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Evaluation of Botanicals, Bio Agents and Fungicides against Stem Bleeding of Coconut caused by *Thielviopsis paradoxa* under *in vitro* Conditions

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A B S T R A C T

Stem bleeding disease caused by *Thielaviopsis paradoxa* is one of the major diseases of coconut in almost all the coconut growing regions of Karnataaka. Bioefficacy of five botanicals *viz. Allium sativa*, *Nerium olender*, *Tinospora cordifolia*, *Osimum sanctum* and *Aegle marmelos*, Seven isolates namely *Trichoderma viridae* (GKVK), *Trichoderma harzianum* (GKVK), *Trichoderma asperillum* (GKVK), *Trichoderma harzianum* (HRS, NBAII, KRN and MYS) and ten systemic and three contact fungicides were evaluated under *in vitro* conditions against *Thielviopsis paradoxa.* Among the five botanicals evaluated only *Allium sativa* found significantly superior and showed complete inhibition of the growth of the pathogen at 15% and 20% concentration followed by *Osimum sanctum* found effective and has recorded eighty five per cent inhibition at 20% concentration. Among the antagonists tested *Trichoderma viridae (GKVK)* was found superior over all other bio agents by recording maximum inhibition of 78.00 per cent followed by *Trichoderma asperillum (GKVK)* which has recorded 74.00 per cent. Among the thirteen fungicides evaluated standard check Carbendazim 50%WP @ 0.1%, Difenoconazole 25% EC @ 0.1%, Propiconazole 25% EC@0.1%, Tebuconazole 25.9% EC @ 0.15% and Thiophanate Methyl 70%WP @ 0.28% has recorded cent per cent inhibition and showed superior over other fungicides .The Azoxystrobin 23%SC @ 0.1% and Pencycuron 22.9%SC @ 0.1% were recorded least per cent inhibition on 9 DAI.

Introduction

Stem bleeding disease of coconut caused by *Thielviopsis paradoxa* (de seyness) von Hohnel is a debilitating disease and is widely prevalent in all coconut growing areas of the country. The disease was first reported from Sri Lanka (Petch, 1906) and later reported in India (Sundararaman, 1922) and other countries. In India, the disease is prevalent in almost all coconut growing states. The disease has been found to occur in all soil types, but more in laterite soils and sandy soils on seashore or backwater areas (Nambiar, 1994). 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 or reddish brown liquid exudates from the longitudinal cracks present on the stem bark. Subsequently, the liquid dries up and turns black; the tissues below the lesions become water soaked and get discolored. The lesion spread upwards as the disease progresses. In the advanced stages, stem gets tapered and crown size gets reduced leading to gradual reduction in yield. The earliest recommendation for the control of the disease was chiseling of the affected tissues and application of hot coal tar. This did not always results in giving effective control of the disease (Nambier and Kalpana sastry, 1988). Soil drenching with calixin 0.1% (Radhakrishnan, 1990) and root feeding with Bavistin 5% or Calixin 5% (Ramanujam *et al*, 1993), have been found to reduce the disease to some extent. Biological control through the use of antagonistic microorganism and locally available botanicals for the development of integrated management strategy against the disease has emerged as available option (Alvindia and Natsuaki, 2008). *Trichoderma* spp., is considered to be antagonistic to many soil borne and plant pathogenic fungi (Prasad *et al*., 2002): Ramanujam *et al*., 2005 and Ranjana Chakrabarty *et al*., 2013). Soil application of Neem cake to the affected palms was found to reduce the population of *T. paradoxa* and encourage the antagonistic population in soil (Sanal Kumar *et al*., 1990). Locally available botanicals secrete antifungal metabolites that substantiate their action against certain fungi. IDM practices used to develop a combination of specific chemicals with bio-agents and botanicals with an aim to reduce the non-availability of bioagents and botanicals at specific period of time sand which are also environmental friendly. Since the pathogen is soil borne, it is essential to adopt an integrated approach involving antagonistic organisms, fungicides and acqueous plant extract for effective disease management. The present study was carried out to investigate the role of all three components in inhibiting the growth of the fungus in *in vitro* and subsequent formulation of IDM practices against the pathogen.

Materials and Methods

The study was carried out at Horticulture Research Station, Arsikere under All India coordinated research project. The fungus *Thielviopsis paradoxa* was isolated from diseased sample collected from stem bleeding affected coconut palm on potato dextrose agar. The six botanicals viz. *Alliumsativa, Nerium olender, Tinospora cordifolia, Osimum sanctum, Aegle marmelos* and *Azadirecta indica* were conducted at four concentrations by employing food poison technique (Nene, 1971). Fresh plant part materials were collected and washed first in tap water and then in distilled water. Aqueous plant extract was prepared by grinding 100 g fresh samples with 100 ml distilled water (w/v) using a blender and filtrate was collected by passing through double layered muslin cloth. Finally filtrate thus obtained was used as stock solution. The seven bio control agents which were isolated from the soil samples of coconut rhizosphere from the different location i.e. *Trichoderma viridae (GKVK), Trichoderma harzianum (GKVK), Trichoderma asperillum (GKVK), Trichoderma harzianum (HRS), Trichoderma harzianum (NBAII), Trichoderma harzianum (KRN)* and *Trichoderma harzianum (HRS)* were evaluated by using dual culture method. Radial growth of *Thielviopsis paradoxa* was measured at different days after inoculation and the per cent inhibition was calculated. While ten systemic and three contact fungicides namely Azoxystrobin 23%SC@0.1%, Carbendazim50%WP @ 0.1%, Carboxin 37.5 + Thiram 37.5% @DS @ 0.3%, Copper oxy chloride 70%WP @ 0.3%, Difenoconazole 25%EC@0.1%,

Pencycuron 22.9% SC@0.1 %, Propiconazole 25% EC@0.1%, Tebuconazole 25.9% EC @0.15%, Tebuconazole + Trifloxystrobin 75%WG@0.04%, Tetraconazole 3.8%w/w EW @0.1%, Tetraconazole 3.8%w/w EW @ 0.15%, Thiophanate Methyl 70%WP @ 0.28% and standard check Hexaconazole 5% SC@0.1% were evaluated at their recommended concentration using food poison technique. The radial growth of the test fungal colony recorded on 10th day when maximum growth was observed in untreated control plates. The percent inhibition of the mycelia growth over control was calculated using formula given by Vincent (1942).

$$I = \frac{C - T}{C} \times 100$$

I = % inhibition of mycelia growth
C= radial growth of fungus in control
T = radial growth of fungus in treatment

Results and Discussion

Influence of botanicals on mycelial growth of *T. paradoxa* was presented in Table 1.

The results clearly reveal that among the six botanicals evaluated only *Allium sativa* found significantly superior and showed complete inhibition of the growth of the pathogen at 15% and 20% concentration followed by Osimum *sanctum* found effective in inhibiting the growth of *Thielviopsis paradoxa* at same concentration and they have recorded 65 - 79 per cent inhibition (Table 1).

Seven bio control agents which were collected from the different locations were tested against *Thielviopsis paradoxa* by dual culture method. The results revealed that, all the antagonists significantly reduced the growth of *Thielviopsis paradoxa* by recording mycelial inhibition ranging from 61.33 to 78.00 per cent (Table 2).

Among the antagonists tested *Trichoderma viridae (GKVK)* was found superior over all other bio agents by recording maximum inhibition of 78.00 per cent followed by *Trichoderma asperillum (GKVK)* which has recorded 74.00 per cent. Least inhibition of 61.33 per cent was recorded in *Trichoderma harzianum (KRN).*

Experiment on *in vitro* evaluation of ten systemic and three contact fungicides were carried out against *Thielviopsis paradoxa* (Table 3) and it was noticed that among the thirteen fungicides evaluated Among the thirteen fungicides evaluated standard check Carbendazim 50% WP @0.1%, Difenoconazole 25% EC@0.1%, Propiconazole 25% EC@0.1%, Tebuconazole 25.9% EC@0.15% and Thiophanate Methyl 70%WP @ 0.28% has recorded cent percent inhibition and showed superior over other fungicides .The Azoxystrobin 23%SC@0.1% and Pencycuron 22.9%SC @ 0.1% were recorded least per cent inhibition on 9 DAI..

The results in conformity with Ramanujam *et al*., 2005 and Ranjan Chakrabarty *et al*., 2013 where *Allium sativum* extract completely inhibited the growth of the pathogen.

Trichoderma viridae was found to be most effective with 61.62% inhibition followed by *T. harzianum* and *T. virens* with 60.80 and 59.49 per cent inhibition respectively, over control after 96h of incubation period.

The fungicides, Calixin (Tridemorph 80EC) @ 0.3% showed 100 per cent inhibition over control whereas Ridomil MZ-72, Blitox-50 and Bavistin showed 92.00, 91.55 and 91.44 per cent inhibition over control after 144h of incubation respectively.

Table.1 *In vitro* evaluation of different botanicals against stem bleeding of coconut caused by *Thielviopsis paradoxa*

Trt No.	Treatment	Part used	% Inhibition of *Thielviopsis paradoxa*											
			Concentration											
			5%			10%			15%			20%		
			3DAI	6DAI	9DAI	3DAI	6DAI	9DAI	3DAI	6DAI	9DAI	3DAI	6DAI	9DAI
T1	*Allium sativa*	Clove	77.63 (61.77)	70.94 (57.40)	73.33 (58.91)	80.48 (63.78)	79.81 (63.30)	81.56 (64.58)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T2	*Tinospora cordifolia*	Leaf	2.85 (8.58)	5.77 (13.77)	0.00 (0.00)	1.90 (7.10)	18.19 (25.75)	18.44 (25.43)	0.49 (1.80)	20.48 (26.90)	29.78 (33.05)	2.94 (8.72)	21.13 (27.29)	0.00 (0.00)
T3	*Nerium olender*	Leaf	3.33 (10.37)	6.75 (14.68)	1.56 (4.44)	24.29 (29.51)	21.43 (27.57)	19.78 (26.39)	27.10 (31.35)	23.27 (28.83)	10.67 (19.06)	27.57 (31.64)	25.14 (30.06)	21.42 (19.82)
T4	*Osimum sanctum*	Leaf	48.08 (43.90)	33.71 (35.46)	4.22 (11.74)	58.10 (49.67)	57.87 (49.53)	49.11 (44.49)	74.88 (59.92)	75.47 (60.32)	76.00 (60.67)	77.34 (61.58)	77.90 (61.97)	85.06 (63.92)
T5	*Aegle marmelos*	Leaf	3.80 (11.10)	12.54 (20.51)	0.00 (0.00)	5.24 (10.89)	25.43 (30.26)	11.78 (20.02)	13.28 (21.32)	24.49 (29.64)	1.78 (7.45)	13.79 (21.76)	23.00 (28.65)	18.43 (18.84)
T6	*Azadirecta indica*	Leaf	48.09 (43.91)	39.56 (38.97)	11.33 (19.62)	49.05 (44.45)	43.74 (41.40)	12.89 (12.22)	59.10 (50.25)	66.45 (54.61)	65.56 (54.07)	69.43 (56.46)	66.86 (54.86)	60.37 (60.07)
SEm±			1.17	1.36	1.28	1.82	0.51	0.56	0.91	0.37	0.57	1.10	0.60	0.36
CD @ 1%			4.70	5.47	5.13	7.30	2.03	2.24	3.66	1.49	2.28	4.40	2.43	1.45

Table.2 *In vitro* evaluation of Bio control agents against stem bleeding of coconut caused by *Thielviopsis paradoxa*

Treatment No.	Bio control agents	% Inhibition of *Thielviopsis paradoxa*		
		3DAI	6DAI	9DAI
T1	*Trichoderma viridae(GKVK)*	39.28 (38.79)	73.96 (59.33)	78.00 (62.03)
T2	*Trichoderma harzianum (GKVK)*	24.12 (29.24)	73.67 (59.14)	64.67 (53.53)
T3	*Trichoderma asperillum(GKVK)*	22.16 (28.00)	70.51 (57.11)	74.00 (59.36)
T4	*Trichoderma harzianum (HRS)*	23.16 (28.69)	75.47 (60.32)	72.89 (58.62)
T5	*Trichoderma harzianum (NBAII)*	22.21 (27.95)	61.97 (51.94)	67.56 (55.28)
T6	*Trichoderma harzianum (KRN)*	19.21 (25.82)	58.19 (49.72)	61.33 (51.55)
T7	*Trichoderma harzianum (MYS)*	23.98 (29.29)	64.05 (53.16)	70.22 (56.96)
	SEm±	0.73	0.33	0.43
	CD(P=0.01)	11.56	2.93	3.83

Table.3 *In vitro* evaluation of fungicides against stem bleeding of coconut caused by *Thielviopsis paradoxa*

Treatment No.	Fungicide	Dosage (%)	% Inhibition of *T. paradoxa*		
			3DAI	6DAI	9DAI
T1	Azosystrobin 23%SC	0.1	35.73 (36.60)	4.00 (8.98)	1.56 (6.21)
T2	Carbendazim50%WP	0.1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T3	Carboxin 37.5 + Thiram 37.5%DS	0.3	100.00 (90.00)	100.00 (90.00)	85.37 (67.53)
T4	Copper oxy chloride50%WP	0.3	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Difenoconazole 25%EC	0.1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6	Hexaconazole 5%SC	0.1	100.00 (90.00)	78.13 (62.13)	67.37 (55.17)
T7	Pencycuron 22.9%SC	0.1	32.80 (34.93)	0.00 (0.00)	1.82 (7.52)
T8	Propiconazole 25%EC	0.1	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T9	Tebuconazole 25.9%EC	0.15	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T10	Tebuconazole+ Trifloxystrobin75%WG	0.04	77.76 (61.89)	82.13 (65.00)	81.18 (64.32)
T11	Thiophanate Methyl 70%WP	0.28	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T12	Tetraconazole3.8% w/w(4%w/v)EW	0.1	100.00 (90.00)	74.40 (59.61)	63.46 (52.81)
T13	Tetraconazole3.8% w/w(4%w/v)EW	0.15	100.00 (90.00)	76.00 (60.69)	67.10 (55.01)
	SEm±		0.67	1.07	0.63
	CD(P=0.01)		5.08	8.18	4.77

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