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Biological control of stem bleeding disease caused by *Thielaviopsis paradoxa* in coconut

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ABSTRACT

Stem bleeding disease caused by *Thielaviopsis paradoxa* is also one of the important diseases in coconut in Andhra Pradesh. Bio efficacy of native fungal and bacterial bioagents on stem bleeding pathogen *Thielaviopsis paradoxa* under *In vitro* conditions revealed that *Trichoderma asperellum* (Ambajipeta) was significantly superior with 79.17% growth inhibition followed by *T.reesei* (Ambajipeta) with 78.33%. Field evaluation of bio-control agents against stem bleeding disease in coconut indicated that cake application of *T. harzianum* and *T. reesei* formulation completely brought down the disease index by 100 % within 50 days of cake application. Paste application of bioagents viz., *T. harzianum*, *T. reesei* and chemical fungicide copper oxychloride application also substantially reduced the disease.

Key words : *bleeding disease caused, Coconut, Thielaviopsis paradoxa*

Introduction

Coconut palm (*Cocos nucifera*, L.) is an important plantation crop of India and often described as 'Kalpavriksha' because of the multifarious uses of every part of it in the commercial sector. Coconut palms are successfully grown in the tropical countries of the world and are hence referred to as "King of the tropical palms." The South Pacific and South Africa are often cited as possible centre of origin. Coconut provides food, drink, shelter and industrial raw materials. Coconut is grown in almost 94 countries in the world of which 90% of the production comes from Asian and Pacific countries.

Coconut plays a vital role in rural horticulture economy with a total Production of 13411 T tonnes in India and 1112T tonnes as share of 8.27% and ranks 4th in Andhra Pradesh with a area and production (1.15 lakh ha; 1378 million nuts) after Kerala, Karnataka and Tamil Nadu with a productivity of 11957 nuts/ha 2021-22). In Andhra Pradesh, East Godavari (50,490 ha), West Godavari (21, 818 ha), Srikakulam (14,753 ha) and Visakhapatnam (7300 ha) districts occupy major area in forefront in coconut cultivation (NHB, 2021-22).

Stem bleeding of coconut is a debilitating disease and is prevalent in all coconut growing regions in the tropics. The disease was first reported from Srilanka (Petch, 1906) and later reported in India (Sundararaman, 1922) and other countries. The disease is caused by a fungal pathogen, *Thielaviopsis paradoxa* (de Seynes) von Hohnel. The disease has been found to occur in all soil types, but more in laterite soils and sandy soils on the seashore or back-

water areas (Nambiar, 1994). Stem bleeding disease on coconut recorded up to 15% in Andhra Pradesh (Srinivasulu *et al*., 2005). The pathogen is a soil borne pathogen and enters the plant through growth cracks present on the stem and causes cortical decay. 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. These streaks may coalesce and form larger lesions. No oozing is seen from old lesions. The exudates eventually dry up to form black encrustations with brownish orange margins. The tissues beneath the discoloured 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. The outer whorl of leaves becomes yellow rather prematurely, droop and finally dry up. The trunk gradually tapers towards the apex and the crown size is reduced. The bleeding patches spread spirally about half way up the stem and sometime reach the crown and cause the death of palms. In severe cases the bleeding patches reach the crown and kill the palm (Plate No.1, 2&3).

Materials and Methods

Isolation and identification of antagonistic fungi from rhizospheric region of coconut: Soil samples were collected from rhizospheric region of coconut in mandals of Dr.B.R.Ambedkar Konaseema district, Andhra Pradesh. Serial dilution and plate count method was used for isolation of antagonistic fungi. The collected soil samples were subjected to serial dilutions using sterile distilled water and 0.5 ml of each sample at 10–3 and 10–4 dilutions were spread on petridishes containing Trichoderma specific medium (TSM) (Elad and Chet, 1983). Two plates were maintained for each dilution. The plates were then incubated at 28 °C and were examined after four days. Hyphal tip method was adopted for pure culture of organisms. The isolated antagonistic fungi were identified up to the level of genus or species based of growth, color, philides characters on PDA medium.

Isolation and identification of antagonistic bacteria: Samples were serially diluted and 0.1 ml of sample was spread on plates containing King's B medium. The isolate was purified by streaking and was maintained further. Identification of bacterial bioagent was made as per the description and physiological status suggested by Hilderband *et al*. (1992).

***In vitro* antagonism on fungal pathogens of coconut:** Dual cultures of the fungal and bacterial antagonists and the test pathogen were prepared by inoculating PDA discs from the growing margins of fresh fungal cultures on to petri dishes containing PDA (Gams *et al*., 1980) and incubating them. The dual cultures were observed for antibiosis and agar blocks from the regions where the colonies merged were observed for typical interactions under the light microscope. In case of bacterial antagonists, 8 mm mycelia discs of the pathogens were placed individually at the center of the plates and bacterial strain was streaked at three positions 2 cm away from edge of the petri plates with PDA medium and incubated. The mycelia growths of the test pathogens were measured at 48 hrs and subsequently one week after incubation (Nandakumar *et al*., 2000). Mycoparasitism of test pathogen isolates by fungal antagonists was studied using the dual culture technique developed by Dennis and Webster (1971) and described by Sanchez *et al.* (2007). The pathogen growth was measure after 4 ±days of incubation in both the cases at 29.1°c and percent inhibition was calculated by using the formula as given by Vincent (1947).

$$\% \text{ inhibition} = \frac{\{\text{Mean growth in control} - \text{Mean growth in treatment}\}}{\text{Mean growth in control}} \times 100$$

***Trichoderma* coir pith cake [TCPC]:** *Trichoderma reesei* [Ambajipeta] and *Trichoderma harzianum* [Ambajipeta] in were used for the study. The trichoderma coir pith cakes were prepared following the Chandra Mohanan *et al.* (2013) with slight modifications. Trichoderma biomass was prepared using potato dextrose broth. The broth was distributed into 500 ml conical flasks with a quantity of 250 ml and plugged tightly with cotton. This was autoclaved at 15 p.s.i. for 20 minutes, cooled to room temperature and inoculated with 5 mm culture disc of three days old Trichoderma inoculated flasks were incubated for 7 days at room temperature in slanting position so as to get the highest surface area of the medium for fungal growth. Seven days after inoculation, the spent medium with fungal biomass

was blended in an electric mixer for 1-2 minutes to homogenize the contents. This fungal biomass slurry was used for the preparation of coir pith cake formulation. Fine Coir pith after removing fibre from husk was collected from a coir industry. It was mixed with neem cake @ 25% and moistened with water to a moisture level of 70-75 per cent. The substrate was taken in a polypropylene bag sealed with electric sealing machine and were autoclaved at 20 p.s.i for 30 minutes and allowed to cool at room temperature. Maida flour was used as a nutrient-cum-binding material for Trichoderma coir pith cake preparation. For this, maida flour was boiled in water to get a sticky thick paste. 250 g of maida flour was used to mix 250 ml of sterile water to get a thick paste like consistency and it was autoclaved at 20 p.s.i for 30 minutes and allowed to cool at room temperature. The sterilized coir pith neem mixture @1000g, Trichoderma biomass slurry@ 250 ml and sterilised maida paste @250 ml were thoroughly mixed in a plastic tray. This mixture was used for making the product.

To prepare the formulation as a solid cake, 50 g mixture was compressed manually using chakodi maker which was usually used in Indian kitchen for snacks preparation. Such an apparatus with provision for making more cakes at a time is being made locally with 5 cm dia. To prepare a single solid cake, 50 g mixture was used and then it was compressed to get solid cake. The cakes thus prepared were dried in an oven at 38-40 °C for 4 days (provided ventilation for first two days for the moisture to escape). The colony forming units of Trichoderma in the cake was determined after 4 days of drying by dilution plate technique (Pramer and Schmidt, 1956). The dried Trichoderma coir pith cakes (TCPC) were packed in polythene bags and stored at room temperature (26-30 °C). The treatments were arranged in a completely randomized block design with 3-5 replications in field. The data were analyzed statistically.

Talc formulation of *Trichoderma* spp: Talc formulation of native *Trichoderma* spp was prepared. Potato dextrose broth was prepared and sterilized by autoclaving at 15 PSI (121.6 °C) for 15 minutes. Eight mm diameter mycelial discs of antagonist was inoculated and incubated at 28° C for 7 days. The homogenate (1 x 10^8 spores/ml) was mixed with talc powder at 1: 2 ratio along with 0.5% carboxy methyl cellulose and dried in shade, following the method described by Jayarajan *et al.* (1994) with slight modification. The product was used for soil application studies.

Field evaluation of *Trichoderma* formulations under natural conditions

A field experiment was conducted at Dr. YSRHU-HRS, Ambajipeta, Dr. B.R. Ambedkar Konaseema district of Andhra Pradesh by imposing treatments along with untreated control in coconut. The experiment on evaluation of cake formulations of bio agent, Trichoderma was tested against stem bleeding disease of coconut. Effect of Trichoderma cake formulation and paste application along with positive control (paste application of copper oxychloride) was tested against stem bleeding disease of coconut. In case of cake application the treatment was given only once during the study period. In case of paste application, the paste application was carried out every month. Cake formulations were used to place on the slightly scraped portion of the bleeding patches. The bleeding patches are covered with fresh leafs of any available broad leaf and tightening with tread or trash (Plate 7&8). Sprinkling of water was done to moist the cake so as to proliferate the Trichoderma coir pith cake. Talc formulations of Trichoderma and Copper oxy chloride were smeared on the bleeding patches as another treatment for comparison. Bleeding patches of nearly equal size were selected for the palms and perimeter of the bleeding patches was taken into account for judging the degree of disease incidence and one conspicuous bleeding patch was selected for each palm imposing treatments on the stem keeping in view the chances of appearance of more than one bleeding patch on the same stem. Initial perimeter on the bleeding patches was recorded prior to imposing the treatments and subsequent observations were made at monthly intervals. The efficacy of the treatments was determined by comparing the reduction in the perimeter of the bleeding patch after recording the final observations. Every month the treated palms were observed for the disease symptom and the percent recovery of the treated palms were observed. The palms were well managed with regular package of practices. Treatments were imposed along with untreated control in Randomized Block Design with SIX replications.

Results and Discussion

Isolation of coconut pathogens: The disease symp-

tom of stem bleeding caused by *Thielaviopsis paradoxa* is depicted in Plate 1, 2 &3. The infected stem portion of the palm where bleeding symptoms were conspicuous was chiselled out and surface sterilized with 0.1% sodium hypochlorite followed by 3 washes in sterilized distilled water (SDW) and then the stem bits were placed on Potato Dextrose Agar (PDA) media plates for *Thielaviopsis paradoxa*. The plates were then incubated for three days at 29 ± 1°C and the test pathogen was isolated by purification.

In vitro antagonism of microbial bioagents on coconut stem bleeding pathogens

The results indicated in Table 1 on *in vitro* antagonism of biocontrol agents on coconut stem bleeding disease pathogen *Thielaviopsis paradoxa* (Plate.4,5&6) it was observed that inhibition of *Thielaviopsis paradoxa* ranged from 47.22 to 79.17 % reduction over control. It was observed that significantly maximum growth inhibition of *Thielaviopsis paradoxa* were observed with *Trichoderma asperellum* [Ambajipeta] to a percent inhibition of 79.17 followed by *T.reesei* (Ambajipeta), *Pseudomonas fluorescens* (Ambajipeta), *Pseudomonas striata* [Ambajipeta], *Pseudomonas aeruginosa* (Tirupathi), *Bacillus subtillis-1* [Tirupathi], *T.reesei* (Tirupathi) to 78.33%, 68.06%, 68.33%, 52.00%, 49.44% and 47.22% respectively. However, no growth inhibition of *Thielaviopsis paradoxa* was observed with *Bacillus subtillis*-2 (Tirupathi). The results are in corroboration with earlier workers who reported the potential of biocontrol agent against coconut pathogens (Tapwal1 *et al*., 2011). (Plate 4, 5 & 6).

Field evaluation of bio-control agents against Stem bleeding disease in coconut

As per the data in the Table 2 the results of the experiment conducted during 2018-19 and 2019-20, application of *T. harzianum* cake formulation completely brought down the disease index of 6.20 and 7.96 to 0 per cent and reduced the disease by 100 % in respective years within 50 days of cake application. Disease index was reduced to 32.17% and 20.52% in case of paste application of copper oxychloride, in respective years and disease index was reduced to 18.81% and 100 % in case of paste application of *T. reesei* against stem bleeding disease of coconut during 2018-19 & 20119-20 respectively (Plate No.7, 8, 9 &10).

The experiment conducted during the year 2020-21 and 2021-22 with *T. reesei* cake formulation and *T. reesei* paste application against stem bleeding disease of coconut (Table 3), the experiment results revealed that application of *T. reesei* cake formulation disease index of 15.07 % 11.28 was brought down to 0.00 per cent within 50 days of cake application. Whereas paste application of *Trichoderma reesei* as 19.50 and 10.14 was reduced to 1.66 (91.48% reduction over control) and 1.64 (83.82% reduction over control) respectively. In case of copper oxychloride application disease was reduced by 66.93% and 72.53 % over control. However, the treatments differ significantly at 50 DAT.

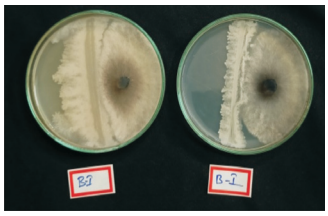
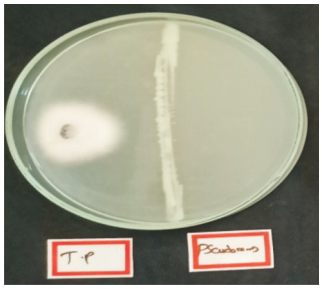
Field evaluation of bio-control agents against Stem bleeding disease in coconut

Trichoderma asperellum produces several groups of

Table 1. ***In vitro*** evaluation of bioagents against *Thielaviopsis paradoxa*

Tr.	*Code*	*Thielaviopsis paradoxa*	% Reduction over control
T1	*Trichoderma reesei –[Ambajipet]*	19.50 (26.18) *	78.33
T2	*T. reesei –[Tirupathi]*	47.50 (43.54)	47.22
T3	*T. harzianum-[Ambajipeta]*	26.25 (30.77)	70.83
T4	*T. asperellum – [Ambajipeta]*	18.75 (25.63)	79.17
T5	*Pseudomonas fluorescens –[Ambajipeta]*	28.75 (32.40)	68.06
T6	*Pseudomonas striata-[Ambajipeta]*	28.50 (32.24)	68.33
T7	*Pseudomonas aeruginosa–[Tirupathi]*	52.00 (46.13)	42.22
T8	*Bacillus subtillis- 1[Tirupati]*	45.50 (42.39)	49.44
T9	*Bacillus subtillis- 2[Tirupati]*	90.00 (71.53)	0.00
T10	Control	90.00 (71.53)	0.00
	Sem±	0.87	
	CD (1%)	2.52	

*Values in the parenthesis are angular transformed values

Sl.No.	Plate	Title
Plate 01		Stem bleeding infection at bottom portion of the stem
Plate 02		Stem bleeding infection at top portion of the stem
Plate 03		Initiation of stem bleeding at bottom potion of stem
Plate 04		Dual culture test *T. Paradoxa* Vs *Bacillus subtillis-I* (Tirupathi) *T. Paradoxa* Vs *Bacillus subtillis-II* Tirupathi)
Plate 05		Dual culture test *T. Paradoxa* Vs *Pseudomonas fluorescens* (Ambajipeta)

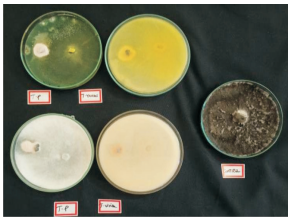
Sl.No.	Plate	Title
Plate 06		Dual culture test *T. Paradoxa* Vs *T. asperellum T. Paradox*a Vs *T. reesei*
Plate 07		Chiselling/scraping of stem bleeding patch
Plate 08		Wrapping with fresh leaf on trichoderma coir pith cake
Plate 09		Paste application of Copper oxy chloride on stem bleeding infected patch
Plate 10		Paste application of *Trichoderma* paste on stem bleeding infected patch

Table 2. Evaluation of *Trichoderma* formulations against stem bl*eeding* disease causing pathogen *Thielaviopsis paradoxa* under natural field conditions

Treatments		* Stem Bleeding Disease Index (PDI)					
		2018-19			2019-20		
		Pre treatment data	3 MAT	Red over. initial	Pre treatment data	3MAT	% Red over. initial
T1.	*T.harzianum* cake application	6.20	0.00	100	7.96(17.21)	0.00*(0.00)	100.00
T2.	*T. reesei* paste application	6.59	5.35	18.81	6.95(15.81)	0.00(0.00)	100.00
T3.	Copper oxychloride paste application	6.90	4.68	32.17	6.82(15.43)	5.42(13.80)	20.52
T4.	Control	8.35	15.61	-	11.70 (21.07)	15.70(23.79)	-
	SEm±	1.12	5.37		2.29	2.52	
	CD@5%	3.33	15.45		**6.76**	**7.46**	

*Values in the parenthesis are angular transformed values

Table 3. Field evaluation of bio-control agents against Stem bleeding disease in coconut 2020-21 and 2021-22

Treatments		* Stem Bleeding Disease Index (PDI)					
		2020-21			2021-22		
		Pre treatment data	3 MAT	Red over. initial	Pre treatment data	3 MAT	%Red over. initial
T1.	*T. reesei* cake application	15.07 (21.35)	0.00 (0.00)	100	11.28 (19.52)*	0.00 (0.00)	100.00
T2.	*T. reesei* paste application	19.50 (25.88)	1.66 (6.39)	91.48	10.14 (18.52)	1.64 (7.31)	83.82
T3.	Copper oxychloride paste application	22.17 (27.97)	7.33 (15.49)	66.93	12.16 (20.58)	3.34 (10.05)	72.53
T4.	Control	15.63 (22.18)	21.16 (27.79)	-	11.09 (19.38)	12.76 (20.87)	-
	SEm±	*NS*	*2.28*		**NS**	**0.83**	
	CD@5%	***NS***	***6.90***		**NS**	**2.52**	

*Values in the parenthesis are angular transformed values

antibiotics, toxins and then the growth of the pathogen is inhibited (Eziashi *et al*., 2010). Also it can inhibit or reduce the growth of the pathogen through competition for space, nutrients or oxygen. Priya *et al*., 2012 reported Pseudomonas fluorescens, a potential inhibitory biocontrol agent against Gnanoderma under in vitro conditions. The inhibition of mycelial growth of the pathogen by *Pseudomonas fluorescens* may be due to the production of antibiotics. Production of antibiotics HCN, pyrrolnitrin, phenazine and 2, 4-diacetyl phloroglucinol and lytic enzymes by *Pseudomonas* fluorescens against fungal pathogens were reported by many workers (Ramamoorthy *et al.*, 2002; Saravanakumar *et al*., 2008).

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