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Evaluation of biocontrol agents and fungicides against stem bleeding disease of coconut

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With increase in area and intensification of coconut cultivation in many parts of Tamil Nadu, problems due to diseases also increased leading to extensive crop losses. Stem bleeding of coconut is a debilitating disease and is prevalent in all coconut growing regions in the tropics (Garofalo and McMillan, 2004). Stem bleeding disease has also been called as trunk-rot, black scorch, dry basal-rot and heart-rot (Alfieri, 1994). All these names describe symptoms which may or may not be expressed in a given case.

The disease is caused by a fungal pathogen, *Thielaviopsis paradoxa*. The disease has been found to occur in all soil types, but more in laterite soils and sandy soils on the seashore or backwater areas. The disease is characterized by development of dark brown patches appearing at the basal portion of the trunk. A dark reddish brown liquid exudes from the longitudinal growth cracks present on the stem bark and form irregular streaks of exudation. The exudates eventually dry up to form black encrustations with brownish orange margins. The tissues beneath the discolored patch show decay. As the decay progresses, the tissues become black and fibrous. As a result of this, cavities are formed from which liquid comes out, when the bark is pressed. Severe infection may lead to reduced yield and death of young palms. Symptoms also occur on crown region. Bleeding symptoms are severe during rainy season. The bleeding patches spread spirally about half way up the stem and some time reach the crown and cause the death of palms.

T. paradoxa is a soil-borne fungus and it enters the palm through wounds and causes the disintegration of the trunk and/or bud and root-rot. The fungus can also enter through the spear-leaf, young leaf bases, the inflorescence, mechanical damage, growth cracks and leaf pruning cuts. Biological control through the use of antagonistic microorganisms has recently emerged as a viable disease management strategy (Alvindia and Natsuaki, 2008). The main modes of action of the bio-control agent include competition for nutrients (Ugur and Altintas, 2008) and space (Ruano Rosa and Herrera, 2009), production of cell wall degrading enzymes, production of antifungal diffusible and volatile metabolites (Tahia *et al*., 2004) and mycoparasitism. *Trichoderma* spp. are considered to be antagonistic to many plant pathogenic fungi including *Botrytis cinerea*, *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides* (Sivakumar *et al*., 2000) and soil borne fungi including *Rhizoctonia*, *Sclerotinia*, *Pythium* and *Fusarium* (Knudsen and Bac, 2007; Suleman *et al*., 2008). *Trichoderma* species are ubiquitous, relatively easy to isolate, grow quickly on many substrates and produce metabolites with demonstratable antibiotic activity. In recent years, there has been much success in biological control of soil-borne diseases with the use of antagonistic fluorescent pseudomonads (Meena *et al.*, 2001). Hence, the present study was undertaken to manage the stem bleeding disease of coconut using fungicides and antagonistic organisms.

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Isolation of *T. paradoxa*

The stem bleeding pathogen, *T. paradoxa* was isolated from the stem portion of the infected palm where bleeding symptoms were conspicuous. The infected tissues were chiseled out and surface sterilized with 0.1% sodium hypochlorite followed by three washes in sterile distilled water and then the stem bits were placed on potato dextrose agar (PDA) medium. The plates were incubated for five days at 28±2°C and the pathogen was purified, identified and maintained on PDA slants.

In vitro* screening of fungicides against *T. paradoxa

The effect of fungicides in inhibiting the mycelial growth of the pathogen was assessed *in vitro* by poisoned food technique (Nene and Thapliyal, 1982). The fungicides were individually mixed with potato dextrose agar medium. The medium (20 mL) was poured into Petridishes and inoculated with mycelial disc (8 mm diameter). Four replications were maintained for each treatment. The Petridishes were incubated for seven days at room temperature (28±2 °C). Suitable control was also maintained for comparison. The growth was measured after seven days of incubation as colony diameter and the results were expressed as per cent inhibition over control.

In vitro* screening of bioagents against *T. paradoxa

The bioagents were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. *In vitro* inhibition of mycelial growth of *T. paradoxa* by antagonistic organisms was assayed by dual culture technique. The fungal antagonistic cultures were plated and bacterial antagonsists were streaked at one side of Petridishes (1 cm away from the edge) containing PDA medium. Simultaneously, an 8-mm diameter mycelial disc cut from a 7-day-old culture of *T. paradoxa* was placed on the opposite side in the Petridish perpendicular to the bacterial streak (Meena *et al.,* 2001). PDA medium inoculated with the fungus alone served as control. The plates were incubated at room temperature (28±2 °C) for five days. At the end of incubation period, the radial growth of the fungus was measured. Four replications were maintained for each treatment.

Talc formulation of bio-control agents

Talc formulation of bio-control agents *viz., Trichoderma viride, T. harzianum, T. hamatum, Pseudomonas fluorescens* and *Bacillus subtilis* were developed under laboratory conditions by multiplying *Trichoderma* sp. on molasses yeast broth, *P. fluorescens* on King's B broth and *B. subtilis* on nutrient broth, respectively. The cultures were transferred to talc powder (carrier material) at 1:2 ratio along with 1% carboxy methyl cellulose (Vidhyasekaran and Muthamilan, 1995). The formulation (35% moisture content) was packed in polythene bags, sealed and stored at room temperature.

Field evaluation of bio-control agents against stem bleeding disease

Field experiment on the effect of bio-control agents against stem bleeding disease of coconut was laid out in the farmer's field at Subbeagoundanpudur, Pollachi Taluk, Coimbatore district during 2011. There were nine treatments with three replications. Ten palms were maintained for each replication. The cultivar used was West Coast Tall. The experiment was laid out in the randomized block design. The antagonists *viz.*, *T. viride, T. harzianum* and *T. hamatum* were applied as basal at the rate of 100 g per palm per year. Smearing of talc formulation of *Trichoderma* spp. at the rate of 50 g per palm as a paste was done in the bleeding patches two times at 45 days interval. Neem cake was applied basally at the rate of 5 kg per palm per year. Pre-treatment and post-treatment observations were made on the perimeter of the bleeding (exudation) patch and the reduction in the bleeding patch was recorded and the data were statistically analysed.

The results presented in Table 1 clearly indicated that there was significant difference among the systemic fungicides in inhibiting the mycelial growth of *T. paradoxa*. Among the tested fungicides, propiconazole (0.1%) was highly effective in inhibiting the fungus which recorded 86.4 per cent inhibition. In the control, maximum mycelial growth of 8.8 cm was observed (Table 1). Radhakrishnan (1990) reported that tridemorph applied as soil drench was effective in controlling stem bleeding disease of coconut. Thiophanate methyl was found to be effective in curing stem

Table 1. Inhibition of growth of *T. paradoxa* by fungicides

Fungicides	Mycelial growth of the pathogen* (cm)	Per cent inhibition over control
Hexaconazole (0.1%)	1.8	79.5
Propiconazole (0.1%)	1.2	86.4
Tebuconazole (0.1%)	2.1	76.1
Triadimefon (0.1%)	3.4	61.4
Tridemorph (0.1%)	2.1	76.1
Carbendazim (0.1%)	1.5	82.9
Control	8.8	-
CD (P=0.05)	0.6	

* Mean of four replications

Table 2. *In vitro* screening of antagonists against *T. paradoxa*

Antagonists	Mycelial growth of the pathogen* (cm)	Per cent inhibition over control
Trichoderma viride	2.5	71.6
T. harzianum	2.8	68.2
T. hamatum	3.1	64.7
Pseudomonas fluorescens	4.9	44.3
Bacillus subtilis	5.9	32.9
Control	8.8	-
CD (P=0.05)	1.2	

*Mean of four replications

lesions and in preventing seedling infections of *T. paradoxa* (Garofalo and McMillan, 2004). Vijaya *et al*. (2007) found carbendazim (0.1%) and propiconazole (0.1%) were most effective in inhibiting the growth of *Ceratocystis paradoxa* causing sett rot of sugarcane.

In vitro studies revealed that in dual culture technique, *Trichoderma* spp. were found to be effective in inhibiting the radial growth of *T. paradoxa* (Table 2). Among the antagonists screened, *T. viride* was highly effective in inhibiting the pathogen which recorded 71.6 per cent inhibition. This was followed by *T. harzianum* which recorded 68.2 per cent inhibition. *Bacillus subtilis* was the least effective in inhibiting the pathogen (32.9 per cent inhibition over control) (Table 2). The antagonistic effect of *T. viride* and *P. fluorescens* against *C. paradoxa* in sugarcane was observed by Vijaya (2006). Eziashi *et al*. (2006) observed that the growth of *C. paradoxa* causing black seed rot in oil palm sprouted seeds was significantly reduced in the presence of metabolites produced by *T. viride* and *T. polysporum.*

The results presented in Table 3 revealed that smearing of talc formulation of *T. viride* (50 g palm^{-1}) on the bleeding patches was found to be effective in reducing the exudation patch (6.8 cm).

Table 3. Management of stem bleeding disease of coconut

Sl. No.	Treatment	Perimeter of the exudation patch* (cm)		
		Initial	Final	Decrease in exudation
1.	Basal application of Neem cake – 5 kg palm^{-1} year^{-1}	7.8	5.7	2.1
2.	Basal application of *T. viride* (100 g) + Neem cake – 5 kg palm^{-1} year^{-1}	10.1	4.7	5.4
3.	Basal application of *T. harzianum* (100 g) + Neem cake – 5 kg palm^{-1} year^{-1}	8.2	3.1	5.1
4.	Basal application of *T. hamatum* (100 g) + Neem cake – 5 kg palm^{-1} year^{-1}	7.4	3.2	4.2
5.	Smearing of talc formulation of *T. viride* – 50 g per palm on the bleeding patches	9.2	2.4	6.8
6.	Smearing of talc formulation of *T. harzianum* - 50 g per palm on the bleeding patches	7.7	1.6	6.1
7.	Smearing of talc formulation of *T. hamatum* - 50 g per palm on the bleeding patches	9.4	4.3	5.1
8.	Smearing of fungicide – Tridemorph 0.1%	11.3	6.2	5.1
9.	Untreated control	11.6	14.7	-3.1
	CD (P=0.05)			1.8

*Mean of three replications

This was followed by smearing of *T. harzianum* (50 g palm^{-1}) on the bleeding patch which recorded a reduction of 6.1 cm. Basal application of *T. viride* at the rate of 100 g along with neem cake at the rate of 5 kg per palm per year recorded a decrease of 5.4 cm in the exudation of bleeding patch. Basal application of neem cake alone at the rate of 5 kg per palm per year was least effective in reducing the exudation of bleeding patch (2.1 cm). In the untreated control, there was an increase of 3.1 cm in the exudation of bleeding patch. Application of *T. harzianum* successfully decreased the stem rot incidence and increased the growth of groundnut plants (Ganesan *et al.,* 2007). *T. harzianum* reduced the severity of symptoms of black scurf and stem canker of potato caused by *Rhizoctonia solani* (Wilson *et al*., 2008). Ha (2010) reported that *Trichoderma* strains reduced diseases caused by fungal pathogens including *Phytophthora palmivora*, *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii* and *Pythium* spp.

Wijesinghe *et al.* (2010) demonstrated the potential value of using the antagonistic fungus *T. asperellum* to control black rot disease of pineapple (*T. paradoxa)*. Coil formation is a common form of mycoparasitism leading to the death of parasitized fungus (Sivakumar *et al*., 2000). Kubicek and Druzhinina (2007) reported that *Trichoderma* produces cell wall degrading enzymes, which degrade the cell walls of the parasitized fungus. Hence, the results of the present study established the biological management of stem bleeding disease of coconut.

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