, Contaf, Tilt and Bavistin were found to inhibit the fungi up to 100%. This was followed by Calixin. Moderate inhibition effect of Antracol and lower inhibition of fungi by Tag cop 50 have been observed. As such, very high-mean inhibition in growth of all the three pathogens (99.5%–100%) at lowest concentration itself has been noticed with four fungicides. The ability of these fungicides in inhibiting all the three major pathogens of leaf rot in a higher order in general and particularly in lower concentration is a point of importance. Concentrations of certain fungicides also influenced the extent of inhibition of fungi depending upon the species involved. For example, cent per cent inhibition of *E. rostratum* with Calixin was obtained even at lowest concentration tested (0.025%), but the same level of inhibition of *C. gloeosporioides* could be achieved at 0.05% concentration of the fungicide, and for *F. solani* at 0.3% level. With Tag cop 50, cent percent inhibition of all the three fungi could be achieved only at 0.3%. Antracol inhibited all the three fungi at lesser level only even at the highest concentration tested. *F. solani* was less inhibited by Antracol, Tag cop 50 and Calixin as compared to other fungi. The mean inhibition in growth on 5<sup>th</sup> day of incubation also revealed cent percent inhibition of all three fungi by Indofil M-45, Contaf, Tilt and Bavistin. Fungal recovery was possible from discs treated with Antracol and Tag cop 50 when re-transferred to fungicide free medium.

The effect of fungicides on the pathogens was recorded in 5<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> day of incubation in the second experiment. Contaf, Tilt, Bavistin and Indofil M-45 inhibited all the

major pathogens heavily even at the lowest concentration. Almost cent percent inhibition of fungi by these fungicides was recorded on the 9<sup>th</sup> day of incubation (data not shown). The effects of varying concentrations of the fungicides on 9<sup>th</sup> day of incubation are given in Table 2. Higher inhibitory effect of Indofil M-45, Contaf, Tilt and Bavistin on the pathogens was evident throughout. Almost cent percent inhibition of all the three fungi by these fungicides on 9<sup>th</sup> day of incubation was thus confirmed. Moderate inhibitory effect of Antracol on various pathogens of leaf rot was also made out. The fungal recovery was not possible on re-transfer of completely inhibited discs to a fresh medium. However, in the case of Antracol fungal recovery was possible when treated discs were re-transferred to fungicide-free medium and incubated (Fig. 1-2).

Wide variations in efficacy of fungicides on plant pathogens have been reported by workers. Shailbala and Tripathi (2004) reported that the application of Tilt (0.1%) resulted in very effective control of urd bean (*Vigna mungo*) web blight caused by *Rhizoctonia solani* followed by Bavistin and Dithane M-45; Antracol performed comparatively less effectively than others. Effectiveness of Tilt and Bavistin in control of various other diseases also has been reported elsewhere by various researchers. Ramachandran and Kalappanavar (2004) reported Tilt, Contaf and Mancozeb as most effective fungicides in inhibiting the growth of *Exserohilum hawaiiensis*, which causes blight of wheat. Saha and Kaiser (2004) observed higher inhibitory effect of Bavistin on *F. semitectum*. Very effective control of cardamom leaf spot (*Phyllosticta elettariae*) with Carbendazim 50 WP (0.1%) was recorded by Arasumalliah and Shivakumar (2004). Akhilesh Singh *et al.* (2003) reported Tilt, Contaf and Dithane M-45 as effective fungicides in reducing the severity of Taphrina leaf spot of turmeric, there Antracol was found to be less effective.

**Table 1. Effect of fungicides on growth of major pathogens of leaf rot in first experiment\***

S. No.	Fungicide *	Fungicide Conc. (%)	Percent Inhibition in growth on 5 <sup>th</sup> day of incubation			Mean percent inhibition in growth on 5 <sup>th</sup> day of incubation (Mean for concentrations)				Mean (C.D. at 1% .09.0)
			<i>C. gloeosporioides</i>	<i>E. rostratum</i>	<i>F. solani</i>	<i>C. gloeosporioides</i>	<i>E. rostratum</i>	<i>F. solani</i>		
1	Propineb 75% WP (Antracol)	0.1	71.8	91.6	67.9	84.2	95.1	77.5	85.6	
		0.2	81.8	93.7	68.6					
		0.3	85.9	95.7	80.1					
		0.4	89.8	96.7	83.3					
		0.5	91.8	97.7	87.6					
2	Copper oxy chloride (Tag cop 50)	0.025	20.7	34.0	11.1	49.6	69.0	49.1	55.9	
		0.05	30.5	60.0	23.2					
		0.1	45.6	74.0	29.6					
		0.2	51.2	77.0	81.5					
		0.3	100.0	100.0	100.0					
3	Mancozeb 75%WP (Indofil M-45)	0.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
		0.2	100.0	100.0	100.0					
		0.3	100.0	100.0	100.0					
		0.4	100.0	100.0	100.0					
		0.5	100.0	100.0	100.0					
4	Hexaconazole 5%EC (Contaf)	0.025	98.9	98.7	98.4	99.7	99.6	99.5	99.6	
		0.05	99.4	99.2	99.1					
		0.1	100.0	100.0	100.0					
		0.2	100.0	100.0	100.0					
		0.3	100.0	100.0	100.0					
5	Propiconazole 5%EC (Tilt)	0.025	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
		0.05	100.0	100.0	100.0					
		0.1	100.0	100.0	100.0					
		0.2	100.0	100.0	100.0					
		0.3	100.0	100.0	100.0					
6	Carbendazim 50%WP (Bavistin)	0.025	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
		0.05	100.0	100.0	100.0					
		0.1	100.0	100.0	100.0					
		0.2	100.0	100.0	100.0					
		0.3	100.0	100.0	100.0					
7	Tridemorph 80% EC (Calixin)	0.025	87.8	100.0	62.9	97.6	100.0	79.6	92.4	
		0.05	100.0	100.0	72.0					
		0.1	100.0	100.0	77.8					
		0.2	100.0	100.0	85.2					
		0.3	100.0	100.0	100.0	90.2	94.8	86.5	(Mean)	

\* Mean of three replications

Table 2. Effect of fungicides on growth of major pathogens of leaf rot in second experiment in relation to period of incubation\*

S. No.	Fungicide	Mean colony diameter in relation to duration of incubation (mm)**											Percent inhibition on 9 <sup>th</sup> day of incubation**			
		Cg (C.D. for mean at 1%: 1.7)*				Er (C.D. for mean at 1%: 0.6) <sup>b</sup>				Fs (C.D. for mean at 1%: 1.5) <sup>c</sup>			Cg	Er	Fs	
		5 <sup>th</sup> day	8 <sup>th</sup> day	9 <sup>th</sup> day	Mean <sup>a</sup>	5 <sup>th</sup> day	8 <sup>th</sup> day	9 <sup>th</sup> day	Mean <sup>b</sup>	5 <sup>th</sup> day	8 <sup>th</sup> day	9 <sup>th</sup> day				Mean <sup>c</sup>
1	Propineb 75% WP (Antracol)	7.7	10.0	11.9	9.9	1.9	2.7	4.3	2.9	6.9	9.2	11.7	9.3	82.4	92.7	78.7
2	Mancozeb 75% WP (Indofil M-45)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
3	Hexaconazole 5% EC (Contaf)	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	99.6	99.7	99.5
4	Propiconazole 5% EC (Tilt)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
5	Carbendazim 50% WP (Bavistin)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0
	Mean	1.6	2.1	2.4	-	0.4	0.6	0.9	-	1.4	1.9	2.4	-	-	-	-
	Control	49.0	55.1	67.7	-	39.3	48.0	58.1	-	30.6	45.0	55.0	-	-	-	-

\* Mean of three replications

\*\* Mean of five concentrations (See text for details)

Cg – *Colletotrichum gloeosporioides*, Er – *Exserohilum rostratum*, Fs – *Fusarium solani*.

Table 3. Effect of fungicides on growth of antagonistic organisms on 5<sup>th</sup> day of incubation\*

S. No.	Fungicide	Concentration of fungicide	Growth rate of bacterial antagonists**		Growth rate of fungal antagonist, <i>Trichoderma harzianum</i>	
			<i>Bacillus subtilis</i>	<i>Pseudomonas fluorescens</i>	Colony diameter (mm)	Percent inhibition (over control)
1	Propineb 75% WP (Antracol)	0.1	+++	++	11.0	82.5
		0.2	+++	+	10.0	84.1
		0.3	+	+	08.0	87.3
		0.4	-	-	06.0	90.5
		0.5	-	-	00.0	100.0
2	Mancozeb 75%WP (Indofil M-45)	0.1	++	+	02.5	96.0
		0.2	+	+	00.0	100.0
		0.3	+	-	00.0	100.0
		0.4	-	-	00.0	100.0
		0.5	-	-	00.0	100.0
3	Hexaconazole 5%EC (Contaf)	0.025	++	+	03.0	95.2
		0.050	+	-	00.5	99.2
		0.10	+	-	00.0	100.0
		0.20	-	-	00.0	100.0
		0.30	-	-	00.0	100.0
4	Propiconazole 5%EC (Tilt)	0.025	+	-	00.0	100.0
		0.050	-	-	00.0	100.0
		0.10	-	-	00.0	100.0
		0.20	-	-	00.0	100.0
		0.30	-	-	00.0	100.0
5	Carbendazim 50%WP (Bavistin)	0.025	+	-	00.0	100.0
		0.050	-	-	00.0	100.0
		0.10	-	-	00.0	100.0
		0.20	-	-	00.0	100.0
		0.30	-	-	00.0	100.0
Control			+++	+++	63.0	-

\* Mean of three replications

\*\* (-): No growth, (+): Low growth, (++) Moderate growth, (+++): Good growth.

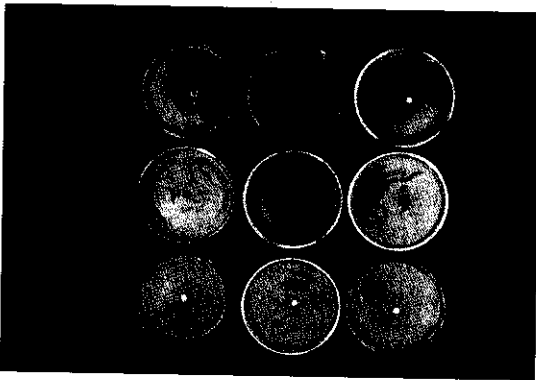


Fig. 1. Inhibition of leaf rot pathogens by Contaf (Top row: *Colletotrichum gloeosporioides*; Middle: *Exserohilum rostratum*; Bottom: *Fusarium solani*)

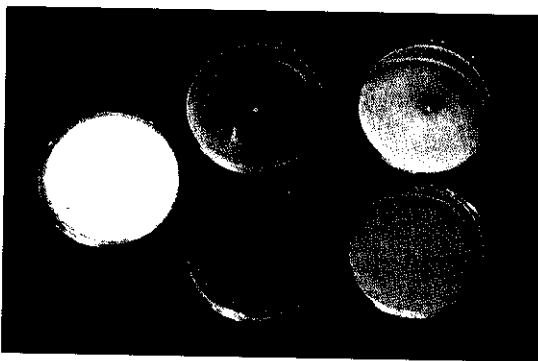


Fig. 2. Inhibition of leaf rot pathogen (*Colletotrichum gloeosporioides*) by Tilt (Growth of the pathogen in control petri dish)

Superior control of ground nut rust and late leaf spot (Sheela and Vimala, 2004), grape vine powdery mildew (Ashwathanarayana *et al.*, 2004) and rice sheath blight (Rajbir Singh and Sinha, 2004) with Hexaconazole (Contaf) have been reported by various workers. Baranwal *et al.* (2003) also recorded maximum suppression of *Stemphylium* blight of onion with Hexaconazole. Srinivasan and Gunasekaran (1998) reported the effectiveness and broad-spectrum potential of Hexaconazole (Contaf) against leaf rot pathogens of coconut that aided a sound base for widespread utility of the fungicide in the management of the disease complex in the diseased region (Srinivasan and Gunasekaran, 2000b, 2003). Among contact fungicides tested in the earlier study, Indofil M-45 performed most effectively

against the pathogens. While results of the current study are in conformity with such earlier findings, very high inhibition potential of certain other systemic fungicides such as Tilt as evaluated in the bioassay would be useful in the field management of leaf rot. Concentration of Antracol or Tag cop 50 (both contact fungicides) had direct relation on the extent of inhibition of leaf rot fungal growth. These results are supported by various reports as fore mentioned.

### Effect of fungicides on antagonists

Results of the *in vitro* effect of fungicides on antagonistic organisms as recorded on the 5<sup>th</sup> day of incubation are given in Table 3. The extent of inhibition of antagonists also depended on the fungicides. Complete inhibition of bacterial antagonists was not seen in 0.1%-0.3% concentrations of Antracol, though at 0.4%-0.5% growth of both *B. subtilis* and *P. fluorescens* were completely suppressed. *B. subtilis* was less affected similarly by lower concentrations of Indofil M-45 (0.1%-0.3%) as compared to *P. fluorescens*. Likewise, in lower concentrations of Contaf (0.025%-0.10%) growth of *B. subtilis* was less affected as compared to *P. fluorescens*. In Tilt or Bavistin amended medium *P. fluorescens* failed to grow even in the lowest concentration (0.025%); *B. subtilis* could tolerate the lowest concentration of these fungicides. Compatibility of *B. subtilis* with lower concentrations of Contaf, Indofil M-45 etc. are significant. The fungal antagonist, *T. harzianum* was completely inhibited even at lowest concentration of Tilt and Bavistin (cent per cent inhibition, incompatible). In Indofil M-45 or Contaf amended medium, slow growth of *T. harzianum* was observed in the lowest concentration while cent percent inhibition occurred at higher concentrations. The fungus was not completely inhibited was completely inhibited at 0.1%-0.4% concentration of Antracol, but at 0.5% concentration. Thus the fungal antagonist was found compatible with lower concentrations of Antracol (Fig. 3-4).

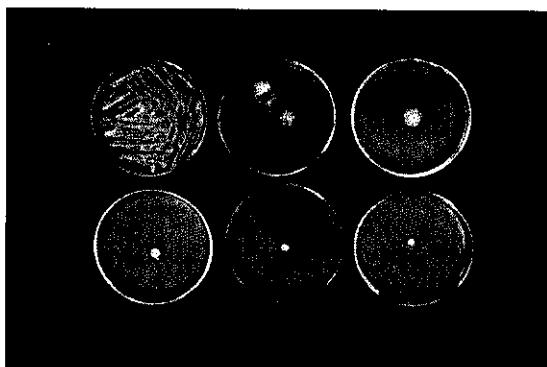


Fig. 3. Growth - viability of bacterial antagonists in Contaf amended medium (lower concentration), where leaf rot pathogen (*Colletotrichum gloeosporioides*) inoculated inhibited (Top row: *Bacillus subtilis*; Bottom: *Pseudomonas fluorescens*)



Fig. 4. Growth - viability of bacterial antagonists in Contaf amended medium (lower concentration), where leaf rot pathogen (*Fusarium solani*) inoculated inhibited (Top row: *Bacillus subtilis*; Bottom: *Pseudomonas fluorescens*)

Little information is available about the effect of fungicides on antagonistic organisms. Mathew (2003) studied about compatibility of *P. fluorescens* with certain fungicides besides insecticides. More compatibility was seen with Mancozeb and Carbendazim as compared to Hexaconazole, based on visual observation. Laha and Venkataraman (2001) reported compatibility of Carbendazim with *P. fluorescens*. Poddar *et al.* (2004) evaluated certain systemic fungicides against *T. harzianum* – significant variation in the fungicidal sensitivity among isolates of the fungus was found and among them one isolate was relatively less sensitive to Carbendazim. Variable response of *B. subtilis* and *P.*

*fluorescens* to fungicides, as observed in the current study, underlines the need and scope for the use of consortium of antagonists in the event of chemical treatment along with bio agents in the process of disease control. *B. subtilis* and *P. fluorescens* are independently inhibitory to various plant pathogens, as reported by various workers (Reddy *et al.*, 2003). *B. subtilis* being less affected (as compared to the more sensitive *P. fluorescens*) by fungicides results of the present study are significant. Similarly, higher sensitivity of *T. harzianum* to several fungicides also provides a clue for the need for rational use of fungicides along with bio agent. The compatibility of bacterial and fungal antagonists to fungicides like Antracol is advantageous for their combined application to the integrated disease management. This is validated by the combined application of fungicide and antagonist that has resulted in very effective control of plant diseases. As for example, Nallathampi and Thakore (2003) recorded that combined application of fluorescent *Pseudomonas* with a fungicide (thiophenate methyl, Captan or alcidine) resulted in effective suppression of fruit rot of ber (*Ziziphus mauritiana*) caused by *Alternaria alternata*. Similarly integration of *T. viride* and fungicide (Thiram, Vitavax or Captaf) was more effective in control of collar rot (*Macrophomina phaseolina*) of French bean – *Phaseolus vulgaris* (Dubey, 2002). Integration of biocontrol agent with fungicides has been found to lead to significantly higher disease control than that attained by either biocontrol agent or fungicide alone in several other crops also (Deepak Kumar and Dubey, 2001; Vyas, 1994). Integrated use of biocontrol agents with fungicides is thus possible in the field-control of leaf rot disease.

#### Field efficacy of *Pseudomonas fluorescens* on leaf rot

Results on the effect of application of talc-based preparation of *P. fluorescens* on leaf rot in field palms are presented in Table 4. Treatment of palms with the bacterial antagonist lead to a decline of disease index in newly emerged leaves in various rounds of observations as

compared to the disease status in control palms which received no antagonist material. The mean disease index of treated palms, which stood as 14.71 (pre-treatment level) at the beginning (April, 2001) of the experiment, gradually declined over the rounds of treatment application to the level of 8.00 at the fifth (final) round (September, 2003). However, in control palms, the pre-treatment disease index, which stood at 15.68 gradually, increased over the period of time and attained the level of 27.50 at the final round of observation. Consequently, at the last round of treatment the mean disease index in the antagonist-treated palms was less by 45.62% over the pre-treatment and also less by 70.91% in comparison with control palms. In the control, the mean disease index increased to 75.38% from pre-treatment level and consequently the disease level worked out to be 243.75% in comparison with treated palms. Comparison of disease index of newly emerged leaves of palms for all rounds of observations also revealed consistent effect of the antagonist in the suppression of leaf rot. The mean disease index for the rounds (after pre-treatment) for treated and control palms were computed as 9.91 and 23.25, respectively; reduced levels of disease in treated palms over own pre-treatment (32.66%) and also control palms (55.28%) *vis-à-vis* increased levels of disease in control palms over own pre-treatment (48.28%) and treated palms (149.46%) are evident. Thus the effectiveness of *P. fluorescens* in controlling leaf rot was brought out.

Several bacterial antagonists, particularly fluorescent *Pseudomonads* have been used as most promising bio agents for the management of different plant diseases of fungal, bacterial, viral etiology (Reddy *et al.*, 2003). For example, Baranwal *et al.* (2003) recorded lesser index of *Stemphylium* blight of onion by the application of *P. fluorescens*. Similarly, Rajbir Singh and Sinha (2004) brought out effective management of sheath blight of rice using different strains of *P. fluorescens* besides certain commercial preparation of the bio agent. Live culture and culture filtrate of *P. fluorescens* significantly inhibited the growth of various pathogens of leaf rot *in vitro* (Srinivasan, 2003; Gunasekaran *et al.*, 2003). Consistent effect of *P. fluorescens* in the suppression of leaf rot leading to amelioration of

disease in coconut palms as observed in the current study is of great importance (Srinivasan, 2003; Joseph *et al.*, 2003). *P. fluorescens* has been reported elsewhere to induce systemic resistance in plants against diseases caused by different biotic agents. Hence, further work on elucidating induced systemic resistance against root (wilt)-leaf rot complex is in progress.

#### **Effect of individual and combined effect of antagonists and fungicides on pathogens**

Compatibility between antagonistic bacteria (*B. subtilis* and *P. fluorescens*) was tested *in vitro*. These bacterial species were grown independently and co-cultured in petri dishes. The behaviour of the organisms towards one another was evaluated at 24 hr interval for 5 days with appropriate incubation. It was found that both the species were able to co-exist in culture without exerting any deleterious effect on each other, a feature that may be regarded as valuable in the bio control of plant diseases. In a preliminary study *B. subtilis* (63%-74%) and *P. fluorescens* (61%-69%) inhibited leaf rot pathogens when the antagonists were individually assayed *in vitro* (CPCRI, 2003). In a further replicated experiment, the bacterial species were grown both independently and in combination, and resultant effect on major pathogens of leaf rot was studied (Table 5). Inhibition of the pathogens by the bacterial species, both individually and in combination, was consistently evident at varying periods of incubation. On the 5<sup>th</sup> day of incubation *B. subtilis* inhibited *C. gloeosporioides*, *E. rostratum* and *F. solani* to the extent of 71.66%, 87.38% and 84.70%, respectively; *P. fluorescens* also inhibited the fore-mentioned fungi to the extent of 60.55%, 59.84% and 31.13%. Thus the two species of bacteria maintained an intrinsic capacity on inhibition of the leaf rot pathogens. Even as the bacterial species independently inhibited the pathogens, almost cent percent inhibition of all the pathogens could be achieved by using their combination, thus proving the efficacy of the consortium approach. A similarity in trend of inhibition of all the pathogens by the antagonists' combination was also seen.

Table 4. Effect of application of talc-based preparation of *Pseudomonas fluorescens* on leaf rot disease on coconut palms\*

S. No.	Category of palms	Pre-treatment Disease Index** (April/May 2001)	Disease Index (D.I.) ***					Mean of Rounds
			Round I Sept. 2001	Round II May 2002	Round III Sept. 2002	Round IV May 2003	Round V Sept. 2003	
1	Palms treated with <i>P. fluorescens</i>	14.71	12.65	11.80	08.83	08.25	08.00	9.91
	Improvement over pre-treatment (Decrease in D. I.)	-	(-) 14.00%	(-) 19.78%	(-) 39.97%	(-) 43.92%	(-) 45.62%	(-) 32.66%
	Improvement over control (Decrease in D. I.)	-	(-) 32.75%	(-) 41.35%	(-) 63.99%	(-) 67.39%	(-) 70.91%	(-) 55.28%
2	Control palms	15.68	18.81	20.12	24.52	25.30	27.50	23.25
	Deterioration over pre-treatment (Increase in D. I.)	-	(+) 19.96%	(+) 28.32%	(+) 56.38%	(+) 61.35%	(+) 75.38%	(+) 48.28%
	Deterioration over treatment (Increase in D. I.)	-	(+) 48.69%	(+) 70.50%	(+) 177.69%	(+) 206.67%	(+) 243.75%	(+) 149.46%

\*Mean of 20 palms in each category.

\*\*For leaves in whole crown.

\*\*\*For leaves emerged subsequent to pre-treatment.

**Table 5. Effect of bacterial antagonists on growth of major pathogens of leaf rot *in vitro* \***

S. No.	Pathogen	Treatment	Colony diameter of fungus (mm) after different days of incubation*			Per cent Inhibition in growth on 5 <sup>th</sup> day of incubation
			3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	
1	<i>Colletotrichum gloeosporioides</i>	<i>Bacillus subtilis</i>	08.5	12.0	17.6	71.66
		<i>Pseudomonas fluorescens</i>	16.5	20.5	24.5	60.55
		<i>B. subtilis</i> + <i>P. fluorescens</i>	0	0	0	100.00
		Control	48.5	56.0	62.1	-
2	<i>Exserohilum rostratum</i>	<i>Bacillus subtilis</i>	02.5	07.0	07.7	87.38
		<i>Pseudomonas fluorescens</i>	11.5	18.0	24.5	59.84
		<i>B. subtilis</i> + <i>P. fluorescens</i>	00.5	01.0	01.0	98.36
		Control	47.0	54.0	61.0	-
3	<i>Fusarium solani</i>	<i>Bacillus subtilis</i>	00.8	03.5	08.8	84.70
		<i>Pseudomonas fluorescens</i>	19.5	33.0	39.6	31.13
		<i>B. subtilis</i> + <i>P. fluorescens</i>	0	0	0	100.00
		Control	46.5	52.0	57.5	-

\*Mean of three replications

In a preliminary study *T. harzianum* was found to inhibit *C. gloeosporioides* and *E. rostratum* to the level of 9-11%; conspicuously it did not inhibit *F. solani*. A reverse effect of *F. solani* (inhibiting *T. harzianum*) could even be seen (CPCRI, 2003). In the subsequent replicated experiment also the antagonistic fungus affected the growth of *C. gloeosporioides* and *E. rostratum* generally at lesser extent (Table 6) on

3<sup>rd</sup> and 5<sup>th</sup> days of incubation (14.10% and 21.31% inhibitions on the 5<sup>th</sup> day of observation for the respective fungi). A clear indication of radial movement of an antifungal substance, secreted by *F. solani* was evident that appeared to affect the growth of *T. harzianum* in the medium and consequently an inhibition zone developed.

The combined effect of bacterial antagonist with fungicide (Contaf), tested against the pathogens of leaf rot, also brought out interesting results. A preliminary study revealed that the fungicide alone or its combination with *B. subtilis* inhibited all the major pathogens of leaf rot upto 100%. In fungicide plus antagonist combination, the inhibition of leaf rot pathogens was generally stronger. Contaf in concentrations of 0.025%, 0.050% and 0.100% did not affect the antagonist much, but the organism was affected in 0.200% concentration. Nevertheless, the effect of antagonist-fungicide compatibility potential in inhibiting *C. gloeosporioides* and *E. rostratum* stood higher and there existed a potential compatibility of *B. subtilis* with lower concentrations of Contaf (CPCRI, 2003). Thus in

continuation of such a study the compatibility effect of Contaf (in various concentrations) with *P. fluorescens* in comparison with *B. subtilis* (individually), besides the individual effects of antagonists and fungicide concentrations, on pathogens of leaf rot was further investigated in a comparative experiment (Table 7). Biocontrol potential of both the bacterial species was confirmed. Fungicide alone or in its combination with the antagonists was proved to be inhibiting the pathogens totally and a synergism was observed in the combination treatment against *C. gloeosporioides* and *E. rostratum*. Hence this feature could be made use of in formulating integrated disease management (IDM) measures for leaf rot.

**Table 6. Effect of fungal antagonist -*Trichoderma harzianum* -on major pathogens of leaf rot *in vitro* \***

S. No.	Pathogen	Treatment	Colony diameter of leaf rot fungus (mm) after different days of incubation*			Per cent Inhibition in growth on 5 <sup>th</sup> day of incubation
			3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	
1	<i>Colletotrichum gloeosporioides</i>	<i>Trichoderma harzianum</i>	16.5	17.5	26.8	14.10
		Control	24.3	28.0	31.2	-
2	<i>Exserohilum rostratum</i>	<i>Trichoderma harzianum</i>	22.5	25.0	24.0	21.31
		Control	23.5	27.0	30.5	-
3	<i>Fusarium solani</i>	<i>Trichoderma harzianum</i>	23.5	28.0	30.0	4.17
		Control	23.3	26.0	28.8	-

\* Mean of three replications

**Table 7. Effect of fungicide and bacterial antagonists on major pathogens of leaf rot *in vitro* \***

Treatment (Antagonist/Fungicide concentration)	Per cent inhibition in growth on 5 <sup>th</sup> day of incubation		
	<i>Colletotrichum gloeosporioides</i>	<i>Exserohilum rostratum</i>	<i>Fusarium solani</i>
I. <i>Bacillus subtilis</i>	63	74	68
II. <i>Pseudomonas fluorescens</i>	62	69	62
III. Hexaconazole (Contaf):			
0.025%	75	100	63
0.050%	85	100	74
0.100%	100	100	100
0.200%	100	100	100
IV. <i>Bacillus subtilis</i> + Hexaconazole (Contaf):			
0.025%	100 <sup>A</sup>	100 <sup>A</sup>	90 <sup>A,C</sup>
0.050%	100 <sup>A</sup>	100 <sup>A</sup>	90 <sup>A,C</sup>
0.100%	100 <sup>A</sup>	100 <sup>A</sup>	100 <sup>B</sup>
0.200%	100 <sup>B</sup>	100 <sup>B</sup>	100 <sup>B</sup>
V. <i>Pseudomonas fluorescens</i> + Hexaconazole (Contaf):			
0.025%	100 <sup>A</sup>	100 <sup>A</sup>	75 <sup>A,C</sup>
0.050%	100 <sup>B</sup>	100 <sup>B</sup>	80 <sup>B</sup>
0.100%	100 <sup>B</sup>	100 <sup>B</sup>	100 <sup>B</sup>
0.200%	100 <sup>B</sup>	100 <sup>B</sup>	100 <sup>B</sup>

\* Mean of three replications

A: Antagonist not inhibited by fungicide.

B: Antagonist inhibited by fungicide.

C: Inhibition Zone – Between Fungus & Antagonist – Interaction effect.

In the combined effect of antagonist and fungicide against the major pathogens of leaf rot, three situations were encountered during the course of experimentation, depending upon the fungicide concentration, antagonist species and pathogen species involved in the combination:

A). *Antagonist not inhibited by fungicide:*

1. *B. subtilis* was not inhibited by Contaf in 0.025%-1.000% concentrations under interaction with *C. gloeosporioides* or *E. rostratum*; but with *F. solani* similar interaction of no inhibition occurred only in the concentration range of 0.025%-0.050%.
2. *P. fluorescens* was not inhibited by Contaf in 0.025% concentration, irrespective of the species of pathogen involved under interaction.

(B). *Antagonist inhibited by fungicide:*

1. *B. subtilis* was inhibited by Contaf at 0.200% concentration under interaction with *C. gloeosporioides* or *E. rostratum* and also at 0.001%-0.200% concentrations with *F. solani*.
2. *P. fluorescens* was inhibited by Contaf at concentrations above the level of 0.025%, irrespective of the pathogen under interaction.

(C). *Appearance of inhibition zone between pathogen and antagonist:*

This situation could be encountered only in the case of interaction with *F. solani*. Even though *B. subtilis* and *P. fluorescens* were not inhibited at certain lower concentrations of Contaf under interactions with *C. gloeosporioides* and *E. rostratum*, a clear zone developed between colony of *F. solani* and bacterial growth, affecting bacterial growth. A diffusible material originating from *F. solani* seemed to have affected the growth of *B. subtilis* and *P. fluorescens* only in presence of the fungicide. Because such an inhibition zone did not develop under individual interaction of

antagonists with *F. solani*. The fore-mentioned observation bears importance while advocating the delivery of fungicide-antagonist treatment against the disease of complex nature (Fig. 5 – 11).

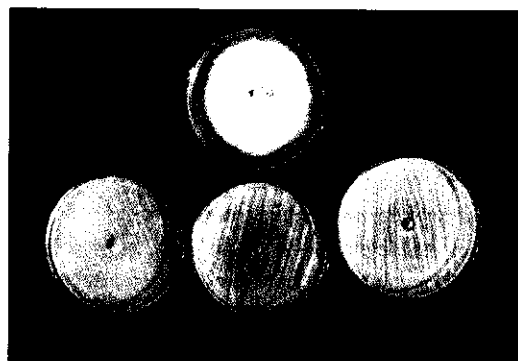


Fig. 5. Growth – viability of *Bacillus subtilis* in Contaf amended medium (lower concentrations), where leaf rot pathogen (*Colletotrichum gloeosporioides*) inoculated suppressed (Growth of the pathogen in control petri dish)

Biocontrol efficacy of *B. subtilis*, *P. fluorescens*, *T. harzianum* etc. individually against plant diseases have been reported by various other workers (e.g., Jeyalakshmi *et al.*, 1998; Naik and Sen, 2003; Kavitha *et al.*, 2003; Rama S. Singh *et al.*, 2003; Radhajealakshmi *et al.*, 2003; Renuka *et al.*, 2003; Saju *et al.*, 2003). However, variable inhibition potential among these bioagents on pathogens is inevitable (Amruta *et al.*, 2003). Inductions of systemic resistance in plants against diseases particularly by *P. fluorescens* and *B. subtilis* have been well documented (Shylaja *et al.*, 2003; Sible *et al.*, 2003). Nevertheless, mixtures of plant growth promoting rhizobacteria have been shown to enhance biological control of multiple pathogens (Raupach and Kloepper, 1998). Ramandeep Kaur *et al.* (2003) reported a synergistic effect in biocontrol of Fusarial wilts of chick pea by combined treatment of non-pathogenic *Fusarium* and fluorescent *Pseudomonas*. Saju *et al.* (2003) found the usefulness of combining *P. fluorescens* with *T. harzianum* for the suppression of *Phytophthora capsici* and also for the promotion of growth of black pepper.

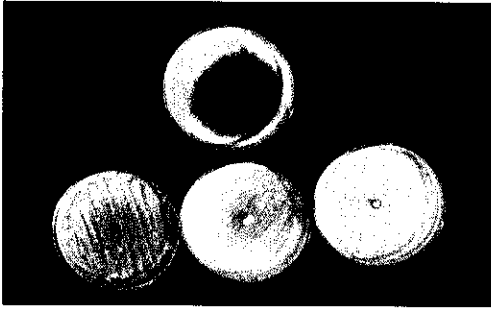


Fig. 6. Growth – viability of *Bacillus subtilis* in Contaf amended medium (lower concentrations), where leaf rot pathogen (*Exserohilum rostratum*) inoculated suppressed (Growth of the pathogen in control petri dish)

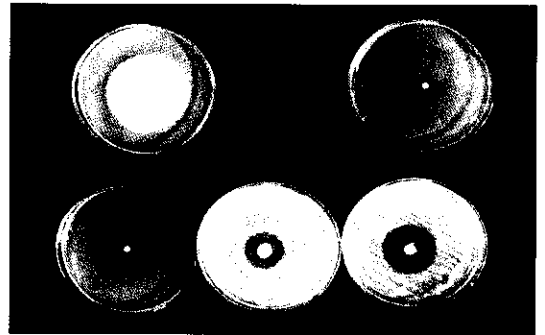


Fig. 7. Growth – viability of *Bacillus subtilis* in Contaf amended medium (lower concentrations), where leaf rot pathogen (*Fusarium solani*) inoculated suppressed (Growth of the pathogen in control petri dish) – Note inhibition zone in bacterial lawn around the pathogen – See text for more details

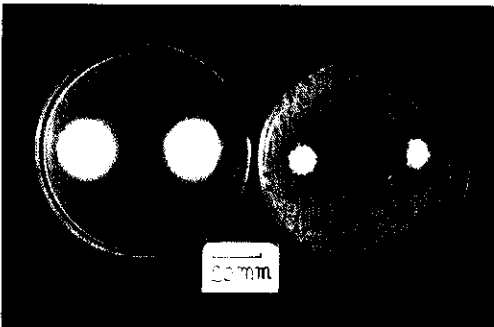


Fig. 8. Inhibition of *Fusarium solani* by *Bacillus subtilis* in medium, where no fungicide amended (No inhibition zone in bacterial lawn around the pathogen appeared)

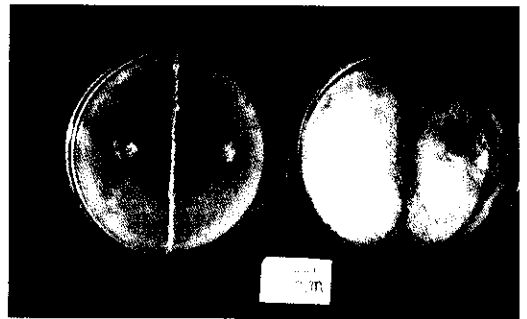


Fig. 9. Inhibition of leaf rot pathogen, *Colletotrichum gloeosporioides* by *Bacillus subtilis* (Right: Control)

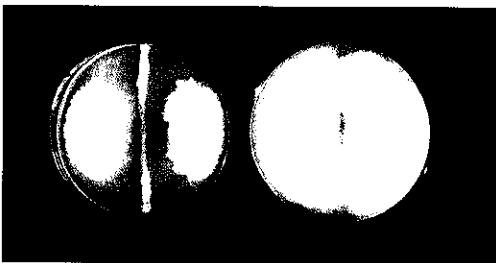


Fig. 10. Inhibition of leaf rot pathogen, *Colletotrichum gloeosporioides* by *Pseudomonas fluorescens* (Right: Control)



Fig. 11. Reverse effect of leaf rot pathogen, *Fusarium solani* on *Trichoderma harzianum* (See inhibition of the antagonist; Right: Pathogen – control)

Thankamani *et al.* (2003) suggested the use of specific strain of *P. fluorescens* alone or in combination with other biocontrol agents like *T. harzianum* and *Glomus fasciculatum* for the eco-friendly management of black pepper diseases and also for producing vigorous rooted cuttings of the crop. Hazarika and Phookan (2003) also found synergistic effect by the combined application of *P. fluorescens*, *Glomus fasciculatum* and *T. harzianum* in the suppression of charcoal stump rot of tea as compared to their individual applications. Results of the present study also have shown evidence of synergism by a combination of *B. subtilis* and *P. fluorescens* in the suppression of leaf rot pathogens. This paves a ground for evolving formulation of a consortium of compatible antagonists, with broad spectrum activity coupled with enhanced efficiency in managing leaf rot. Efficacy of Rhizobacteria based bio formulation (bacterial consortium) for diseases management has been demonstrated (Bharathi, 2001). Field experiments have been laid out to assess the efficacy of consortium of bio agents in managing leaf rot. This would help promoting a delivery system, which combines efficiency and ease of application.

#### Assessment of bioagents from coconut phylloplane on pathogens

Bacteria and fungi, isolated from coconut phylloplane were tested for their effect on leaf rot fungi. From them *Bacillus* sp., *Aspergillus* sp. and *Penicillium* sp. were found to be antagonistic to leaf rot pathogens. As *Aspergillus* sp. seemed to exhibit higher extent of inhibition to leaf rot pathogens over *Penicillium* sp., the former only was selected for further studies on biocontrol. Results are presented in Table 8. From this it could be seen that isolate 13 inhibited *C. gloeosporioides* and *E. rostratum* maximum (67-68%) while isolate 6 was effective against *F. solani* ((89%). Similarly among the fungal antagonists, isolate 12 inhibited *C. gloeosporioides* and *E. rostratum* maximum ((61-64%) while isolate 8 was most efficient (89%) against *F. solani* (Fig. 12).

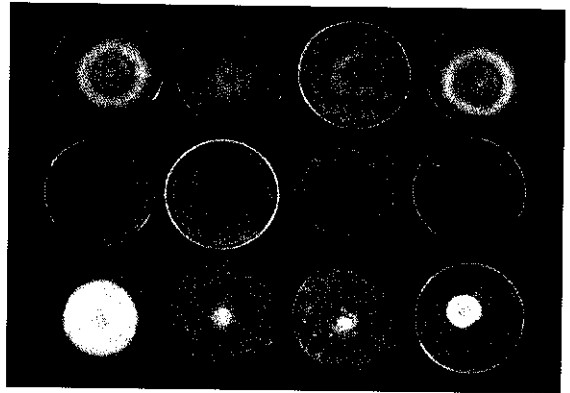


Fig. 12. Variable extent of inhibition of leaf rot pathogens by isolates of coconut phylloplane bacteria (Top row: *Colletotrichum gloeosporioides*; Middle: *Exserohilum rostratum*; Bottom: *Fusarium solani*-Left petri dish in each row control; Bottom: *Fusarium solani* – Left petri dish in each row control).

Phylloplane microorganisms have been implicated elsewhere in the biocontrol of plant pathogens. For example, Samuel (2003) found variability in antagonism among phylloplane bacteria (*Bacillus* sp.) from green gram against bacterial leaf spot pathogen of green gram and developed an efficient bio formulation out of best isolate of antagonists. Susan Thomas *et al.* (2003) observed effective inhibition of *Pythium aphanidermatum* by *Aspergillus* spp. in dual culture and damping off tomato caused by that pathogen. Seasonal variation in the mycoflora of phylloplane/phyllosphere is possible (Jayashree Deshpande and Wanmare, 2003) and also dynamics of phylloplane microflora could be influenced by various other parameters such as leaf age/plant age etc. (Bank *et al.*, 2003). Presence of bio control potential among phylloplane microbial agents of coconut leaves has been indicated in the present study. Further efficient isolates singly and in combination with other bio control agents should be tried so as to enrich the microbial consortium for combating leaf rot.

**Table 8. Efficacy of isolates of coconut phylloplane antagonistic bacteria (*Bacillus* sp.) and fungi (*Aspergillus* sp.) on growth of major pathogens of leaf rot *in vitro* on 5<sup>th</sup> day of incubation\***

Bacterial Isolate No.	Per cent inhibition			Fungal isolate No.	Per cent inhibition		
	<i>Cg</i>	<i>Er</i>	<i>Fs</i>		<i>Cg</i>	<i>Er</i>	<i>Fs</i>
01	36.30	21.6	39.66	01	12.34	14.96	51.94
02	45.21	17.6	13.79	02	30.25	14.28	54.26
03	10.96	20.00	14.66	03	57.41	30.61	68.28
04	52.05	58.40	60.34	04	45.06	45.58	59.69
05	21.23	32.00	44.83	05	39.51	02.04	29.46
06	25.34	16.80	89.65	06	44.44	04.76	44.19
07	04.11	18.40	29.31	07	09.88	25.17	20.93
08	38.36	49.60	82.76	08	59.26	23.18	89.15
09	31.5	44.00	12.93	09	32.10	15.65	36.43
10	15.75	16.00	48.28	10	09.88	09.52	06.98
11	41.78	13.60	27.59	11	49.38	00.68	52.71
12	48.63	43.20	69.83	12	63.58	61.22	76.74
13	67.12	68.00	39.66	13	58.02	04.76	28.68

\*Mean of three replications

*Cg* – *Colletotrichum gloeosporioides*, *Er* – *Exserohilum rostratum*, *Fs* – *Fusarium solani*.

### Conclusion

Inhibitory effect of Hexaconazole (Contaf), Mancozeb (Indofil M-45), Propiconazole (Tilt) and Carbendazim (Bavistin) on pathogens of leaf rot indicates their usefulness in the management of leaf rot. Tolerance of *B. subtilis* (at low concentrations of certain fungicides) lends itself to be used in combination with these fungicides. Management of leaf rot affected palms with *P. fluorescens* is a positive indication in the use of bio pesticides in the field management of the disease. Individual and the synergistic effect of *B. subtilis* and *P. fluorescens* on the pathogens offer scope for evolving highly effective bio formulations. Study of the interactions among the various candidate organisms, fungicides and pathogens could guide the choice of components in a consortium to be used against leaf rot where multiple pathogens are involved. Antagonistic organisms prevalent *in situ* in coconut phylloplane could as well be exploited for enrichment/consortia of bio agents in the disease management system. Bio pesticides in general possess the potential of plant disease control in an eco-friendly manner. It is known that plant growth promoting rhizobacteria induce systemic resistance in plants against diseases not withstanding the level of innate resistance/susceptibility of the host that aids in prevention or reduction of diseases. Hence by combining the technologies of bio pesticides induced systemic resistance could be strategically utilized (from the stage of planting of coconut seed/seedling) as a component of the integrated management package to effectively combat root (wilt)-leaf rot complex.

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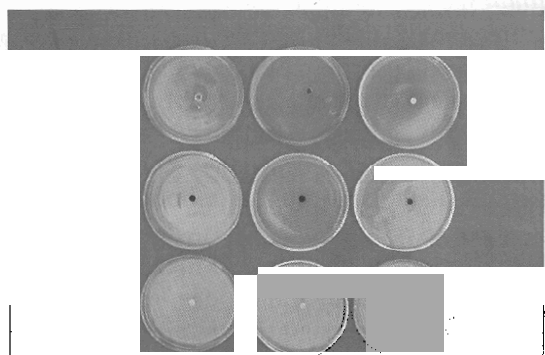


Fig. 1. Inhibition of leaf rot pathogens by Contaf (Top row: *Colletotrichum gloeosporioides*; Middle: *Exserohilum rostratum*; Bottom: *Fusarium solani*)

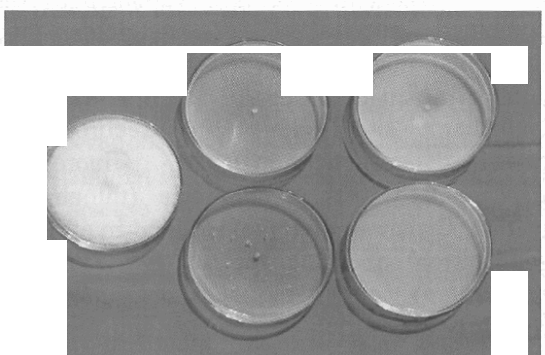


Fig. 2. Inhibition of leaf rot pathogen (*Colletotrichum gloeosporioides*) by Tilt (Growth of the pathogen in control petri dish)

Superior control of ground nut rust and late leaf spot (Sheela and Vimala, 2004), grape vine powdery mildew (Ashwathanarayana *et al.*, 2004) and rice sheath blight (Rajbir Singh and Sinha, 2004) with Hexaconazole (Contaf) have been reported by various workers. Baranwal *et al.* (2003) also recorded maximum suppression of *Stemphylium* blight of onion with Hexaconazole. Srinivasan and Gunasekaran (1998) reported the effectiveness and broad-spectrum potential of hexaconazole (Contaf) against leaf rot pathogens of coconut that aided a sound base for widespread utility of the fungicide in the management of the disease complex in the diseased region (Srinivasan and Gunasekaran, 2000b, 2003). Among contact fungicides tested in the earlier study, Indofil M-45 performed most effectively

against the pathogens. While results of the current study are in conformity with such earlier findings, very high inhibition potential of certain other systemic fungicides such as Tilt as evaluated in the bioassay would be useful in the field management of leaf rot. Concentration of Antracol or Tag cop 50 (both contact fungicides) had direct relation on the extent of inhibition of leaf rot fungal growth. These results are supported by various reports as fore mentioned.

### Effect of fungicides on antagonists

Results of the *in vitro* effect of fungicides on antagonistic organisms as recorded on the 5<sup>th</sup> day of incubation are given in Table 3. The extent of inhibition of antagonists also depended on the fungicides. Complete inhibition of bacterial antagonists was not seen in 0.1%-0.3% concentrations of Antracol, though at 0.4%-0.5% growth of both *B. subtilis* and *P. fluorescens* were completely suppressed. *B. subtilis* was less affected similarly by lower concentrations of Indofil M-45 (0.1%-0.3%) as compared to *P. fluorescens*. Likewise, in lower concentrations of Contaf (0.025%-0.10%) growth of *B. subtilis* was less affected as compared to *P. fluorescens*. In Tilt or Bavistin amended medium *P. fluorescens* failed to grow even in the lowest concentration (0.025%); *B. subtilis* could tolerate the lowest concentration of these fungicides. Compatibility of *B. subtilis* with lower concentrations of Contaf, Indofil M-45 etc. are significant. The fungal antagonist, *T. harzianum* was completely inhibited even at lowest concentration of Tilt and Bavistin (cent per cent inhibition, incompatible). In Indofil M-45 or Contaf amended medium, slow growth of *T. harzianum* was observed in the lowest concentration while cent percent inhibition occurred at higher concentrations. The fungus was not completely inhibited was completely inhibited at 0.1%-0.4% concentration of Antracol, but at 0.5% concentration. Thus the fungal antagonist was found compatible with lower concentrations of Antracol (Fig. 3-4).

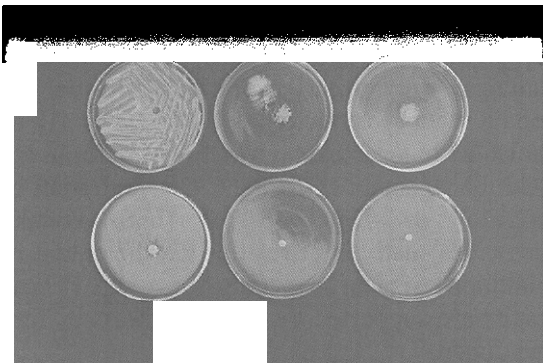


Fig. 3. Growth - viability of bacterial antagonists in Contaf amended medium (lower concentration), where leaf rot pathogen (*Colletotrichum gloeosporioides*) inoculated inhibited (Top row: *Bacillus subtilis*; Bottom: *Pseudomonas fluorescens*)

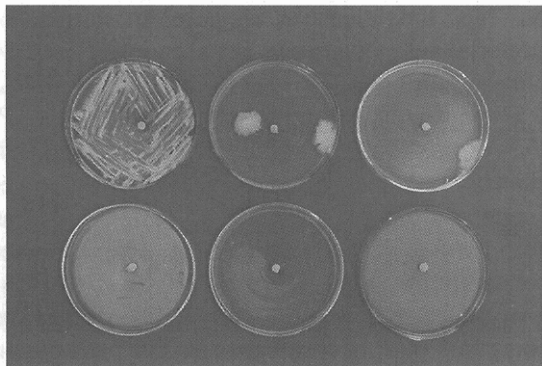


Fig. 4. Growth - viability of bacterial antagonists in Contaf amended medium (lower concentration), where leaf rot pathogen (*Fusarium solani*) inoculated inhibited (Top row: *Bacillus subtilis*; Bottom: *Pseudomonas fluorescens*)

Little information is available about the effect of fungicides on antagonistic organisms. Mathew (2003) studied about compatibility of *P. fluorescens* with certain fungicides besides insecticides. More compatibility was seen with Mancozeb and Carbendazim as compared to Hexaconazole, based on visual observation. Laha and Venkataraman (2001) reported compatibility of Carbendazim with *P. fluorescens*. Poddar *et al.* (2004) evaluated certain systemic fungicides against *T. harzianum* – significant variation in the fungicidal sensitivity among isolates of the fungus was found and among them one isolate was relatively less sensitive to Carbendazim. Variable response of *B. subtilis* and *P.*

*fluorescens* to fungicides, as observed in the current study, underlines the need and scope for the use of consortium of antagonists in the event of chemical treatment along with bio agents in the process of disease control. *B. subtilis* and *P. fluorescens* are independently inhibitory to various plant pathogens, as reported by various workers (Reddy *et al.*, 2003). *B. subtilis* being less affected (as compared to the more sensitive *P. fluorescens*) by fungicides results of the present study are significant. Similarly, higher sensitivity of *T. harzianum* to several fungicides also provides a clue for the need for rational use of fungicides along with bio agent. The compatibility of bacterial and fungal antagonists to fungicides like Antracol is advantageous for their combined application to the integrated disease management. This is validated by the combined application of fungicide and antagonist that has resulted in very effective control of plant diseases. As for example, Nallathampi and Thakore (2003) recorded that combined application of fluorescent *Pseudomonas* with a fungicide (thiophenate methyl, Captan or alcidine) resulted in effective suppression of fruit rot of ber (*Ziziphus mauritiana*) caused by *Alternaria alternata*. Similarly integration of *T. viride* and fungicide (Thiram, Vitavax or Captaf) was more effective in control of collar rot (*Macrophomina phaseolina*) of French bean – *Phaseolus vulgaris* (Dubey, 2002). Integration of biocontrol agent with fungicides has been found to lead to significantly higher disease control than that attained by either biocontrol agent or fungicide alone in several other crops also (Deepak Kumar and Dubey, 2001; Vyas, 1994). Integrated use of biocontrol agents with fungicides is thus possible in the field-control of leaf rot disease.

#### Field efficacy of *Pseudomonas fluorescens* on leaf rot

Results on the effect of application of talc-based preparation of *P. fluorescens* on leaf rot in field palms are presented in Table 4. Treatment of palms with the bacterial antagonist lead to a decline of disease index in newly emerged leaves in various rounds of observations as

In the combined effect of antagonist and fungicide against the major pathogens of leaf rot, three situations were encountered during the course of experimentation, depending upon the fungicide concentration, antagonist species and pathogen species involved in the combination:

A). *Antagonist not inhibited by fungicide:*

1. *B. subtilis* was not inhibited by Contaf in 0.025%-1.000% concentrations under interaction with *C. gloeosporioides* or *E. rostratum*; but with *F. solani* similar interaction of no inhibition occurred only in the concentration range of 0.025%-0.050%.
2. *P. fluorescens* was not inhibited by Contaf in 0.025% concentration, irrespective of the species of pathogen involved under interaction.

(B). *Antagonist inhibited by fungicide:*

1. *B. subtilis* was inhibited by Contaf at 0.200% concentration under interaction with *C. gloeosporioides* or *E. rostratum* and also at 0.001%-0.200% concentrations with *F. solani*.
2. *P. fluorescens* was inhibited by Contaf at concentrations above the level of 0.025%, irrespective of the pathogen under interaction.

(C). *Appearance of inhibition zone between pathogen and antagonist:*

This situation could be encountered only in the case of interaction with *F. solani*. Even though *B. subtilis* and *P. fluorescens* were not inhibited at certain lower concentrations of Contaf under interactions with *C. gloeosporioides* and *E. rostratum*, a clear zone developed between colony of *F. solani* and bacterial growth, affecting bacterial growth. A diffusible material originating from *F. solani* seemed to have affected the growth of *B. subtilis* and *P. fluorescens* only in presence of the fungicide. Because such an inhibition zone did not develop under individual interaction of

antagonists with *F. solani*. The fore-mentioned observation bears importance while advocating the delivery of fungicide-antagonist treatment against the disease of complex nature (Fig. 5 – 11).

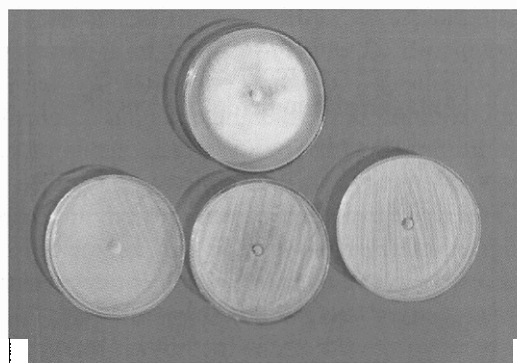


Fig. 5. Growth – viability of *Bacillus subtilis* in Contaf amended medium (lower concentrations), where leaf rot pathogen (*Colletotrichum gloeosporioides*) inoculated suppressed (Growth of the pathogen in control petri dish)

Biocontrol efficacy of *B. subtilis*, *P. fluorescens*, *T. harzianum* etc. individually against plant diseases have been reported by various other workers (e.g., Jeyalakshmi *et al.*, 1998; Naik and Sen, 2003; Kavitha *et al.*, 2003; Rama S. Singh *et al.*, 2003; Radhajeyalakshmi *et al.*, 2003; Renuka *et al.*, 2003; Saju *et al.*, 2003). However, variable inhibition potential among these bioagents on pathogens is inevitable (Amruta *et al.*, 2003). Inductions of systemic resistance in plants against diseases particularly by *P. fluorescens* and *B. subtilis* have been well documented (Shylaja *et al.*, 2003; Sible *et al.*, 2003). Nevertheless, mixtures of plant growth promoting rhizobacteria have been shown to enhance biological control of multiple pathogens (Raupach and Kloepper, 1998). Ramandeep Kaur *et al.* (2003) reported a synergistic effect in biocontrol of Fusarial wilts of chick pea by combined treatment of non-pathogenic *Fusarium* and fluorescent *Pseudomonas*. Saju *et al.* (2003) found the usefulness of combining *P. fluorescens* with *T. harzianum* for the suppression of *Phytophthora capsici* and also for the promotion of growth of black pepper.

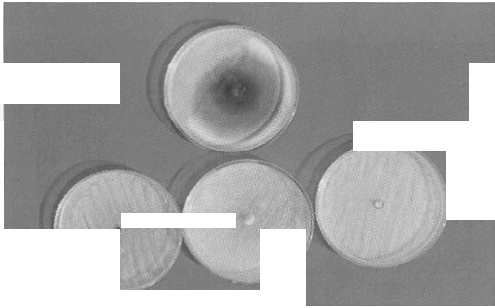


Fig. 6. Growth – viability of *Bacillus subtilis* in Contaf amended medium (lower concentrations), where leaf rot pathogen (*Exserohilum rostratum*) inoculated suppressed (Growth of the pathogen in control petri dish)

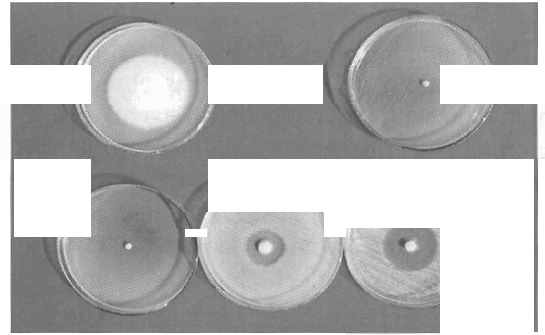


Fig. 7. Growth – viability of *Bacillus subtilis* in Contaf amended medium (lower concentrations), where leaf rot pathogen (*Fusarium solani*) inoculated suppressed (Growth of the pathogen in control petri dish) – Note inhibition zone in bacterial lawn around the pathogen – See text for more details

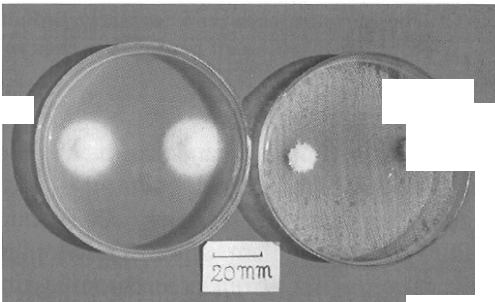


Fig. 8. Inhibition of *Fusarium solani* by *Bacillus subtilis* in medium, where no fungicide amended (No inhibition zone in bacterial lawn around the pathogen appeared)

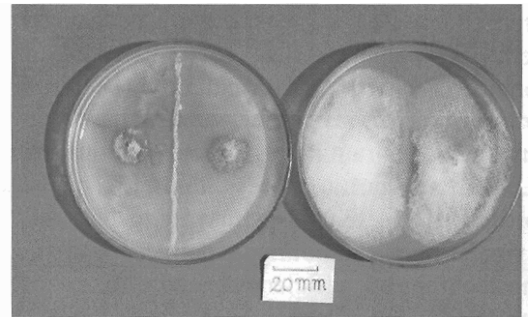


Fig. 9. Inhibition of leaf rot pathogen, *Colletotrichum gloeosporioides* by *Bacillus subtilis* (Right: Control)

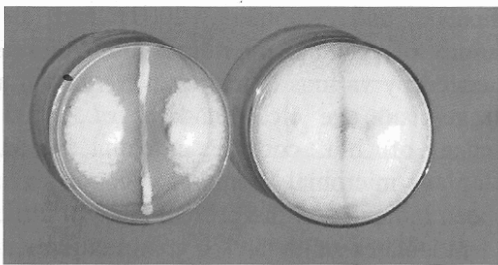


Fig. 10. Inhibition of leaf rot pathogen, *Colletotrichum gloeosporioides* by *Pseudomonas fluorescens* (Right: Control)

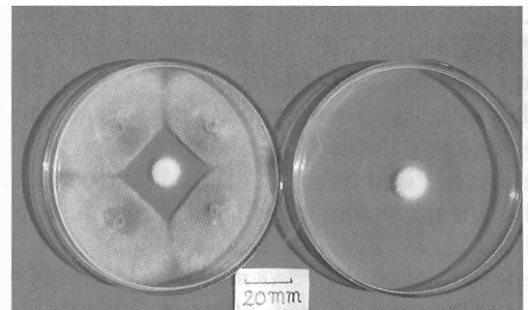


Fig. 11. Reverse effect of leaf rot pathogen, *Fusarium solani* on *Trichoderma harzianum* (See inhibition of the antagonist; Right: Pathogen – control)

Thankamani *et al.* (2003) suggested the use of specific strain of *P. fluorescens* alone or in combination with other biocontrol agents like *T. harzianum* and *Glomus fasciculatum* for the eco-friendly management of black pepper diseases and also for producing vigorous rooted cuttings of the crop. Hazarika and Phookan (2003) also found synergistic effect by the combined application of *P. fluorescens*, *Glomus fasciculatum* and *T. harzianum* in the suppression of charcoal stump rot of tea as compared to their individual applications. Results of the present study also have shown evidence of synergism by a combination of *B. subtilis* and *P. fluorescens* in the suppression of leaf rot pathogens. This paves a ground for evolving formulation of a consortium of compatible antagonists, with broad spectrum activity coupled with enhanced efficiency in managing leaf rot. Efficacy of Rhizobacteria based bio formulation (bacterial consortium) for diseases management has been demonstrated (Bharathi, 2001). Field experiments have been laid out to assess the efficacy of consortium of bio agents in managing leaf rot. This would help promoting a delivery system, which combines efficiency and ease of application.

#### Assessment of bioagents from coconut phylloplane on pathogens

Bacteria and fungi, isolated from coconut phylloplane were tested for their effect on leaf rot fungi. From them *Bacillus* sp., *Aspergillus* sp. and *Penicillium* sp. were found to be antagonistic to leaf rot pathogens. As *Aspergillus* sp. seemed to exhibit higher extent of inhibition to leaf rot pathogens over *Penicillium* sp., the former only was selected for further studies on biocontrol. Results are presented in Table 8. From this it could be seen that isolate 13 inhibited *C. gloeosporioides* and *E. rostratum* maximum (67-68%) while isolate 6 was effective against *F. solani* ((89%). Similarly among the fungal antagonists, isolate 12 inhibited *C. gloeosporioides* and *E. rostratum* maximum ((61-64%) while isolate 8 was most efficient (89%) against *F. solani* (Fig. 12).

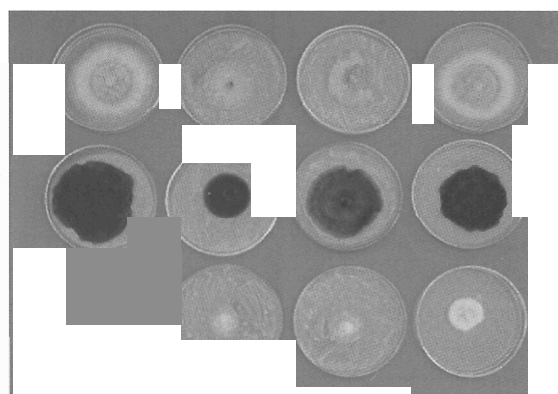


Fig. 12. Variable extent of inhibition of leaf rot pathogens by isolates of coconut phylloplane bacteria (Top row: *Colletotrichum gloeosporioides*; Middle: *Exserohilum rostratum*; Bottom: *Fusarium solani*-Left petri dish in each row control; Bottom: *Fusarium solani* - Left petri dish in each row control).

Phylloplane microorganisms have been implicated elsewhere in the biocontrol of plant pathogens. For example, Samuel (2003) found variability in antagonism among phylloplane bacteria (*Bacillus* sp.) from green gram against bacterial leaf spot pathogen of green gram and developed an efficient bio formulation out of best isolate of antagonists. Susan Thomas *et al.* (2003) observed effective inhibition of *Pythium aphanidermatum* by *Aspergillus* spp. in dual culture and damping off tomato caused by that pathogen. Seasonal variation in the mycoflora of phylloplane/phyllosphere is possible (Jayashree Deshpande and Wanmare, 2003) and also dynamics of phylloplane microflora could be influenced by various other parameters such as leaf age/plant age etc. (Bank *et al.*, 2003). Presence of bio control potential among phylloplane microbial agents of coconut leaves has been indicated in the present study. Further efficient isolates singly and in combination with other bio control agents should be tried so as to enrich the microbial consortium for combating leaf rot.