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Development of Microbial Consortia for the Management of Leaf Blight Disease of Coconut

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The leaf blight disease caused by *Lasiodiplodia theobromae* is an important foliar disease in coconut that results in a yield reduction of 10–24 per cent in Tamil Nadu, India. In the present study, five *Trichoderma viride* isolates, *Pseudomonas fluorescens* and *Bacillus subtilis* strains were isolated from the coconut rhizosphere and tested against *L. theobromae*. *P. fluorescens* Pf1, *B. subtilis* (Km1) and *T. viride* (TNAU) isolates were found highly effective against the leaf blight pathogen under *in vitro* conditions and hence, all the three antagonists were combined together to develop microbial consortia and tested against leaf blight disease under field conditions. Soil application of microbial consortia formulated using talc as a carrier material at 150 g (50 g each) and 300 g (100 g each) doses at different intervals (quarterly, half-yearly and annually) was evaluated for three years from 2011 to 2013. Among the treatments, the fungicide carbendazim was found to be the most effective against coconut leaf blight. Among the treatments with bioagents, soil application of microbial consortia @ 300 g + 5 kg of farm yard manure at quarterly interval/palm/year was the best treatment which was followed by the treatment with TNAU *Bacillus subtilis* (Bs1) mixture in two locations. Confirmatory results were obtained in similar experiments carried out at two different locations during 2013–2014, too.

Keywords: Coconut, *Lasiodiplodia theobromae*, microbial consortia, *Pseudomonas*, *Bacillus*, *Trichoderma*.

Leaf blight disease of coconut caused by *Lasiodiplodia theobromae* (Syn: *Botryodiplodia theobromae*) (Pat.) Griffon and Maubl. is an emerging problem in Tamil Nadu. Recent years, the disease is spreading at a faster rate in Coimbatore, Erode, Dindigul, Tirunelveli, Kanyakumari and other districts of Tamil Nadu. It was reported in India during 1984 by Raju (Raju, 1984), and it is also reported to cause coconut fruit (nut) rot in Brunei, Indonesia and Vietnam (Johnston, 1965). In case of nut infection, the affected nuts were desiccated, shrunk, deformed and dropped prematurely (Warwick et al., 1993; Lakshmanan and Jagadeesan, 2004) and resulting in nut yield loss up to 10 to 25%. Even though several chemicals were found to manage the disease, the factors including devel-

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opment of fungicide resistance, residual toxicity and environmental pollution forced to develop an alternate method, obviously biological management.

Biological control by antagonistic organisms is established as a potential non-chemical tool for crop protection against phytopathogens (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 through various modes of actions.

Fluorescent pseudomonads were known to survive both in rhizosphere (Parke et al., 1991) and phyllosphere (Wilson and Lindow, 1992) and the antagonist *P. fluorescens* significantly inhibited the mycelial growth of *Pestalotia palmarum* (Karthikeyan and Bhaskaran, 1998). *P. fluorescens* isolated from the surface of healthy cocoa pods were antagonistic to *Phytophthora palmivora* both *in vitro* and *in vivo* and were more effective than cupric oxide or chlorothalonil in controlling black pod (Galindo, 1992).

Similarly, *Trichoderma viride* reduced the fruit rotting in banana caused by *L. theobromae* (Mortuza and Ilag, 1999) while, *T. virens* and *T. hamatum* were highly effective against *L. theobromae* by producing volatile metabolites (Swapna and Nagaveni, 2009; Johnson et al., 2014). Swain and Ray (2009) reported that *Bacillus subtilis* was highly effective against the post-harvest pathogens of yam, viz. *L. theobromae* and *Fusarium oxysporum.*

Development of microbial consortium based on their compatibility can reduce the possibilities of failure of potential microbial inoculants in the rhizosphere. Application of more than one biocontrol agent is a reliable means of reducing the variability and increasing the reliability of biological control. The use of several antagonists with different mode of action may improve biocontrol efficacy under wide range of environmental conditions (Grosch et al., 2011). The three-way mixture of plant growth-promoting rhizobacteria strains INR7 (*B. pumilus*), GB03 (*B. subtilis*) and MEI (*Curtobacterium flaccumfaciens*) as a seed treatment showed increased plant growth promotion and reduction of cucumber diseases (Raupach and Kloepper, 1998) under field conditions. Use of different bacterial species of *P. aeruginosa, S. marcescens, Bacillus* spp., *B. subtilis, P. fluorescens* etc. in biological control of plant pathogens have been reported by several workers (Asaka and Shoda, 1996; Someya et al., 2000; Kim et al., 2009; Suryadi et al., 2011). Moreover, *B. subtilis* and *P. fluorescens* have been successfully formulated and commercially available for the biological management of crop diseases (Jayaraj et al., 2005; Mathivanan et al., 2005). Hence, the present investigation was carried out with different biocontrol agents having different modes of action to develop a cheap, easy to use and beneficial strategy for the farmers and to reduce the hazardous effects of pesticides.

Materials and Methods

Isolation of pathogen

L. theobromae was isolated from infected coconut leaves and further 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 of rhizosphere microbes

The *Pseudomonas fluorescens*, *Trichoderma viride* and *Bacillus subtilis* were isolated from the rhizosphere soil collected from various locations using the specific media, viz. King's B (King et al., 1954), *Trichoderma* specific media (TSM) (Elad and Chet, 1983) and Nutrient agar (NA), respectively. The *Pseudomonas* and *Bacillus* isolates were characterized based on standard biochemical tests (Schaad, 1992; Hildebrand et al., 1994) and the *Trichoderma viride* was characterized based on by microscopic examination of the morphological and reproductive characters. *P. fluorescens (*Pf1) and *T. viride* (TNAU) were obtained from culture collection centre, Dept. of Plant Pathology, TNAU, Coimbatore. *B. subtilis* (Km1) was isolated from Kambalapatti.

Dual culture 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 diam. 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*/*Bacillus 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 replicated plates were maintained for each treatment. The plates were incubated together with the experimental controls (pathogen without antagonists) at 28 ± 2 °C for 7 days, and the radial growth (mm) of the pathogen mycelium was recorded. Per cent inhibition of mycelial growth of the pathogen was calculated.

Field experiment

Field experiments were carried out during 2011 to 2014 in a randomized block design at four different locations, viz. Angalakurichi, Pethanaickanur and N.M. Sungam areas of Pollachi taluk and Ravanapuram village of Udumalpet taluk, Coimbatore district, Tamil Nadu, India. *P. fluorescens* Pf1, *B. subtilis* (Km1) and *T. viride* (TNAU) isolates were used for field experiments. Three and five days after inoculation of bacterial and fungal cultures in the liquid broths, respectively, were mixed with @ 400 ml/kg of talc powder along with 5 g of carboxy methyl cellulose (except for *B. subtilis* formulation) as

sticking agent. Talc-based formulations of all the antagonistic organisms were developed individually and mixed together at equal quantity for field evaluation. 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:

Treatments:

1. SA of 150 g of MC along with 5 kg of FYM/palm at quarterly interval.
2. SA of 150 g of MC along with 5 kg of FYM/palm at half yearly interval.
3. SA of 150 g of MC along with 5 kg of FYM/palm once in a year.
4. SA of 150 g of MC along with 5 kg of FYM + 5 kg NC per palm per year.
5. SA of 300 g of MC along with 5 kg of FYM/palm at quarterly interval.
6. SA of 300 g of MC along with 5 kg of FYM/palm at half yearly interval.
7. SA of 300 g of MC along with 5 kg of FYM/palm once in a year.
8. SA of 300 g of MC along with 5 kg of FYM + 5 kg NC per palm per year.
9. SA of TNAU *Bacillus subtilis* (Bs1) mixture @ 300 g + FYM 5 kg/palm at quarterly interval (Standard).
10. SA of TNAU *Bacillus subtilis* (Bs1) mixture @ 300 g + FYM 5 kg/palm at quarterly interval + NC 5 kg/palm/year.
11. SA of 5 kg of NC/palm/year
12. Chemical check (RF of carbendazim 2 g + 100 ml water) for 3 times at 3 months interval.
13. Control.

Abbreviations: FYM: farm yard manure (SA = soil application; MC = microbial consortia; NC = neem cake; RF = root feeding)

(150 g = 50 g each of *P. fluorescens* (Pf1), *B. subtilis* (Km 1) and *T. viride* (TNAU) are talc-based formulations. Similarly equal quantities of all the three are mixed together for 300 g, too)

Pre- and post-treatment observations were carried out on leaf blight incidence on about 25 leaflets from the lower 10 leaves in each palm selected at random and the disease severity was scored based on a score chart of 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

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{No. of leaves examined}} \times \frac{100}{\text{Maximum grade available in the score chart (5)}}$$

Statistical analysis

In vitro experiments were repeated three times. Field experiments were conducted at Angalakurichi and Pethanaickanur villages for two years during 2011–12, 2012–13 and confirmed at N.M. Sungam and Ravanapuram villages during 2013–2014. In each location, trees showing leaf blight symptoms were randomly selected and 10 trees were

labelled for each treatment and scored. The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI) Biometrics unit, the Phillippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease indices were arcsine transformed. Means ± standard errors are indicated. Data were subjected ANOVA at $P < 0.05$ significant level and means were compared by Duncan's multiple range test (DMRT).

Results

In vitro *studies*

Five *Trichoderma viride* isolates, and isolates of *P. fluorescens* and *Bacillus* sp. (n = 10 each) 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) and *Bacillus subtilis* (Kambalapatti) and the fungi *T. viride* (TNAU) were found highly effective against the leaf blight pathogen and recorded an inhibition zone of 12.7 ± 0.35 mm, 6.7 ± 0.37 mm and 6.5 ± 0.22 mm, respectively (Fig. 1). Hence, all the three antagonists are combined together to develop microbial consortia and evaluated under field conditions.

In vivo *studies*

Field experiments were carried out in farmers' fields at Angalakurichi, Pethanaickanur and N. M. Sungam villages of Pollachi taluk, Coimbatore and Ravanapuram village of Udumalpet taluk, Tirupur during 2011 to 2014 to find out the efficacy of developed microbial consortia against leaf blight disease.

The chemical treatment T_{12} (root feeding of fungicide Carbendazim @ 2 g/100 ml water for 3 times at 3 months interval) was found to be the best treatment among the others including microbial consortia and resulted in 16.67 ± 0.73 per cent reduction in leaf blight disease incidence in both the trials conducted at Angalakurichi and Pethanaickanur villages during 2011–2012 (Table 1). Efficacy of treatment T_{12} was followed by that of T_5 (soil application of microbial consortia @ 300 g + FYM 5 kg/palm at quarterly interval) and T_{10} (TNAU *B. subtilis* (Bs1) mixture @ 300 g + FYM 5 kg/palm at quarterly interval + neem cake 5 kg/palm/year) in both the trials and showed a disease reduction of 14.53 ± 1.40, 14.40 ± 0.60 per cent in Angalakurichi trial and 14.40 ± 1.13 and 14.13 ± 0.70 per cent in Pethanaickanur trial, respectively (Table 1).

Similarly, during 2012–2013 season, the chemical treatment T_{12} was found to be the best treatment among the others and recorded 8.67 ± 0.76 per cent and 9.47 ± 1.01 per cent reduction in leaf blight disease incidence in Angalakurichi and Pethanaickanur trials, respectively. The efficacy of treatment T_{12} was followed by those of T_5, T_9 and T_{10} in both the trials and showed 8.00 ± 1.37, 8.07 ± 0.69, 8.00 ± 0.89 per cent and 8.27 ± 1.27, 8.13 ± 0.87, 8.00 ± 0.63 per cent reduction in the disease incidence both in Angalakurichi and Pethanaickanur trials, respectively (Table 1).

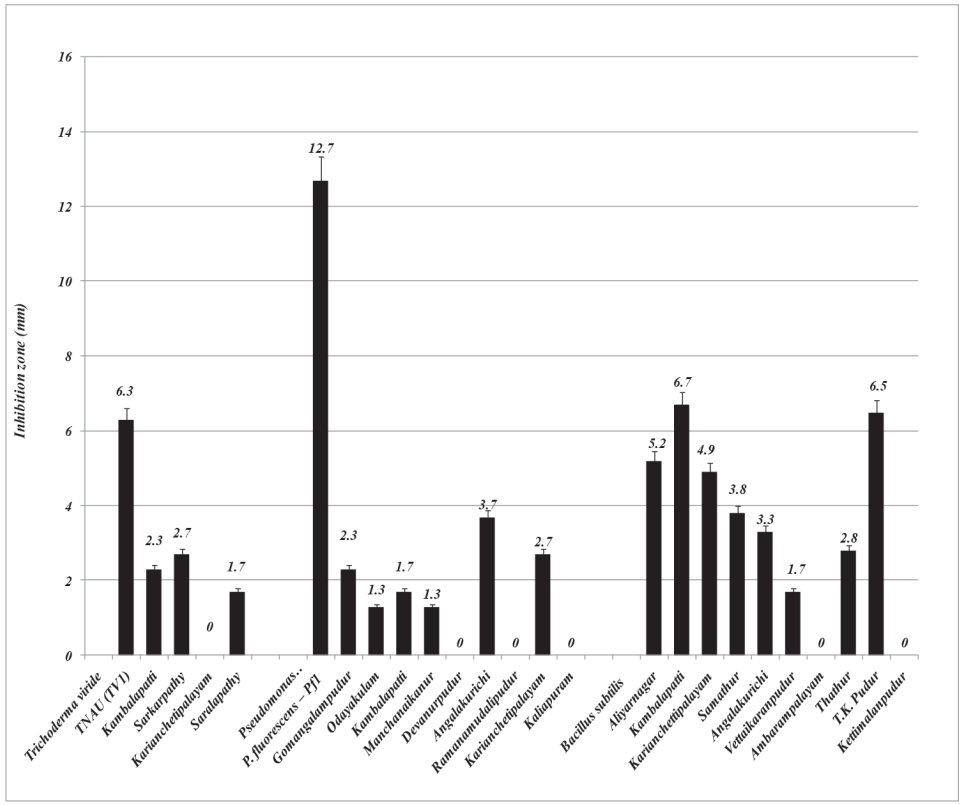

Fig. 1. Mycelial growth inhibition of *Lasiodiplodia theobromae* by bacterial and fungal antagonists (mm). Bars represent mean ± standard errors of three independent experiments.
"0" Value means represent there is no inhibition of mycelia growth observed *in vitro* assay

During 2013–2014, similar results were observed from trials laid out at N.M. Sungam, Pollachi taluk, Coimbatore district and Ravanapuram, Udumalpet taluk, Tirupur district. But in the trial laid out at NM Sungam, the treatment T_1 (soil application of microbial consortia @ 150 g + FYM 5 kg at quarterly interval) resulted in 9.84 ± 1.54 per cent reduction in leaf blight incidence and found on par with T_5, T_9 and T_{10} which showed 11.44 ± 0.69, 11.04 ± 0.56 and 11.52 ± 0.81 per cent decrease in symptoms, respectively (Table 1).

Pooled analysis:

Observations recorded in all the six trials carried out at different locations from 2011–2014 were pooled together and analyzed using IRRISTAT programme. The pooled analysis data (Table 2) clearly indicated that chemical treatment T_{12} (root feeding of Carbendazim @ 2 g/100 ml water for 3 times at 3 months interval) was found to be the best among the others and yielded 13.09 ± 2.53 per cent reduction in leaf blight disease

Table 1

Effect of microbial consortia against leaf blight disease of coconut at different locations during 2011–2014

Treatments	Treatment details	Reduction in disease severity					
		2011–2012		2012–2013		2013–2014	
		Angalakurichi	Pethanaickanur	Angalakurichi	Pethanaicka-nur	N.M. Sungam	Ravanapuram
T_1	MC @ 150 g + FYM 5 kg at quarterly interval	11.99 ± 1.01^{bc} (20.20)	10.80 ± 1.30^{cde} (19.02)	6.00 ± 0.80^{bc} (13.98)	5.73 ± 0.62^{bc} (13.77)	9.84 ± 1.54^{bc} (17.84)	6.16 ± 1.10^{cde} (14.01)
T_2	MC @ 150 g + FYM 5 kg at half yearly interval	9.80 ± 0.31^{cd} (18.23)	9.60 ± 0.64^{def} (18.01)	4.13 ± 0.87^{cd} (11.57)	4.27 ± 1.00^{cd} (11.70)	7.08 ± 0.66^{de} (15.28)	4.96 ± 0.25^{def} (12.84)
T_3	MC @ 150 g + FYM 5 kg once in a year	6.85 ± 0.66^{e} (15.10)	5.73 ± 0.73^{g} (13.75)	3.02 ± 0.67^{d} (9.75)	3.07 ± 1.04^{de} (9.49)	4.84 ± 0.89^{f} (12.24)	3.36 ± 0.55^{f} (10.31)
T_4	MC @ 150 g + FYM 5 kg + NC 5 kg once in a year	8.73 ± 1.59^{de} (16.96)	8.23 ± 2.36^{fg} (15.99)	2.73 ± 0.51^{d} (9.08)	3.20 ± 0.43^{de} (10.01)	5.78 ± 0.51^{ef} (13.78)	3.76 ± 0.36^{f} (11.08)
T_5	MC @ 300 g + FYM 5 kg at quarterly interval	14.53 ± 1.40^{ab} (22.32)	14.40 ± 1.13^{ab} (22.24)	8.00 ± 1.37^{ab} (16.28)	8.27 ± 1.27^{ab} (16.48)	11.44 ± 0.69^{bc} (19.70)	8.56 ± 1.59^{bc} (16.54)
T_6	MC @ 300 g + FYM 5 kg at half yearly interval	11.80 ± 1.11^{bc} (20.00)	11.60 ± 1.29^{bcd} (19.80)	5.90 ± 1.02^{bc} (13.84)	5.87 ± 1.33^{bc} (13.62)	8.88 ± 0.58^{cd} (17.27)	6.88 ± 0.42^{bcd} (15.15)
T_7	MC @ 300 g + FYM 5 kg once in a year	9.63 ± 0.35^{cd} (18.07)	8.53 ± 1.35^{efg} (16.75)	3.80 ± 0.74^{cd} (11.07)	3.87 ± 0.76^{cd} (11.09)	6.64 ± 0.45^{de} (14.86)	4.40 ± 0.98^{ef} (11.69)
T_8	MC @ 300 g + FYM 5 kg + NC 5 kg once in a year	10.68 ± 0.79^{cd} (19.03)	9.87 ± 0.77^{def} (18.25)	3.98 ± 0.41^{ab} (11.46)	3.47 ± 0.87^{de} (10.28)	6.96 ± 0.53^{de} (15.21)	4.48 ± 0.62^{ef} (11.79)
T_9	TNAU Bs1 mixture – (300 g) + FYM 5 kg @ quarterly interval	13.73 ± 0.83^{b} (21.71)	13.73 ± 0.89^{abc} (21.71)	8.07 ± 0.69^{ab} (16.39)	8.13 ± 0.87^{ab} (16.52)	11.04 ± 0.56^{bc} (19.36)	8.64 ± 0.71^{b} (16.99)
T_{10}	TNAU Bs1 mixture (300 g) + FYM 5 kg @ quarterly interval + NC 5 kg (once in a year)	14.40 ± 0.60^{ab} (22.28)	14.13 ± 0.70^{ab} (22.06)	8.00 ± 0.89^{ab} (16.38)	8.00 ± 0.63^{ab} (16.34)	11.52 ± 0.81^{b} (19.76)	8.56 ± 0.89^{b} (16.85)
T_{11}	NC alone – 5 kg/ palm/year	2.27 ± 0.66^{f} (8.25)	3.03 ± 0.53^{h} (9.88)	1.20 ± 0.27^{e} (6.20)	1.90 ± 0.83^{e} (7.14)	2.32 ± 0.41^{g} (8.47)	1.28 ± 0.22^{g} (6.31)
T_{12}	Carbendazim (2 g/100 ml water) RF 3 times at 3 months interval	16.67 ± 0.73^{a} (24.07)	16.67 ± 1.02^{a} (24.06)	8.67 ± 0.76^{a} (17.06)	9.47 ± 1.01^{a} (17.90)	14.96 ± 0.86^{a} (22.70)	12.08 ± 1.11^{a} (20.19)
T_{13}	Control	4.67 ± 0.97(+)	4.93 ± 0.42 (+)	2.80 ± 0.67(+)	3.03 ± 1.08 (+)	1.92 ± 0.61 (+)	2.96 ± 0.69(+)

MC: Microbial consortia; FYM: Farm Yard Manure; NC: Neem Cake; RF: Root Feeding

Means ± standard errors are shown. Values marked with different letters are significantly different at $P < 0.05$, based on Duncan's multiple range test. In control (+) indicates the disease progression

Table 2

Pooled analysis of reduction in leaf blight disease severity from all the locations over the years from 2011 to 2014

Treatments	Treatment details	Means of three-year pooled data of leaf blight disease severity
T_1	MC @ 150 g + FYM 5 kg at quarterly interval	8.42 ± 1.77^c
T_2	MC @ 150 g + FYM 5 kg at half yearly interval	6.64 ± 1.81^d
T_3	MC @ 150 g + FYM 5 kg once in a year	4.48 ± 1.86^e
T_4	MC @ 150 g + FYM 5 kg + Neem Cake 5 kg once in a year	5.41 ± 1.89^e
T_5	MC @ 300 g + FYM 5 kg at quarterly interval	10.87 ± 1.93^b
T_6	MC @ 300 g + FYM 5 kg at half yearly interval	8.49 ± 1.94^c
T_7	MC @ 300 g + FYM 5 kg once in a year	6.15 ± 2.02^d
T_8	MC @ 300 g + FYM 5 kg + Neem Cake 5 kg once in a year	6.57 ± 2.13^d
T_9	BS1 mixture – TNAU (300 g) + FYM 5 kg @ quarterly interval	10.56 ± 2.26^b
T_{10}	BS1 mixture – TNAU (300 g) + FYM 5 kg @ quarterly interval + Neem cake 5 kg (once in a year)	10.77 ± 2.38^b
T_{11}	Neem cake alone – 5 kg/palm/year	2.00 ± 0.45^f
T_{12}	Carbendazim (2 g/100 ml water) RF 3 times at 3 months interval	13.09 ± 2.53^a
T_{13}	Control	3.39 ± 0.52 (+)

Means of pooled disease severity ratings from 2011 to 2014 ± standard errors are shown. Values marked with different letters are significantly different at $P < 0.05$. based on Duncan's multiple range test.

incidence. The efficiency of treatment T_{12} was followed by that of T_5 (soil application of microbial consortia @ 300 g + FYM 5 kg/palm at quarterly interval) that showed 10.87 ± 1.93 per cent reduction in the disease incidence. The treatments T_{10} (TNAU Bs1 mixture - @ 300 g + FYM 5 kg/palm at quarterly interval + neem cake 5 kg/palm/year) and T_9 (TNAU Bs1 mixture consortia @ 300 g + FYM 5 kg/palm at quarterly interval) showed 10.77 ± 2.38 and 10.56 ± 2.26 per cent reduction in the disease incidence, respectively and were on par with T_5.

Discussion

In all the three years of evaluation, soil application of microbial consortia T_5 (*P. fluorescens* Pf1+ *T. viride* TNAU *B. subtilis* + Km1) @ 300 g + farm yard manure 5 kg/palm at quarterly interval was found effective against leaf blight disease next to the standard check Carbendazim (root feeding @2 g/100 ml water for 3 times at 3 months interval). Hence, the microbial consortia developed may be included as a component in the integrated diseases management strategies for leaf blight disease management.

Sharma et al. (2009) reported the inhibitory potential of *P. fluorescens* against citrus stem end root rot pathogen *Botryodiplodia theobromae*, while Srinivasulu et al. (2008) reported that spraying of 10 to 15-day-old culture filtrate of *P. fluorescens* twice at 30 days interval on crown region and on nuts of coconut reduced the bud rot incidence.

While Bokhari et al. (2008) reported that the application of *T. harzianum* along with Topsin-M effectively controlled the pathogens of guava decline disease, viz. *B. theobromae, Fusarium oxysporum* f. sp. *psidii*. Srinivasulu and Raghava Rao (2009) reported that the application of *Trichoderma* spp. caused lysis of the mycelium of *Ganoderma lucidum* which causes basal stem rot/Thanjore wilt disease in coconut. Furthermore, they have found that the application of *T. harzianum*/*T. viride*/*T. hamatum* pasted over bleeding patches and soil application of the bioagents @ 50 g in 5 kg neem cake has reduced the perimeter of the stem bleeding patches on coconut trees.

Moreover the type and rate of organic amendment can strongly influence the quality and subsequent crop performance in the field as well as rhizosphere bacterial communities (Allison et al., 2011). Srinivasan (2009) reported that certain bacteria (*P. fluorescens*, *B. subtilis*) and fungi (*T. viride*) have been found to kill or prevent the growth of the major pathogens of leaf rot and hence amelioration of leaf rot disease is possible with the use of biocontrol agents. Such instances of using bacteria and fungi are common in the control of various plant diseases (Ramjegathesh et al., 2013).

Application of two biocontrol agents together, a yeast (*Pichia guilermondii*) and a bacterium (*Bacillus mycoides)* resulted in better suppression of *Botrytis cinerea*, and also reduced the variability of disease control (Guetsky et al., 2001). Hence, application of more than one biocontrol agent is suggested as a reliable means of reducing the variability and increasing the reliability of biological control (Shtienberg and Elad, 2002). The bacterial antagonists may have various modes of actions which includes substrate competition, production of siderophore (Scher and Baker, 1982), antibiotics (Shanahan et al., 1992), enzymes, viz. chitinase, gluconase, cellulose, pectinase, protease etc. (Raupach et al., 1996; Van Loon et al., 1998; Vidhyasekaran et al., 2001; Ramamoorthy and Samiyappan, 2001) and enhance the plant growth and yield either directly or indirectly by production of plant growth regulators, hormones like indole acetic acid, gibberlic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993) and solubilization of minerals and nutrients (Gaur, 1990; Cattelan et al., 1999).

Multiple strain mixtures of microbial agents such as mixtures of fungi, mixtures of bacteria and combination of bacteria with fungi had been employed to enhance the consistancy of control (Duffy and Weller, 1995; Schisler et al., 1997; Nandakumar et al., 2001). Use of several antagonists with different mode of action may improve biocontrol efficacy under wide range of environmental conditions; however; the success of biological control of plant diseases depends up on the availability of effective formulations of biocontrol agents, survival during storage as well as rapid multiplication and colonization after inoculation. Karthikeyan et al. (2008) reported that the talc-based formulation of mix cultures containing *B. subtilis*, *P. fluorescens*, *Gliocladium virens* and *Trichoderma* spp. could significantly reduced the leaf blight incidence and increased growth of onion in both glass house and field trials.

Consortia of native rhizosphere bacteria *Bacillus megaterium* with *T. harzianum* are able to manage root-knot nematode *Meloidogyne incognita* infestation in *Bacopa monnieri*. L effectively and even performed better than chemical nematicides as they colonize root and have direct interaction making impact on plant health and nematode management (Gupta et al., 2014).

Beneficial rhizosphere microbes increased the root growth resulting in greater root surface area which enables the plant to access more nutrients from soil (Patten and Glick, 2002; Suzuki et al., 2004). Many PGPRs stimulated plant growth either by improving plant nutrition, by releasing plant growth regulators or by suppressing pathogenic organisms (Solano et al., 2008). The rhizosphere antagonists including strains of PGPR are associated with (1) fixing atmospheric nitrogen and supplying it to plants (2) synthesizing various phytohormones including auxins and cytokinins (3) providing mechanisms for the solubilization of minerals such as phosphorous (4) antibiotic synthesis (Haas and Defago, 2005). Certain compatible rhizosphere antagonists mixtures enhanced the plant defense by inducing peroxidase (PO), polyphenol oxidase (PPO), PR proteins, lignifiaction, superoxide dismutase and phenolic compounds. Therefore, these beneficial microorganisms can significantly contribute to plant health and function as major component in integrated disease management systems.

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