



Research Article

Identification and confirmation of hotspot areas and management of root (wilt) disease in coconut

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Abstract

Phytoplasma malady associated with root (wilt) disease in coconut is a serious threat and causes heavy economic loss to the coconut farmers in Kerala and Tamil Nadu in India. A roving survey was carried out during 2016 -2019 to assess the disease incidence in Coimbatore, Tirupur, Dindigul, Theni, Tenkasi, Tirunelveli and Kanyakumari districts. From the results, Cumbum block in Theni district, Tenkasi block in Tirunelveli (Tenkasi) district and Kuruthancode block in Kanyakumari district were identified as hot spot area for root (wilt) incidence. The disease is generally recognized based on leaf symptoms, but the development of foliar symptoms in coconut palms is very slow and there is a time lag between infection and symptom expression. To the confirmation of the latent infection of root (wilt) affected palms, Direct Antigen Coated (DAC) indirect ELISA has been standardized earlier. Since this ELISA system requires 24 hours, a modified protocol was developed using leaf disc as test antigen that was simple, rapid and provide results within 7 hours. The test yielded 98% more sensitivity with respect to the visual observations. The modified protocol is used for the routine screening of coconut palms for selection of disease-free mother palms and for developing root (wilt) resistant varieties. Besides this, the test is used for confirmation of root (wilt) disease in the early stages of the infection and in newly affected areas. The result of the field experiment on root (wilt) management revealed that the soil application of microbial consortia (*Trichoderma viride* (Tv)+ *Pseudomonas fluorescens* (Pfi)+ *Bacillus subtilis*) at 100 + FYM 5 kg + phosphobacteria at 200 g at quarterly intervals along with copper sulphate at the concentration of 75 g at three months intervals was effective in reducing the disease incidence from 13.33 to 8.13% after two years of treatment.

Keywords: disease assessment, phytoplasma, coconut, DAC-ELISA, management, root (wilt) disease

Introduction

In India, the coconut production is mainly carried out in the southern areas namely Andhra Pradesh, Telunkana, Kerala, Karnataka and Tamil Nadu. Recently the production slowly declined due to various factors. Among these factors, biotic diseases are one of the major considering that coconut is affected by more than 50

diseases worldwide. Among these 'the root (wilt) is the most important and it was first observed in Kerala in 1882 (Butler, 1908). Recently, it has become the most serious disease of coconut in the southern parts India (Kerala and Tamil Nadu) and also worldwide with different names. In most of the coconut plantations of Kerala and nearby border districts of Tamil Nadu, it

causes 35% yield reduction and the losses may extend up to 80% in the severe cases. The disease is associated with phytoplasma presence and its insect vectors such as planthopper and lace wing bug were demonstrated by Rajan and Mathen (1985). The main symptom of the disease is flaccidity, yellowing, wilting, drooping of leaves, necrosis of leaflets and rotting of roots. Foliar yellowing and marginal necrosis of the older leaves were observed in advanced disease stages. Coconut root (wilt) disease must be continuously monitored in the coconut growing areas and also the level and pattern of the disease spread needs a detailed methodical study in order to recognize and work out the appropriate management measures. The disease detection was achieved by ELISA technique (Solomon *et al.*, 1999). The current management methods for containing the severity of the disease does not provide expected levels of control because the disease is complex and its progress is also highly variable. It depends on several factors *viz.* different biotypes of the pathogen, variable concentration of the phytoplasma in the host plants, insect vectors and environmental circumstances. As a result, there is no single and permanent control strategy to be adopted for other phytoplasma associated diseases (Osler and Carraro, 2004). The available methods including insecticides, antibiotic (oxytetracycline hydrochloride) and integrated nutrient management practices were quite ineffective under field conditions, because it is not possible to eradicate the insect vectors from environment, moreover they are very expensive.

Exploitation of mixtures of various plant growth promoting *Rhizobacteria* (PGPR) strains were shown to provide superior protection than a single organism (Thomashow and Weller, 1998), and also direct antagonistic activity by the production of various microbial metabolites (Gumedde, 2008), induction of systemic resistance (ISR) by biocontrol agents as *Trichoderma*, *Pseudomonas fluorescens* and *Bacillus subtilis* against most of the pathogens were applied (Ramos Solano *et al.*, 2008). PGPR strains belonging to the genera *Pseudomonas* and *Bacillus* have been reported to elicit growth promotion and ISR in many crops (Gutierrez Manero *et al.*, 2001). Some *Trichoderma* strains establish long lasting colonization of plant roots and produce or release compounds which induce

localized or systemic plant resistance response (Harman *et al.*, 2004).

Micro nutrients involved in the suppression of plant diseases by systemic acquired resistance (SAR) were also tested. The disease severity reduction has been reported in many crops after the application of H_3BO_3 , $CuSO_4$, $ZnSO_4$, $MnCl_2$ or $KMnO_4$ either by foliar or soil which provided systemic protection against many pathogens (Dordas, 2008). Phytoplasmas are highly susceptible to tetracyclines (Ishii *et al.*, 1967) which gave a remission from symptoms but only when the treatment is continued. The tetracycline group of antibiotics suppresses the symptom development in coconut palms only if applied before the expression of systemic foliar yellowing.

Presently, integrated nutrient management, quarantine, sanitation, conventional plant protection measures, and use of biocontrol agents have been advocated for the management of phytoplasma diseases. With this as background, the current study was carried out to monitor root (wilt) disease in nearby Kerala border districts of Tamil Nadu, with the confirmation of its presence through ELISA tests and an attempt was made to manage the disease with microbial consortia, micro nutrients and bio fertilizers.

Materials and Methods

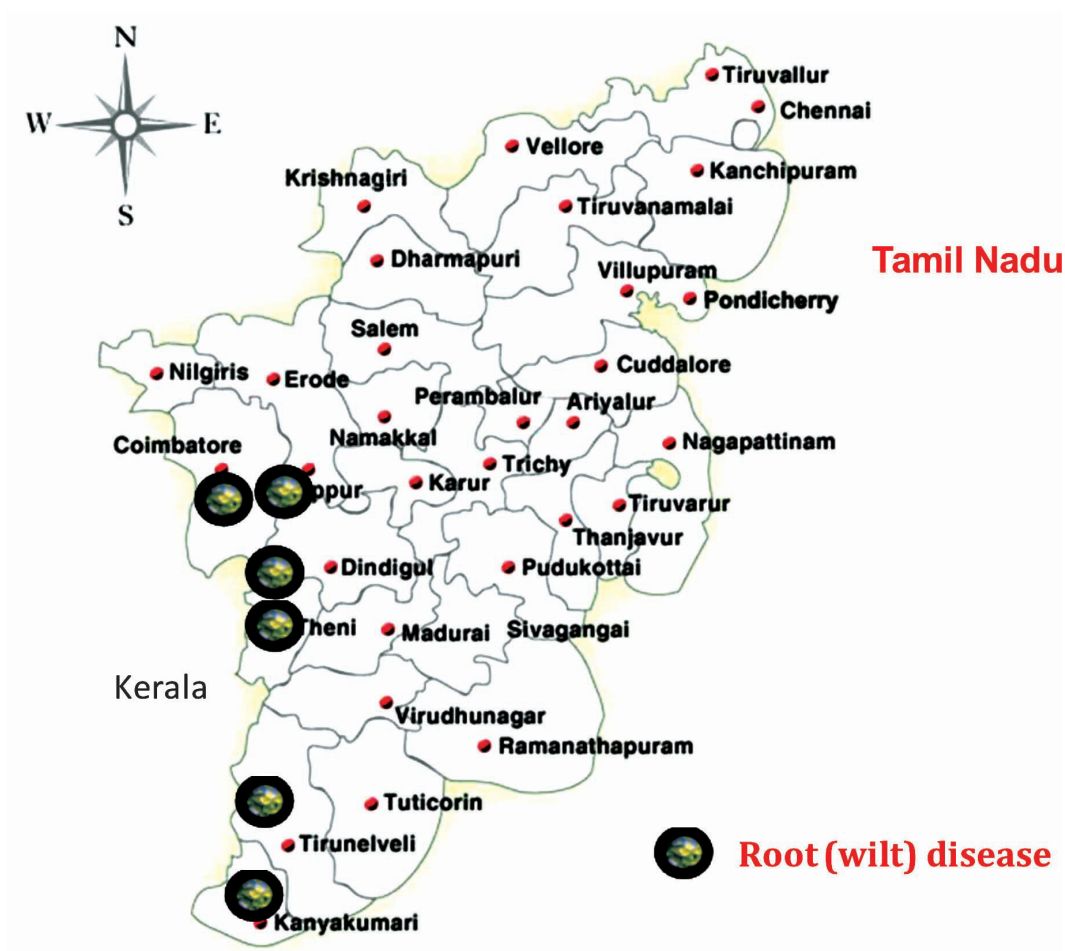
Roving survey

To monitor the spread of root (wilt) incidence, a roving survey was carried out from 2016 to 2019 in the districts of Coimbatore, Dindigul, Kanyakumari, Tirupur, Theni and Tirunelveli of Tamil Nadu, India (Figure 1). In each district five blocks were selected for the survey. Each block had five villages comprising of ten gardens with a minimum of 75 palms/garden. The incidence of root (wilt) disease was recorded on the expression of major foliar symptoms in the palms like flaccidity, yellowing and necrosis. The percentage of the disease incidence was calculated by using the formula.

$$\text{Disease incidence} = \frac{\text{Number of palms infected}}{\text{Total number of palms observed}} \times 100$$

Confirmation of root (wilt) disease presence was performed through protein based detection methods.

Figure 1. Survey for the incidence of coconut root (wilt) disease. The surveyed areas are circled in black.



Forty samples were collected from root (wilt) diseased symptomatic palms and correspondingly healthy palm of the hot spot areas of Tamil Nadu; the disease index was recorded in the same palms as described from George and Radha (1973). Spindle leaf samples were collected from healthy and diseased elite coconut palms in the disease endemic areas of Tamil Nadu like Coimbatore, Theni, Tirunelveli and Kanyakumari districts at different locations.

Preparation of samples

Non chlorophyllous tissues containing leaf samples were collected from the spear leaves of diseased and healthy coconut palms. Ten mg of leaf discs were taken and cut into small pieces and grinded in carbonate bicarbonate buffer (0.05 M, pH 9.6) for DAC ELISA.

A protocol developed by Sasikala *et al.* (2005) was used with some modifications. Instead of leaf grind

being used as antigen, leaf discs were cut from the spindle leaves of diseased and healthy palms and added into each well of the microtitre plate (Nunc, Denmark) preloaded with 50 µl of carbonate bicarbonate buffer. After the antigen was loaded, 50 µl of the same buffer were added. Additives like gelatin 1% and ovalbumin 0.2% were added to the antigen coating buffer. The negative control was taken from healthy palms and added 0.05 M carbonate bicarbonate buffer. After the addition of spindle leaf discs, all the sample wells were sequentially loaded with blocking agent (bovine albumin fraction V; Hi Media, Bombay, India), unfractionated root (wilt) phytoplasma specific antiserum, enzyme conjugate (goat antirabbit IgG conjugated with horseradish peroxidase; Genei, Bangalore, India) and substrate (tetramethyl benzidine/hydrogen peroxidase). The plate was incubated for 90 minutes at 37°C after loading the antigen for getting

improved adsorption of antigen molecules to the wells of the ELISA plate. In a like experiment subsequent to loading the antigen the plate was incubated at 37°C for 90 minutes followed by incubation at 4°C overnight. Then the plates were washed with wash buffer (PBST pH 7.4) for 3 to 5 times. The plates were incubated for 60 minutes at 37°C and washed three times with wash buffer. After that, the substrate was added and the plates were kept at room temperature until the development of bright blue color in the positive control. Afterwards the reaction was stopped by using 1 N H₂SO₄ and the absorbance measured using 450 nm filter in a VERSA max microplate reader.

Field experiments

Field experiments were laid out in farmer's field at Aathupollachi village of Pollachi Taluk in Coimbatore district. The field experiment was set in a randomized block design with eight treatments comprising three replications. Each treatment had 15 palms in the age group of 35 years. The recommended doses of fertilizers of 1.3 kg urea, 2.0 kg super phosphate, 3.5 kg of muriate of potash and 1.0 kg of magnesium sulphate along with treatments were evaluated for three years from 2017 to 2019 (Table 1).

Observations were recorded on the individual palms for major foliar symptoms of root (wilt) disease viz., flaccidity, yellowing and necrosis. The disease index for each palm was calculated based on the following formula. Pre (before treatments) and post treatment (after treatments) observations were also made on individual palm at yearly interval.

$$\text{Disease Index} = \frac{F+Y+N}{L} \times 10$$

The scale assigned to F-flaccidity (0-5), Y-yellowing (0-

3), N-necrosis (0-2), and L is the total number of leaves (George and Radha, 1973).

As part of the study, awareness programmes on identification, symptomatic and management measures of root (wilt) disease were organized to the Agricultural Officers, Assistant Agriculture Officers, Horticultural officers and farmers in the surveyed districts. All the data were analyzed statistically and prior to the statistical analysis, the values of the disease index were arcsine transformed. Data were subjected ANOVA at P<0.05 significant level and means were compared by LSD.

Results and Discussion

The results of the roving survey conducted on the occurrence of root (wilt) disease in the different blocks containing villages of nearby Kerala border districts of Tamil Nadu during 2016-2019 are presented in Table 2. A total of 30 blocks of above districts were surveyed and among these, the Theni district recorded the highest mean incidence of 16.15% followed by Tirunelveli district (15.77%). Among the five blocks surveyed in the Coimbatore district, the root (wilt) incidence was observed in four blocks viz., Pollachi South (10.58%), Periyanaikanpalayam (4.00%), Anamalai (3.80%) and Pollachi North (1.62%) and it was not observed in the Kinathukadavu block. The status of root (wilt) was surveyed in five blocks of Tirupur district and the disease was observed only in Tirupur and Gudimangalam blocks. In the Theni district the occurrence of root (wilt) was recorded in Uthamapalayam and Cumbum blocks and it was observed in the 53.00 and 27.75%, respectively. The root (wilt) disease was absent in the villages of Periyakulam and Bodinayakkanur. The maximum incidence of the

Table 1. Treatment schedule for root (wilt) disease management.

Test number	Treatment details	Frequency
T ₁	SA of MC 300 g + FYM 5 kg + Phosphobacteria 200 g / palm / year	three months interval
T ₂	T ₁ + SA of copper sulphate (75 g)	three months interval
T ₃	T ₁ + SA of magnesium sulphate (75 g)	three months interval
T ₄	T ₁ + SA of zinc sulphate (75 g)	three months interval
T ₅	T ₁ + Coconut micronutrient mixture 1 kg / palm / year	three months interval
T ₆	RF of tetracycline 1,000 ppm / palm - 100 ml / palm	three months interval
T ₇	Untreated control	

SA, soil application; MC, microbial consortia including *Pseudomonas fluorescens* (Pf1), *Trichoderma viride* (Tv1) and *Bacillus subtilis*; RF, root feeding.

Table 2. Incidence of coconut root (wilt) disease in different districts of Tamil Nadu (India).

District	Blocks	Root (wilt) incidence (%)*
Coimbatore	Pollachi South	10.58
	Pollachi North	1.62
	Anaimalai	3.80
	Periyanaikanpalayam	4.00
	Kinathukadavu	0.00
	Mean	4.00± 2.33
Dindigul	Palani	0.00
	Oddanchatram	0.00
	Reddiarchatram	0.00
	Nilakottai	0.00
	Batlagundu	0.00
	Mean	0.00±0.00
Kanyakumari	Rajakkamangalam	4.10
	Thiruvattaru	5.48
	Kurunthancode	14.63
	Munjerai	1.18
	Thovalai	1.00
	Mean	5.27±2.87
Theni	Cumbum	53.00
	Uthamapalayam	27.75
	Periyakulam	0.00
	Bodinaickanur	0.00
	Theni	0.00
	Mean	16.15±12.58
Tirupur	Gudimangalam	0.06
	Udumalpet	0.00
	Tirupur	0.05
	Avinashi	0.00
	Pongalur	0.00
	Mean	0.02±0.03
Tirunelveli	Thenkasi	30.9
	Kadayanallur	13.7
	Vasudevanallur	0.00
	Shencottah	18.5
	Kadayam	15.75
	Mean	15.77±6.4

*Values represent mean ± standard error.

disease was observed in the Thenkasi block (30.90%) and the disease was not found in Vasudevanellur block of Tirunelveli district. A total of five blocks were surveyed for assessing the status of root (wilt) incidence in Kanyakumari district. Among these, the disease incidence was recorded in all blocks and maximum in Kurunthancode (14.63%), Thiruvattaru (5.42%) and Rajakkamangalam (4.10%) blocks.

Solomon *et al.* (1999) observed that the root (wilt) disease was first noticed in Theni district followed by Tirunelveli and Kanyakumari districts, later the disease

spread to remaining Kerala border districts of Tamil Nadu in Coimbatore and Tirupur districts. Ramjegathesh *et al.* (2018) revealed that the maximum incidence of the disease was observed in Gudalur village of Cumbum block in Theni district. Based on the previous and present study the disease spreading was very slow and only some new areas were identified. Eradication of root (wilt) affected palm to prevent the spread of the disease within this area or nearby areas can be successful when a continuous monitoring of the disease is performed, however the monitoring programme was not followed properly and can't achieve the goal of disease-free gardens in Tamil Nadu.

For confirmation of root (wilt) disease presence the DAC ELISA test was conducted using polyclonal antiserum which is highly specific to phytoplasmas. Forty samples were collected from coconut trees showing typical symptoms of root (wilt) from different villages of Coimbatore, Kanyakumari, Theni and Tirunelveli. In this modified assay, after adding the substrate the plate was incubated for 5 to 10 minutes at room temperature the bright blue color appears in the symptomatic samples and there was no color in healthy samples. The infected samples turned to bright yellow color when the reaction was stopped. Based on the absorbance values, all the 40 samples collected from the above districts showed positive reaction except samples from Pudhukudy village of Tirunelveli district and Hanumanthanpatti village of Theni district (Table 2). All the positive samples were recorded with higher OD value than healthy samples. Similar finding were obtained by Sasikala *et al.* (2005, 2010); the modified method is highly helpful for screening coconut palms for developing root (wilt) resistant varieties. The test was used here for the confirmation of the root (wilt) disease presence in the coconut palms observed in the new areas. The result of the present study revealed that the sampling of non chlorophyllus spindle leaves of coconut appears to be the best target for protein based detection of phytoplasmas in diseased palms.

A field experiment was laid out at Aathupollachi village of Anamalai block with seven treatments and three replications during 2017-2019. The treatments were imposed as per schedule (Table 1) and the observations on root (wilt) incidence was recorded. The results of the experiment revealed that the soil

Table 3. Confirmation of root (wilt) disease presence through DAC ELISA.

Sample number	District	Village	GPS co-ordinates	Disease index-stage of the palm	Number of samples	Reaction	
1.	Coimbatore	Ambarampalayam	10°37'N; 76°57'E	36 - DMS, 28-DMS	2	+	
2.		Manakkadavu	10°39'N; 76°52'E	30-DMS, 24-DMS	2	+	
3.		Jamin Uthukuli	10°40'N; 76°52'E	28-DMS	1	+	
4.		Aathupollachi	10°38'N; 76°55'E	56-DAS, 44-DMS	2	+	
5.		Mannur	10°39'N; 76°55'E	24-DMS, 14-DES, 28-DMS	3	+	
6.		Ayyampalayam	10°39'N; 76°55'E	24-DMS	1	+	
7.		Nanjae goundanpudur	10°38'N; 76°57'E	26-DMS	2	+	
8.	Tirunelveli	Kanakkapillaivalasai	9°005'N; 77°265'E	26-DMS, 22-DMS	2	+	
9.		Ilathur	9°017'N; 77°253'E	20-DMS, 46-DMS	2	+	
10.		Karisalkuduyiruppu	9°027'N; 77°258'E	26-DMS	1	+	
11.		Meenachipuram	9°015'N; 77°23'E	24-DMS	1	+	
12.		Thaenpothai	9°009'N; 77°249'E	52-DAS, 36-DMS	2	+	
13.		Kasitharmum	9°057'N; 77°316'E	28-DMS, 18-DES, 34-DMS	3	+	
14.		Pettai, Kadayanallur	9°086'N; 77°366'E	28-DMS	1	+	
15.	Theni	Pudhukudy	9°070'N; 77°364'E	38-DMS	1	-	
16.		Angoorpalyam	9.6898594; 77.268158	56-DAS, 62-DAS	2	+	
17.		Gudalur	9.719784;77.271746	60-DAS, 28-DMS	2	+	
18.		Hanumanthanpatti	9.787751;77.320362	12-DES	1	-	
19.		Samandipuram	9.695611;77.277542	48-DMS, 24-DMS	2	+	
20.		Kanyakumari	Odayarvilai	8.182828; 77.274584	34-DMS, 26-DMS	2	+
21.			Kottilpadu	8.168488; 77.268474	40-DMS	1	+
22.	Kankalam, Mathicodu		8.228238;77.261283	52-DAS, 10-DES	2	+	
23.	Arumanai		8.363411;77.236196	12-DES	1	+	
24.		Kulithurai	8.309862;77.210753	8-DES	1	+	

DES, disease early stage; DMS, disease middle stage; DAS, disease advanced stage; +, positive; -, negative.

application of microbial consortia at the concentration of 300 g (*Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* each at 100 g) + FYM 5 kg + phosphobacteria + copper sulphate 75 g at three months intervals was effective in reducing the disease incidence from 13.33 to 8.13% after two years. In the same treatment is was also recorded the highest nut yield of 117 per tree per year with a cost/benefit ratio of 1: 3.13. The above treatment was on par with chemical check used for comparison viz., root feeding of tetracycline at the concentration of 1,000 ppm / palm at three months intervals which reduced the disease incidence from 16.0 to 9.11%. In the untreated control plot the incidence increased to 15.56 - 18.89% (Tables 4 and 5).

In order to verify the extend of spread and losses due to root (wilt) disease, about 14 awareness programmes and one brain storming session were organized to agricultural extension workers and farmers to teach the identification of initial symptoms and detection of the disease in order to stop any additional spread of the disease (Table 6). One of the significant features of the

root (wilt) disease is that it is not lethal, but a devastating problem which reacts to perfect management practices. Till now, there were no precise methods available for the management of phytoplasma diseases, but it is possible to reduce its speed of spread and induce a remission of the symptoms. The report on management of root (wilt) disease using biocontrol agents is scanty. The appropriateness of biocontrol agents for the management of this prokaryotes was demonstrated. One promising area is the exploitation of plant growth promoting rhizobacteria through the induction of plants natural defences by SAR against phytoplasma infection using chemical inducing agents. PGPR induce resistance against several plant pathogens but only a few reports are available for phytoplasma-associated diseases under field environments (Poza *et al.*, 2005). In this strategy, induced resistance develops as a result of colonization of plant roots by PGPR (Beckers and Conrath, 2007). Combination of bioagents like *P. fluorescens* (Pf1) along with endophytic *B. subtilis* (EPC 5) and *T. viride* (Tv1) showed enhanced production of

Table 4. Efficacy of biocontrol agents and micronutrients on root (wilt) disease.

Treatment number	Treatment details	O MAA	12 MAA	18 MAA	24 MAA	Difference
T ₁	Soil application of microbial consortia 300 g + FYM 5 kg / palm at 6 months intervals intervals+ phosphobacteria 200 g / palm	16.44 (23.56)	15.33 (23.21)	14.87 (22.67)	14.44 22.30	2.00
T ₂	T ₁ + SA of CuSO ₄ 75 g at 3 months intervals	13.33 (21.31)	11.78 (20.07)	11.11 (19.44)	8.13 (16.87)	5.2
T ₃	T ₁ + SA MgSO ₄ 75 g at 3 months intervals	18.22 (25.22)	16.22 (23.75)	16.0 (23.57)	15.11 (22.85)	3.11
T ₄	T ₁ + SA ZnSO ₄ 75 g at 3 months intervals	17.56 (24.92)	15.56 (23.22)	14.67 (22.45)	13.78 (21.22)	3.78
T ₅	Root feeding of tetracycline 1,000 ppm / palm bimonthly	16.0 (22.42)	12.00 (20.25)	10.4 (18.72)	9.11 (20.01)	6.89
T ₆	Recommended fertilizer alone	14.4 4 (22.30)	15.38 (23.08)	13.7 (21.53)	12.67 (20.95)	1.73
T ₇	Untreated control	15.56 (23.88)	16.67 (23.94)	18.7 (25.60)	18.89 (25.66)	- 3.33
		SEd	CD (P = 0.05)			
	M	0.654	1.311			
	T	0.865	1.734			
	M X T	1.734	3.46			

MAA, months after applications. Values are mean of three replications, data in parenthesis are arc sine transformed values

Table 5. Efficacy of biocontrol agents and micronutrients on nut yield of coconut.

Treatment number	Treatment details	Yield (nuts/year)*	C:B Ratio
T ₁	Soil application of microbial consortia 300 g + FYM 5 kg / palm at 3 months intervals <i>Phosphobacteria</i> 200 g / palm	95	1: 2.14
T ₂	T ₁ + SA of CuSO ₄ 75 g at 3 months intervals	117	1: 3.13
T ₃	T ₁ + SA MgSO ₄ 75 g at 3 months intervals	108	1: 2.67
T ₄	T ₁ + SA ZnSO ₄ 75 g at 3 months intervals	102	1: 2.13
T ₅	Root feeding of tetracycline 1,000 ppm / palm bimonthly	98	1: 2.40
T ₆	Recommended fertilizer alone	86	1: 1.37
T ₇	Untreated control	75	-
	SEd	2.836	
	CD (P = 0.05)	6.18	

*Mean of three replications

Table 6. Awareness programmes in root (wilt) disease.

	Venue	District	Number of programmes	Number of beneficiaries
1.	Kurunthankodu, Athengode, Monday nagar	Kanyakumari	5	230
2.	Aathupollachi, Ambarampalayam, Anaimalai	Coimbatore	3	165
3.	Achampatti, Panpoli, Nainaragaram	Tirunelveli	3	140
4.	Gudalur, Maninagaram	Theni	2	78
5.*	TNAU, Coimbatore	Coimbatore	1	50

*Brain storming session conducted at TNAU, Coimbatore.

defense enzymes in response to root (wilt) pathogen in coconut palms (Ramjegathesh *et al.*, 2016). Srinivasan *et al.* (2010) reported that, soil application of *P. fluorescens*, *B. subtilis* and *T. viride* at the concentration of 300 g / palm / year at 6 months interval is able to manage the root (wilt) associated leaf rot symptoms under field conditions. Srinivasan (1999) observed that

root wilt disease affected palms responded to the oxytetracycline hydrochloride and there was a temporary remission in the disease symptoms. The antibiotic does not necessarily slay the phytoplasma other than just decrease or hold back its concentration in the affected palm to a particular level and it is not damaging the growth of palms. Once all the above

management practices are discontinued, it is possible that the phytoplasma concentration will increase more than before, and all the symptoms re-appear.

Soil application of microbial consortia at the concentration of 300 g + FYM 5 kg + phosphobacteria at 6 months interval and copper sulphate 75 g at three months interval along with appropriate nutrients may be included as components in the integrated disease management for root (wilt) disease. Further, if all the management measures are discontinued, the phytoplasma concentration will increase in the palm leading to symptom re-appearance. A direct biological control of pathogenic organism in the field should be achieved by modifying the indigenous microbial community to favour the reduction of pathogenic inoculum through different mode of actions.

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