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Exploitation of Antibiotics and Biocontrol Agents for the Management of Root (Wilt) Disease in Coconut Palms

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Antibiotics (oxytetracycline hydrochloride) and bioconsortia *Pseudomonas fluorescens strain* (Pf1), *Bacillus subtilis* (EPC 5) and *Trichoderma viride* (Tv1) were used to evaluate their efficacy against the root (wilt) disease in coconut under two different field conditions. Field trial was conducted at hot spot area of the disease with a local 'Tall' variety of coconut. The results revealed that the root feeding of 100 ml of oxytetracycline hydrochloride @ 1000 ppm at monthly intervals up to one year resulted in the remission of root (wilt) disease symptoms in addition to increasing the nut yield compared to untreated control palms. This treatment was followed by the soil application of bioconsortia (Pf1+EPC5+Tv @ 100 g each+ 5 kg FYM/palm). Biochemical and physiological analysis revealed that the chlorophyll and soluble protein content were found to be increased and the stomatal resistance was found to be reduced in the bioconsortia (Pf1+EPC5+Tv) treated palms compared to control palms.

Key words: Antibiotics, Biocontrol agents, Coconut, Phytoplasma, Root (wilt) disease

The production of coconuts in India was decreasing considerably year after year, due to various biotic factors. Among them, coconut root (wilt) disease (RWD) caused by phytoplasma is the most serious disease in Southern India (Kerala, Tamil Nadu and Karnataka) and also worldwide. It is being transmitted by the plant hopper (*Proutista moesta*) and lacewing bug (*Stephanitis typica)* in a propagative and persistent manner. Root (wilt) disease in coconut has high complexity and variability in its progress. Because of this, there is no effective measure of curing phytoplasma plant diseases. MLOs (phytoplasma) were found to be susceptible to tetracycline (Ishiie *et al.* 1967), which gave a remission from symptoms only while treatment continued. Due to the phytoplasma nature of the causal agent of lethal yellowing, tetracycline group antibiotics suppressed symptom development if applied before expression of systemic foliar yellowing. The available methods including chemicals like insecticides and antibiotics (Oxytetracycline hydrochloride) in the management of disease were quite ineffective under field conditions and also were not quite good for the elimination of all insect vectors from the environment and also very expensive. Root (wilt) disease transmitting insect vectors are mostly present on the underside of leaves and application of chemicals is therefore also difficult in coconut gardens. Hence, the adoption of integrated management approaches that involve a combination of agronomic, biological (such as application of Plant Growth Promoting Rhizobacteria (PGPR), fluorescent Pseudomonads or endophytic microbes) and chemical methods that will lead to remission of the root (wilt) symptoms by way of induced systemic resistance in coconut palms.

In the recent years, the biocontrol agents such as *Trichoderma, Pseudomonas fluorescens* and *Bacillus subtilis* etc., were exploited for the management of various diseases of crop plants (Zehnder *et al*. 2001). Besides direct antagonistic activity by the production of various microbial metabolites (Gumede, 2008), induction of systemic resistance (ISR) by biocontrol agents against diseases has been established as a new mechanism by which the plants defend themselves from pathogen attack. ISR has been reported as one of the mechanisms by which PGPR reduces plant disease, thereby altering the physical and biochemical properties of host plants.

Despite decades of research, a complete control for phytoplasma is not yet available, but measures were made to reduce its rate of spread. Current methods include quarantine, sanitation, conventional plant protection measures such as application of pesticides, chemicals, use of resistant cultivars, use of biological control and integrated nutrient management (Maheswarappa and Anithakumari, 2005). The objective of the present study is to evaluate the antibiotics and biocontrol agents for the management of root (wilt) disease in coconut palms.

Materials and Methods

Field Experiments

Field experiments were conducted in coconut root (wilt) endemic location of Pollachi, Coimbatore district, Tamil Nadu, India to test the bioefficacy of microbial consortia formulations along with antibiotics

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and chemicals for coconut root (wilt) disease. Each field experiment was laid out in Randomized Block Design (RBD) with three replications and all the treatments T_1 - Pf1+Tv (each 150 g mixed with 5 kg FYM/palm); T_2 - Pf1+TDK1+KH1 (each 100 g mixed with 5 kg FYM/palm); T_3 - Pf1+EPC5 +Tv (each 100 g mixed with 5 kg FYM/palm); T_4 - Pf1+PY15+AH1 (each 100 g mixed with 5 kg FYM/palm); T_5 - Oxytetracycline hydrochloride (1000 ppm)- 100 ml of water; T_6 - Monocrotophos (10 ml+10 ml of water); T_7 – Control) were applied as every months interval.

Whereas, Pf1, AH1, TDK1, PY15 and KH1- *Pseudomonas fluorescens* strains; Tv - *Trichoderma viride*; EPC5 - *Bacillus subtilis.* All the strains were obtained from the culture collection section, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Method of application in mixture of biocontrol agents

The talc based formulations of bioconsortia were applied at the rate of 300 g/palm as soil application at monthly intervals. Three hundred gram of consortia mixture was mixed with 5 kg of farm yard manure heaped for 25 days, rotating with alternative days with optimum moisture level. Five kg of mixture was applied for each palm.

Disease index

The intensity of root (wilt) disease and yield data (two months interval) were recorded in each palm for before imposing treatments as initial and two year after imposing the treatments. DI = {(F+Y+N)/L} X 10; Where F, Y and N are the grade points assigned to Flaccidity (0-5), Yellowing (0-3), Necrosis (0-2), respectively and L is the total number of fronds for taking observation (George and Radha, 1973). The disease index was expressed as numbers.

Biochemical and Physiological characters

Pigments

The content of total chlorophyll was estimated by adopting the procedure of Witham *et al*. (1971) and the contents were expressed as mg/gram of fresh weight basis.

Total soluble protein

Total soluble protein of fresh leaf samples was determined by Lowry's method and the protein contents were expressed as mg/gram of fresh weight (Lowry *et al*. 1951).

Stomatal resistance

The resistance offered by stomata for the exit of water from leaf surface was measured using a steady state porometer (LI-1600, LICOR, Inc., Nebraska, USA) and expressed in Scm^{-1}.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI) Biometrics unit, the Philippines (Gomez and Gomez, 1984).

Results and Discussion

Effect of different treatments on the intensity of root (wilt) disease

The results showed that, root feeding of 100 ml of oxytetracycline hydrochloride (1000 ppm) significantly reduced the development of symptoms in coconut palms compared to the application of bioagents. The per cent reduction in disease index over initial values was found to be 33.98 at two years after application of oxytetracycline hydrochloride (1000 ppm). This was followed by the application of bioconsortia *viz.,* Pf1+EPC5+Tv (each 150 g + 5 kg FYM/palm) which recorded 25.48 per cent reduction in disease index over the initial level two years after application (Table 1). Nut yield was observed to be higher after treatment for all the treatments. Root feeding of oxytetracycline hydrochloride (1000 ppm) recorded the highest number of nuts (74.82 nuts/palm/year) followed by soil application of Pf1+EPC5+Tv (70.11nuts/palm/year) in the field trials (Fig. 1).

Table 1. Effect of different treatments on the severity of root (wilt) disease of coconut palms

Treatments	Disease index		Per cent reduction over initial
	Initial	Two years after application	
Pf1+Tv	16.30^{d} (23.81)	12.74^{a} (20.91)	21.84^{e}
Pf1+TDK1+AH1	19.02^{b} (25.86)	14.72^{c} (22.56)	22.60^{c}
Pf1+EPC5+Tv	20.99^{a} (27.27)	15.64^{f} (23.30)	25.48^{b}
Pf1+PY15+ KH1	19.66^{b} (26.32)	15.34^{e} (23.06)	19.86^{d}
Oxytetracycline hydrochloride	21.75^{a} (27.80)	14.36^{b} (22.27)	33.98^{a}
Monocrotophos	16.89^{c} (24.40)	15.00^{d} (22.91)	11.19^{f}
Untreated Control	18.97^{b} (25.82)	23.65^{g} (31.30)	- 4.68^{g}
SEd	0.42	0.02	0.02
CD (0.05)	0.91	0.05	0.05

Values are mean of three replications. For statistical analysis the disease index data in parentheses are arc sine transformed values. Means in a column followed by same superscript letters are not significantly different by Duncan's Multiple Range Test at p<0.05.

One of the significant features of the root (wilt) disease is that it is not lethal, but a debilitating malady, which responds to ideal management practices. Accurate methods for phytoplasma diseases are not yet available, but management measures may be taken up to reduce its rate of spread and remission of symptoms. In the present study, root feeding of 1000 ppm oxytetracycline @ 100 ml/ palm was found to reduce the severity of coconut root (wilt) disease, besides increasing the nut yield. Tetracycline antibiotics, which inhibit protein synthesis by binding the 30S ribosomal subunit, are effective against phytoplasma. Himelick (1972) observed that remission of symptoms in lower dose of tetracycline repeated treatments may be necessary to maintain a palm in a state of remission. The treated palms showed full remission of symptoms when treated with oxytetracycline (100 mg/tree) prior to the development of foliar yellowing. Srinivasan *et al*. (2010) observed that root (wilt) disease affected palms responded to the antibiotic oxytetracycline hydrochloride and temporary remission in the disease symptoms. Instead of killing the phytoplasma, the application of antibiotic oxytetracycline reduced the phytoplasma concentration in the palm to a level which is not harmful to the normal growth of palm. When the injection is stopped, the occurrence of disease will gradually increase.

Table 2. Total chlorophyll, stomatal resistance and soluble protein contents in coconut palms treated with different treatments under field conditions.

Treatments	Total chlorophyll* (mg/g)		Soluble protein (mg/g)		Stomatal resistance (S cm^{-1})	
	Initial	Two years after treatment	Initial	Two years after treatment	Initial	Two years after treatment
Pf1+Tv	0.67^{a}	1.32^{c}	0.44^{b}	0.86^{d}	0.84^{a}	0.64^{d}
Pf1+TDK1+AH1	0.61^{d}	1.46^{b}	0.37^{d}	0.94^{bc}	0.85^{a}	0.57^{b}
Pf1+EPC5+Tv	0.57^{d}	1.52^{a}	0.42^{c}	1.01^{a}	0.82^{a}	0.53^{a}
Pf1+PY15+KH1	0.48^{e}	1.32^{c}	0.37^{d}	0.96^{c}	0.85^{a}	0.61^{c}
Oxytetracycline hydrochloride	0.57^{b}	1.44^{d}	0.42^{c}	0.97^{b}	0.84^{a}	0.54^{a}
Monocrotophos	0.59^{c}	1.20^{b}	0.37^{d}	0.74^{e}	0.87^{a}	0.70^{e}
Untreated Control	0.67^{a}	1.01^{e}	0.48^{a}	0.060^{f}	0.87^{a}	0.86^{f}
SEd	0.01	0.02	0.01	0.01	0.01	0.01
CD(0.05)	0.02	0.05	0.02	0.03	0.02	0.02

Values are mean of three replications. Means in a column followed by same superscript letters are not significantly different by Duncan's Multiple Range Test at $p<0.05$.

Because of limited information available for the management of root (wilt) associated disease using biocontrol agents, we studied the suitability of biocontrol agents for the management of the obligate parasite. According to Raupauch and Kloepper (1998), the use of more than one biocontrol agents was more effective when compared to the use of single biocontrol agent. A single biocontrol agent is not active for all soil environments and also against all pathogens. Combinations of certain compatible bioagents activate peroxidase, lignification, super oxide dismutase and results in accumulation of phenolic compounds that gives high plant defense than the plants treated with single antagonist. Combination of bioagents such as P. fluorescens (Pf1) strain along with endophytic B. subtilis (EPC 5) and T. viride (Tv) showed enhanced production of defense enzyme in response to root (wilt) pathogen in coconut palms. By applying PGPR or by defense inducing chemical, the plant's natural defense is induced through Systemic Acquired Resistance (SAR) against phytoplasma infection (Chiesa et al. 2007). PGPR induces resistance against several plant pathogens but few reports are available for phytoplasma-associated disease under field environments.

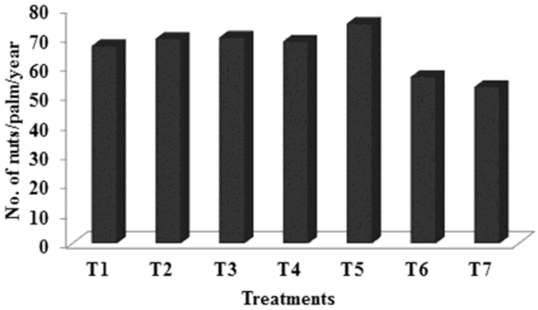
Total chlorophyll and soluble protein

The mixture of biogents Pf1+ EPC5+Tv recorded the highest leaf chlorophyll and total soluble protein content in coconut palms at up to two years after application, followed by the root feeding of oxytetracycline hydrochloride (1000 ppm) and then the bio control consortia Pf1+TDK1+AH1. Lenon *et al*. (1996) reported a reduction in chlorophyll content by 60 per cent in coconut palms affected by lethal yellowing. In plants, which were severely infected with phytoplasma, the chlorophyll content was reduced to 36.1 per cent and these plants exhibited early leaf chlorosis/necrosis symptoms (Junqueira *et al*. 2004). The loss of leaf protein in infected leaves may account for chloroplast damage or may be the result of inhibition of protein synthesis. Among the defense proteins produced by the host plants by the application of bio agents to induce peroxidases, chitinases and β 1,3 glucanase are the major ones. Increase in defense proteins in corn plants infected by the phytoplasma could contribute to the increase in total protein content.

Stomatal resistance

All the treatments significantly influenced stomatal resistance of coconut palm leaves. In both the field trials, mixtures of bio agents Pf1+EPC5+Tv recorded the minimum stomatal resistance followed by oxytetracycline hydrochloride (1000 ppm) and Pf1+TDK1+AH. Reduced stomatal resistance was observed in bioagents applied palms to enhance the remission of root (wilt) symptoms. Abnormal permanent stomatal closure may trigger leaf yellowing and senescence. Stomatal resistance decreases early during symptom development and is almost completely suppressed before the onset of leaf yellowing (Maust *et al*. 2003).

Fig 1. Effect of different treatments on nut yield of coconut under field conditions.



Values are mean of three replications

T1- Pf1+Tv (each 150 g + 5 kg FYM/palm), T2- Pf1+TDK1+KH1 (each 100 g + 5 kg FYM/palm), T3- Pf1+EPC5 +Tv (each 100 g + 5 kg FYM/palm),T4- Pf1+PY15+AH1 (each 100 g + 5 kg FYM/palm), T5- 1000 ppm Oxytetracycline hydrochloride @100 ml/palm, T6- Monocrotophos (10 ml+10 ml of water) and T7- Untreated control.

From the above studies, certain compatible PGPR mixtures, which enhanced the plant defense and plant health as major components in integrated disease management systems could be identified. Thus, the study on the management of root (wilt) disease by root feeding of oxytetracycline hydrochloride resulted in remission of phytoplasma symptoms and increased the yield.

The antibiotic does not necessarily kill the phytoplasma but simply reduces or suppresses the concentration in the palm to a level that is no longer harmful to the normal growth. If the root feeding and all the good management practices are stopped, it is possible that concentration of phytoplasma will increase again and all the symptoms may reappear.

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