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EFFECT OF FUNGICIDES, *TRICHODERMA* AND PLANT EXTRACTS ON MYCELIAL GROWTH OF *THIELAVIOPSIS PARADOXA*, UNDER *IN VITRO* CONDITION

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ABSTRACT
Stem bleeding disease caused by *Thielaviopsis paradoxa* is one of the major diseases of coconut and is prevalent in almost all the coconut growing countries. The present study was carried out to test the efficacy of antagonist, fungicides and plant extracts in inhibiting the pathogen, under *in vitro* condition. *Thielaviopsis* was isolated from young lesion of affected coconut palm of CPCRI, RC, Kahikuchi, Guwahati. Among the three species of *Trichoderma* tested so far, *T. viride* was found to be the 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, Among the forty five plant extracts tested, the aqueous extract (10%) of *Allium sativum,* exerted 100 per cent inhibition of the pathogen over control after 48h, 72h and 96h of incubation compared to other extracts.

INTRODUCTION

Stem bleeding is the most common and well known disease of coconut and is prevalent in almost all the coconut growing countries. It was first reported (Petch, 1906) from Sri Lanka and later from India and other countries. In India, the disease is prevalent in almost all coconut growing states. Survey conducted in different parts of Assam revealed that the per cent disease incidence ranged from 1-16% with maximum incidence in Kamrup district. The consensus of opinion is that *Ceratostomella paradoxa* (de seynes) Dade, or *Thielaviopsis paradoxa* (de Seynes) von Hohnel (the imperfect stage of the fungus), is involved in the disease. Petch (1906) observed that *Thielaviopsis paradoxa* is a weak wound pathogen associated with the disease. The causal organism, *Thielaviopsis paradoxa* is a soil borne pathogen. Infection usually occurs at the basal portion of the trunk and starts with the exudation of dark reddish liquid from the longitudinal cracks in the 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 advance stages, production of bunch is affected, nut shedding takes place. Presence of growth cracks on trunk and severe summer followed by water stagnation, imbalanced nutrition act as predisposing and disease aggravating. Control of the disease through phytosanitation and application of hot coal tar did not offer satisfactory control (Nambiar and Sastry, 1988).

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 a viable option (Alvindia and Natsuaki, 2008). *Trichoderma* spp. is considered to be antagonistic to many soils borne and plant pathogenic fungi (Prasad *et al.,* 2002; Ramanujam *et al*., 2005 and Suleman *et al*., 2008). Locally available botanicals secrete anti-fungal 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 bio-agents and botanicals at specific period of times and which are also environment-friendly. Since the pathogen is soil borne, it is essential to adopt an integrated approach involving antagonistic organisms, fungicides and aqueous 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 vitro* and subsequent formulation of IDM practices against the pathogen.

MATERIALS AND METHODS

The fungus, *T. paradoxa* was isolated from diseased sample collected from stem bleeding affected coconut palm on potato dextrose agar. Bio agents, *Trichoderma harzianum, T. viride* and *T. virens*; six fungicides and aqueous extract of forty five locally available plant species were evaluated *in vitro* for their inhibitory efficacy against *T. paradoxa. Trichoderma* spp. which

was reported as antagonistic to a number of plant pathogens were isolated from the rhizosphere of coconut palms of CPCRI, RC, Kahikuchi farm, Guwahati on *Trichoderma* specific medium with captan (TSMC) (Elad and Chet, 1983). These isolates were purified and tested for their antagonism against the pathogen.

***In vitro* assay of antagonists**

In vitro antagonism of the three species of *Trichoderma* against *T. paradoxa* was tested by dual culture technique on PDA medium (Dhingra and Sinclair, 1985). A Petri plate inoculated only with *T. paradoxa* served as control. All the plates were incubated at room temperature (28 ± 2ºC). Each experiment was replicated three times. Observation on mycelial growth of the pathogen was recorded upto 96h of incubation. The per cent inhibition over control was calculated.

***In vitro* assay of fungicides**

The comparative toxicity of fungicides on the growth of the fungus under *in vitro* condition was evaluated by poisoned food technique (Nene, 1971). Fungicides *viz*., Bavistin (Carbendazim 50% WP), Blitox-50W (Copper Oxychloride 50% WP), Calixin (Tridemorph 80 EC), Captaf (Captan 50%WP), Dithane M-45 (Mancozeb), and Ridomil MZ-72 @ 0.3% were used for *in vitro* assay. Stock solutions of the fungicides were prepared in sterile distilled water and added aseptically to sterilized PDA medium to get the required concentrations and then poured into petriplates. The plates prepared without any fungicides served as control. These plates were inoculated with 7 mm disc of four day old culture of the test fungus and incubated at 28 ± 2ºC for 6 days.

***In-vitro* assay of botanicals**

Forty five locally available botanicals were tested for their antifungal property against *T. paradoxa* by poisoned food technique (Nene, 1971) under *in vitro* condition. Fresh leaves of test plants were taken for preparing crude extracts. The leaves were thoroughly washed with water and fine slurry was prepared by taking 100g leaves with 100 mL of distilled water. The resultant slurry was filtered through muslin cloth and then through Whatman No.1 filter paper and the extracts were used as stock solution. From the stock solution, 10mL was added with 90mL of medium to make 10% concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty mL of medium was poured into sterile Petri plates. Mycelium of seven mm size discs from periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 28 ± 1ºC for 96h and radial growth was measured. The efficacy of plant products or botanicals was expressed as percent of radial growth over the control, which was calculated by using the formula (Vincent, 1947).

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

Where,

I = Percent inhibition over control

C = Radial growth in control

T = Radial growth in treatment

The values obtained in different categories are transformed, wherever necessary and subjected to statistical analysis (Panse and Sukhatme, 1995) for treatment comparison.

RESULTS AND DISCUSSION

Effect of bio-agents on mycelial growth of *T. paradoxa*

Antagonistic fungi *viz., Trichoderma harzianum* (AF1), *T. viride* (AF2) and *T. virens* (AF3) were isolated from the soil samples of coconut rhizosphere. The identification was confirmed according to the identification key (Rifai, 1969) based on the branching of conidiophores, shape of phialides, emergence of phialospores and shape of phialospores. The three fungal cultures isolated from soil were found to have inhibitory effect on the mycelial growth of the pathogen (Table 1) and data showed that degree of inhibition was maximum with *T. viride* (61.62%), followed by *T. harzianum (*60.80*%)* and *T. virens* (59.49%) after 96 h of incubation. No significant difference was observed between *T. viride* and *T. harzianum* for percent inhibition after 96h. However, both these bioagents showed significant result when compared to *T. virens*. Gunasekaran *et al*. (1986) and Bhaskaran (1990) reported that *T. viride* and *T. harzianum* are potentially antagonistic to *Ganoderma lucidum* and can be successfully employed in the bio-control of basal stem rot disease. *Trichoderma virens* (Uduma isolates), *T. harzianum* (Kallangai isolate), *T. viride* (IISR, Calicut isolate)

Table1: Inhibition of mycelial growth of *T. paradoxa* by bio-agents

Bio-agent	Per cent inhibition over control		
	48 h	72 h	96 h
T.viride	35.37	63.91	61.62
T. harzianum	32.89	63.08	60.80
T.virens	32.33	57.77	59.49
C.D. (0.05)	1.05	0.68	0.69
CV (%)	2.27	0.80	0.83

Table 2: Efficacy of fungicides against *Thielaviopsis paradoxa*

S. No.	Fungicides	Per cent inhibition over control		
		48h	96h	144h
1	Bavistin@0.3%	86.95 (9.32)	91.44 (9.56)	91.44 (9.56)
2	Blitox 50@0.3%	100 (10.00)	100 (10.00)	91.55 (9.57)
3	Calixin@0.3%	100 (10.00)	100 (10.00)	100 (10.00)
4	Captaf@0.3%	86.78 (9.31)	91.33 (9.70)	89.67 (9.47)
5	Ridomil@0.3%	100 (10.00)	100 (10.00)	92.00 (9.59)
6	Mancozeb@0.3%	84.24 (9.18)	85.89 (9.27)	66.67 (8.16)
CD (0.05)		0.013	0.067	0.050
CV (%)		0.08	0.39	0.30

*Figure in the parenthesis is transformed value.

Table 3: Efficacy of aqueous extract of botanicals on mycelial growth of *Thielaviopsis paradoxa*, causal organism of stem bleeding of coconut

Family		Botanicals	Per cent inhibition over control		
			48 h	72 h	96 h
Fam: Amaryllidaceae					
	1	*Allium cepa*	45.81 (42.60)	35.78 (36.76)	0.00 (0.25)
	2	*Allium sativum**	100.00 (89.76)	100.00 (89.76)	100.00 (89.76)
Fam: Apocynaceae					
	3	*Calotropis sp.*	0.00 (0.25)	0.00 (0.25)	0.00 (0.25)
	4	*Rauwolfia tetraphyla*	1.86 (8.50)	0.00 (0.25)	0.00 (0.25)
Fam: Araceae					
	5	*Typhonium trilobatum*	0.67 (4.58)	0.00 (0.25)	0.00 (0.25)
Fam: Asteraceae					
	6	*Ageratum houstonianum*	8.37 (16.73)	0.00 (0.25)	0.00 (0.25)
	7	*Bidens pilosa*	0.19 (5.24)	0.00 (0.25)	0.00 (0.25)
	8	*Carthamus oxyacantha*	1.67 (6.14)	0.00 (0.25)	0.00 (0.25)
	9	*Carthamus roseus*	0.00 (0.25)	0.00 (0.25)	0.00 (0.25)
	10	*Stevia*	0.37 (4.14)	0.00 (0.25)	0.00 (0.25)
	11	*Tagetes erecta*	2.23 (9.24)	0.00 (0.25)	0.00 (0.25)
Fam: Brassicaceae					
	12	*Cleome viscose*	65.36 (54.16)	73.11 (36.82)	56.11 (48.53)
Fam: Caeselpinaceae					
	13	*Cassia tora*	14.15 (22.07)	0.00 (0.25)	0.00 (0.25)
Fam: Cupressaceae					
	14	*Thuja occidentalis*	1.67 (5.43)	0.00 (0.25)	0.00 (0.25)
Fam: Dryopteridaceae					
	15	*Dryopteris flix-mas*	0.00 (0.25)	0.00 (0.25)	0.00 (0.25)
Fam: Euphorbiaceae					
	16	*Ricinus communis*	45.06 (42.13)	49.74 (45.01))	0.00 (0.25)
Fam: Fabaceae					
	17	*Crotolaria juncea*	20.66 (22.46)	0.00 (0.25)	0.00 (0.25)
	18	*Dalbargia sisso*	0.00 (0.25)	0.00 (0.25)	0.00 (0.25)
	19	*Gliricidia sepium*	48.79 (44.29)	53.67 (47.08)	26.44 (30.70)
	20	*Pongamia pinnata*	49.72 (44.88)	36.89 (37.40)	0.00 (0.25)
	21	*Cassia fistula*	56.05 (48.64)	35.22 (36.40)	22.55 (28.33)
Fam: Gramineae					
	22	*Vetiveria zizanioides*	1.86 (7.3)	0.00 (0.25)	0.00 (0.25)
Fam: Lamiaceae					
	23	*Leucas aspera*	1.11 (6.58)	0.00 (0.25)	0.00 (0.25)
Fam: Lauraceae					
	24	*Cinnamon zelyanicum*	18.06 (25.1)	0.00 (0.25)	0.00 (0.25)
Fam: Lythraceae					
	25	*Lawsonia inermes*	46.74 (43.38)	35.22 (36.38))	0.00 (0.25)
Fam: Melastomataceae					
	26	*Melastoma malabathricum*	1.30 (6.58)	0.00 (0.25)	0.00 (0.25)
Fam: *Meliaceae*					
	27	*Azadirachta indica*	38.36 (38.20)	23.22 (28.83)	0.00 (0.25)
Fam: Myrtaceae					
	28	*Psidium guajava*	79.14 (62.92)	70.41 (57.23)	50.96 (45.55)
	29	*Syzigium cumini*	2.23 (66.8)	0.00 (0.25)	0.00 (0.25)
Fam: Oxalidaceae					
	30	*Oxalis corniculata*	80.45 (63.80)	74.00 (59.34)	46.55 (42.85)
Fam: Piperaceae					
	31	*Piper longum*	0.37 (3.43)	0.00 (0.25)	0.00 (0.25)
	32	*Piper nigrum*	2.05 (7.9)	0.00 (0.25)	0.00 (0.25)
Fam: Rubiaceae					
	33	*Coffea arabica* (seeds)	0.56 (5.48)	0.00 (0.25)	0.00 (0.25)
Fam: *Rutaceae*					
	34	*Aegle marmelos (lvs)*	65.92 (54.28)	58.55 (49.92)	55.44 (48.10)
	35	*Aegle marmelos* **	58.66 (51.00)	35.11 (36.33)	18.67 (25.60)
	36	*Citrus limon*	8.37 (16.92)	0.00 (0.25)	0.00 (0.25)
Fam: Solanaceae					
	37	*Datura stramonium*	22.57 (24.87)	0.00 (0.25)	0.00 (0.25)
	38	*Solanum khasianum*	0.56 (4.14)	0.00 (0.25)	0.00 (0.25)
	39	*Solanum nigrum*	84.37 (66.80)	78.89 (63.20)	75.55 (60.75)
Fam: Verbenaceae					
	40	*Clerodendron infortunatum*	37.43 (37.68)	35.55 (36.82)	0.00 (0.25)

Table 3: Cont.....

	41	*Clerodendron inerme*	0.00 (0.25)	0.00 (0.25)	0.00 (0.25)
	42	*Lantana cámara*	57.73 (48.18)	34.00 (35.67)	0.00 (0.25)
	43	*Lippia geminata*	0.93 (5.27)	0.00 (0.25)	0.00 (0.25)
Fam: Zingiberaceae					
	44	*Curcuma longa*	44.69 (37.68)	35.00 (36.29)	0.00 (0.25)
	45	*Zingiber officinale*	1.67 (6.69)	0.00 (0.25)	0.00 (0.25)
		CD(0.05)	0.42	0.33	0.16
		CD(0.01)	0.55	0.44	0.21
		CV%	0.96	1.11	1.03

*Figure in the parenthesis is transformed value

and *T. hamatum* (ITCC, New Delhi isolate) were identified as potential antagonists of *T. paradoxa*, the pathogen of stem bleeding disease of coconut, based on *in vitro* screening of several fungi (Ramanujam *et al*., 2000)

Effect of fungicides on mycelial growth of *T. paradoxa*

The effect of fungicides on the growth of the fungus is presented in Table 2. Among the tested fungicides, Calixin @ 0.3% showed 100 per cent inhibition over control and showed significant results as compared to other fungicides tried against the fungus. The other fungicides *viz*., Ridomil MZ-72, Blitox-50, Bavistin, Captaf and Mancozeb @ 0.3% recorded 92.00, 91.55, 91.44, 89.67 and 66.67 per cent inhibition, respectively, over control after 144h of incubation. Ramanujan *et al*., 2005 reported strong inhibitory effect of different fungicides including Tridemorph against the fungus, *Thielaviopsis paradoxa* at very low concentration.

Effect of botanicals on mycelial growth of *T. paradoxa*

Influence of botanicals on mycelial growth of *T. paradoxa* is presented in Table 3. Results revealed that among the forty five plant extracts tested, *Allium sativum* extracts completely inhibited the growth of the pathogen, followed by *Solanum nigrum* and *Cleome viscosa* with 75.55 and 56.11 per cent inhibition over control, respectively. There is significant difference among *A. sativum, S. nigrum and C. viscosa*. Gowda and Nambiar (2006) reported that garlic extract at 5% concentration was found to be fungitoxic to the pathogen of stem bleeding disease of coconut.

The compatibility study of the antagonistic microorganism with plant extracts showed that all extracts are found to be compatible except the extract of *Allium sativum*. Iyer *et al.,* 2004 reported that *Allium sativum* extract completely inhibited the growth of the pathogen and also growth of antagonistic micro organism.

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