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Research Article

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Biological management of leaf blight disease of coconut using rhizosphere microbes

I. Johnson*, B. Meena[1] and K. Rajamanickam

Coconut Research Station, Tamil Nadu Agricultural University, Aliyarnagar – 642 101, Coimbatore, Tamil Nadu, India

[1]Department of Medicinal Plants, HC&RI, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu, India

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Abstract

The production and productivity of coconut is seriously affected by various factors including pest and diseases. Among the foliar diseases, the leaf blight caused by *Lasiodiplodia theobromae* is a serious problem accounting 10-24 per cent yield reduction in Tamil Nadu. Several fungicides were found to be effective for the management of coconut leaf blight disease. Continuous use of fungicides leads to inherent ill-effects like residual toxicity, resistance to fungicide, environmental pollution *etc*. Hence, biological control is the only alternative method which is cheap, easy to use and eco-friendly. In the present study, a total of twenty five fungal (*Trichoderma viride*) and bacterial (*Pseudomonas fluorescens* and *Bacillus subtilis*) antagonists were isolated from the rhizosphere soil and screened against *L. theobromae*. *In vitro* evaluation revealed that the rhizospere bacteria *P. fluorescens* isolate Pf1 was found to be highly effective against *L. theobromae* compared to the other bacterial and fungal antagonists. Further, the best antagonist Pf1 was evaluated as root feeding of 100 per cent culture suspension with different combinations and durations for two years (2010-11 and 2011-12) against leaf blight disease under field condition in three different locations *viz.,* Kambalapatti, Karianchettipalayam and Samathur villages of Pollachi taluk, Coimbatore. Observations were recorded on 0-5 scale and the per cent disease index (PDI) was calculated. Among the treatments imposed, root feeding of *P. fluorescens* culture suspension @ 25 mL at quarterly interval combined with soil application of *P. fluorescens* talc formulation (50 g) + Neem cake 5 kg was found to be the best and significantly reduced the incidence to 12.9, 11.9 and 7.9 per cent during 2010-11 and 8.1, 8.1 and 6.5 per cent during 2011-12 in the locations *viz.,* Kambalapatti, Karianchettipalayam and Samathur, respectively.

Keywords: Coconut, bio-control, *Lasiodiplodia theobromae,* leaf blight, *Pseudomonas fluorescens*

Introduction

Production and productivity of coconut was severely affected by several reasons which includes pest and diseases. Of the diseases, the leaf blight disease of coconut caused by the fungus *Lasiodiplodia* (*Botryodiplodia*) *theobromae* is an emerging serious problem in Pollachi tracts of Tamil Nadu. At present, the disease is spreading at a faster rate in Coimbatore, Erode, Dindigul, Tirunelveli, Kanyakumari and other districts of Tamil Nadu. In India, it has been reported to occur on seed coconuts (Raju, 1984). The fungus causing coconut fruit (nut) rot has also been recorded in Brunei, Indonesia and Vietnam (Johnston, 1965).

Leaf blight causes serious damage in seedlings and adult palms. The pathogen causes damage in leaf and nuts. Generally, the older leaves in the lower 3 to 4 whorls are affected. The affected leaflets start drying from the tip downwards and exhibit a charred or burnt appearance. Dark grey to brown lesions with wavy to undulated margins appear from the apex of the nuts. The fungus enters into the kernel through mesocarp, resulting in decay of the endosperm. The disease causes reduction in

*Corresponding Author: johnsonpath@gmail.com

vigour of the coconut seedlings by lesser leaf production, stunted growth and poor nut yield in adult palms. The affected nuts become desiccated, shrunk, deformed and drop prematurely (Warwick *et al*., 1993; Lakshmanan and Jagadeesan, 2004) resulting in nut yield loss up to 10 to 25 per cent.

Several chemicals were found to inhibit the growth of *L. theobromae* and effectively controlled the leaf blight disease. Continuous use of chemicals leads to inherent ill-effects like residual toxicity, resistance to fungicide and environmental pollution. Hence, as an alternative to chemical methods, biological control of plant diseases is gaining importance. Biological control by antagonistic organisms was established as a potential non-chemical tool for crop protection against phyto-pathogens (Papavizas, 1985). A directed biological control of pathogenic fungi in the field may be achieved by modifying the indigenous microbial community in such a way as to favour the destruction of the pathogen, probably through various modes of actions including lysis, antibiotics, parasitism *etc*.

Since, no reports are available on the biological control of coconut leaf blight disease, this present investigation was attempted as it would be a cheaper, easy to adopt by the farmers and reduce the use of hazardous chemicals.

Materials and methods

Isolation of pathogen

L. theobromae was isolated from infected coconut leaves, purified by the hyphal tip method and identified based on the descriptions of Punithalingam (1976). Cultures were maintained on potato dextrose agar (PDA) at 4 °C.

Isolation and collection rhizosphere microbes

P. fluorescens, *Trichoderma* sp. and *Bacillus* sp. were isolated from the rhizosphere soil collected from various locations using the specific medium *viz*., King's B (King *et al*., 1954), TSM (Elad and Chet, 1983) and nutrient agar medium, respectively. The *Pseudomonas* and *Bacillus* isolates were characterized based on standard biochemical tests (Hildebrand *et al*., 1992 and Schaad, 1992, respectively) and the *Trichoderma viride* was characterized based on by microscopic examination of the morphological and reproductive characters.

In vitro assay

The effectiveness of the bacterial and fungal antagonists *viz*., *Pseudomonas, Bacillus* and *Trichoderma* isolates against mycelial growth of *L. theobromae* was tested by dual culture technique *in vitro* (Dennis and Webster, 1971). For each test, an 8 mm dia. mycelial disc from a 7-day-old culture of *L. theobromae* was placed on the agar surface of a 90 mm Petri dish 1 cm from the edge of the dish. Streaking of *P. fluorescens/B. subtilis* strains or an 8 mm diameter mycelial disc from an actively growing *Trichoderma* sp. culture was done on the agar surface opposite the target pathogen. Three replicate plates were maintained for each treatment. The plates were incubated together with the experimental controls without antagonists) at 28±2 °C for 7 days, and the radial growth (mm) of the pathogen mycelium was recorded. Inhibition of mycelial growth of the pathogen was calculated in percentage.

Field experiment

Field experiments were laid out during 2010 - 2011 and 2011 – 2012 in a randomized block design at three different locations *viz*., Kambalapatti, Karianchettipalayam and Samathur villages of Pollachi taluk, Coimbatore district, Tamil Nadu. Trees showing leaf blight symptoms were randomly selected and each tree was considered as a replication, likewise 10 replications were maintained for each treatment. The treatment schedule as follows:

T_1 – Root feeding of 100% *P. fluorescens* Pf1 liquid culture @ 25 mL at quarterly interval

T_2 – Root feeding of 100% Pf1 liquid culture @ 25 mL at half yearly interval

T_3 – Root feeding of 100% Pf1 liquid culture @ 25 mL once in a year

T_4 – Soil application of Pf1 talc formulation (50 g $palm^{-1}yr^{-1}$) + neem cake (5 kg $palm^{-1}yr^{-1}$)

T_5 – (T1 + T4); T_6 – (T2 + T4); T_7 – (T3 + T4)

T_8 – Soil application of neem cake (5 kg $palm^{-1}yr^{-1}$)

T_9 - Control

Pre and post treatment observations were recorded on leaf blight incidence of about 25 leaflets from the lower 10 leaves in each palm were selected at random and the disease grade was assigned based on score chart; 0-5 scale (0 – No infection; 1 - < 10%; 2 – 11-25%; 3 – 26-50%; 4 – 51-75%; 5 - >75% leaf area infected). The per cent disease index (PDI) was calculated based on the formula

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{No. of leaves examined}} \times \frac{100}{\text{Maximum grade available in the score chart}}$$

Results and discussion

In vitro studies

Five *Trichoderma viride* isolates, 10 isolates each of *P. fluorescens* and *Bacillus* sp. were isolated from the coconut rhizosphere soil and tested against *L. theobromae* by dual plate technique. The *in vitro* evaluation revealed that the rhizosphere bacteria *P. fluorescens* Pf1 (12.7 mm) was found to be highly inhibitory to *L. theobromae* among the 25 bacterial and fungal antagonists evaluated which was followed by *B. subtilis* (6.7 mm) isolate. Hence, the *P. fluorescens* Pf1 isolate was taken to the field for further evaluation against leaf blight disease (Table 1).

Table 1. *In vitro* screening of biocontrol agents against *L. theobromae*

Sl.No.	Antagonistic organism	Inhibition zone (mm)
Trichoderma viride		
1	*Trichoderma viride* (TV1)	6.3
2	Kambalapatti	2.3
3	Sarkarpathy	2.7
4	Karianchetipalayam	0.0
5	Saralapathy	1.7
Pseudomonas fluorescens		
1	*P. fluorescens* – Pf1	12.7
2	Gomangalampudur	2.3
3	Odayakulam	1.3
4	Kambalapatti	1.7
5	Manchanaikanur	1.3
6	Devanurpudur	0.0
7	Angalakurichi	3.7
8	Ramanamudalipudur	0.0
9	Karianchetipalayam	2.7
10	Kaliapuram	0.0
Bacillus subtilis		
1	Aliyarnagar	5.2
2	Kambalapatti	6.7
3	Karianchettipalayam	4.9
4	Samathur	3.8
5	Angalakurichi	3.3
6	Vettaikaranpudur	1.7
7	Ambarampalayam	0.0
8	Thathur	2.8
9	Thappattaikizhavanpudur	6.5
10	Kettimalanpudur	0.0

In vivo studies

Among the nine treatments imposed for the leaf blight management, root feeding of *P. fluorescens* liquid culture (25 mL) at quarterly interval combined with soil application of *P. fluorescens* talc formulation (50 g) + neem cake 5 kg was found to be the best in all the 3 locations and significantly reduced the incidence to 12.9, 11.9 and 7.9 per cent in Kambalapatti, Karianchettipalayam and Samathur respectively during 2010 – 2011 (Table 2) which was on par with T_6 at Kambalapatti and Samathur locations. Similar results were observed in the second year also (2011 - 2012) and recorded 8.1, 8.1 and 6.5 per cent in the aforesaid locations, respectively (Table 3). Root feeding of 25 mL liquid culture of *P. fluorescens* at half yearly interval combined with soil application of *P. fluorescens* talc formulation (50 g) + neem cake 5 kg was the next effective treatment in almost all the trials, during both the years.

Fluorescent pseudomonads are currently considered as the most effective bacteria for biological control of soil and foliar diseases. Several studies have indicated that the plant growth promoting rhizobacteria (PGPR) may stimulate the production of biochemical compounds associated with the plant defense; massive accumulation of phytoalexins and phenolic compounds; increase in the activities of pathogenesis (PR) proteins, defense enzymes and enhanced lignifications *etc*. Among the various biocontrol agents, fluorescent pseudomonads were known to survive both in rhizosphere (Parke *et al*., 1991) and phyllosphere (Wilson and Lindow, 1992). Reports on the control of foliar disease with *P. fluorescens* applied to foliage were available (Gnanamanickam and Mew, 1992) where it significantly inhibited the mycelial growth of *Pestalotia palmarum* (Karthikeyan and

Table 2. Management of leaf blight disease of coconut during 2010 – 2011.

Treatments	Difference between pre and post treatment per cent disease index (PDI)		
	Kambalapatti	Karianchettipalayam	Samathur
T_1 – RF- Culture suspension of *P. fluorescens* Pf1 (25 mL) at quarterly interval	9.07^{b}(17.49)	6.87^{c}(15.18)	6.13^{bc}(14.33)
T_2 – RF- Culture suspension of *P. fluorescens* Pf1 (25 mL) at half yearly interval	7.33^{c}(15.56)	5.87^{d}(13.99)	4.13^{d}(11.72)
T_3 – RF- Culture suspension of *P. fluorescens* Pf1 (25 mL) once in a year	5.73^{cd}(13.78)	4.53^{e}(12.26)	4.15^{d}(11.74)
T_4 – SA-*P. fluorescens* Pf1 talc formulation (50g palm^{-1}yr^{-1}) + Neem cake (5kg palm^{-1}yr^{-1})	5.60^{d}(13.58)	4.13^{e}(11.64)	4.00^{d}(11.43)
T_5 – T1 + T4	12.93^{a}(21.05)	11.87^{a}(20.14)	7.87^{a}(16.13)
T_6 – T2 + T4	10.93^{ab}(19.28)	9.87^{b}(18.30)	6.93^{ab}(15.26)
T_7 – T3 + T4	6.93^{cd}(15.25)	5.47^{d}(13.49)	4.93^{cd}(12.81)
T_8 – SA – Neem cake (5 kg palm^{-1}yr^{-1})	3.20^{e}(10.10)	4.27^{e}(11.90)	2.40^{e}(8.84)
T_9 - Control	4.54 (+)	4.54 (+)	4.80 (+)

RF: Root feeding; SA: Soil application; Values in the parentheses are Arcsine transformed values.
The alphabet represents the treatment significance based on DMRT.

Bhaskaran, 1998). *P. fluorescens* isolated from the surface of healthy cocoa pods were antagonistic to *P. palmivora* both *in vitro* and *in vivo* and were more effective than cupric oxide or chlorothalonil in controlling black pod disease (Galindo, 1992). The inhibitory potential of *P. fluorescens* against citrus stem end rot pathogen *B. theobromae* (Sharma *et al*. 2009) and tomato bacterial wilt pathogen *Ralstonia solanacearum* (Vanitha and Umesha 2011) were reported. Bokharai *et al*. (2008), reported that the

Table 3. Management of leaf blight disease of coconut during 2011 – 2012

Treatments	Difference between pre and post treatment per cent disease index (PDI)		
	Kambalapatti	Karianchettipalayam	Samathur
T_1 – RF- Culture suspension of *P. fluorescens* Pf1 (25 mL) at quarterly interval	6.40^{a}(14.36)	6.27^{ab}(14.30)	4.8^{ab}(12.02)
T_2 – RF- Culture suspension of *P. fluorescens* Pf1 (25 mL) at half yearly interval	4.00^{b}(11.49)	4.00^{bc}(11.20)	3.73^{ab}(11.02)
T_3 – RF- Culture suspension of *P. fluorescens* Pf1 (25 mL) once in a year	1.53^{c}(6.89)	2.27^{cd}(8.60)	2.93^{bc}(9.32)
T_4 – SA-*P. fluorescens* Pf1 talc formulation (50 g palm^{-1}yr^{-1}) + Neem cake (5 kg palm^{-1}yr^{-1})	1.20^{c}(6.20)	1.87^{d}(7.61)	2.73^{bc}(9.23)
T_5 – T1 + T4	8.13^{a}(16.51)	8.14^{a}(16.23)	6.53^{a}(14.49)
T_6 – T2 + T4	6.13^{a}(14.20)	6.00^{ab}(13.67)	4.53^{ab}(11.79)
T_7 – T3 + T4	3.34^{b}(10.05)	2.80^{cd}(9.51)	2.40^{bc}(8.59)
T_8 – SA – Neem cake (5 kg palm^{-1}yr^{-1})	1.07^{c}(5.84)	1.47^{d}(6.75)	0.93^{c}(5.49)
T_9 - Control	3.06 (+)	3.86 (+)	4.00 (+)

RF: Root feeding; SA: Soil application; Values in the parentheses are Arcsine transformed values. The alphabet represents the treatment significance based on DMRT.

in vivo application of *Trichoderma harzianum* along with Topsin-M effectively controlled the pathogens of guava decline disease *B. theobromae* and *F. oxysporum* f.sp. *psidii.*

Induced resistance by fluorescent pseudomonads has broad spectrum activity against several fungal, bacterial and viral diseases through root colonization, antibiotic production, iron chelation by siderophore production and strengthening the epidermal and cortical cell wall with the callose, lignin and phenolics and by activating defense genes encoding chitinase, PAL, POX, PPL *etc* (Hoffland *et al*., 1997; Wei *et al*., 1996; Chen *et al*., 2000 and Zehnder *et al*., 2001). Hence, one or combination of many activities of *P. fluorescens* may be responsible for the *in vitro* and *in vivo* efficacy against *L. theobromae* in the present study.

The present study revealed that the root feeding combined with soil application was found to be the best when compared to application either as root feeding or soil application. Eventhough, the biocontrol agent was effective against leaf blight; the field maintenance was also an important factor for disease management. The results from Kambalapatti and Karianchettipalayam was comparatively more effective than Samathur which was not maintained well. Unlike fungicide application, biocontrol agents should be applied consistently at periodical interval for a long time to reduce the impact of pathogenic spread from nearby fields.

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