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IDENTIFICATION OF POTENTIAL ANTAGONISTIC FUNGI FOR THE BIOCONTROL OF *THIELAVIOPSIS PARADOXA* CAUSING STEM BLEEDING DISEASE OF COCONUT

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Thirty two isolates of fungi belonging to 13 species were evaluated for their antagonistic effect on *Thielaviopsis paradoxa* by dual culture and antibiosis tests. Based on the percentage of inhibition, *Gliocladium virens*, *Trichoderma harzianum*, *T. viride and T. hamatum* were identified as potential antagonists of *T. paradoxa*. *G. virens* (Uduma), *T. harzianum* (Kallangai), *T. viride* (Calicut) and *T. hamatum* (Delhi) showed maximum inhibition of *T. paradoxa* in dual culture and in antibiosis tests. These four antagonists were tested on two other isolates of *T. paradoxa* (T.p-2 and T.p-3) as well. *T. paradoxa* lesion development on coconut petioles was inhibited by G. *virens*, *T. harzianum*, *T. viride* and *T. hamatum* by 69.9, 66.9, 63.4 and 57.7 per cent, respectively.

INTRODUCTION

Stem bleeding disease caused by the soil borne fungus, *Thielaviopsis paradoxa* (de Seynes) von Hohnel is a debilitating disease prevalent in almost all coconut growing areas of the country imparting considerable economic loss. The fungus infects through the growth cracks or wounds that occur on the stem near the ground level and causes extensive bleeding patches. The affected palm shows gradual decline in yield. Control of the disease through phytosanitation and application of hot coal tar and disease management using systemic fungicides like tridemorph (Calixin) and carbendazim (Bavistin) have been reported with limited success (Radhakrishnan, 1990; Ramanujam *et al.*, 1993). Since the pathogen is soil borne, it is essential to adopt an integrated approach involving antagonistic organisms along with other control measures for effective disease management. The present investigations were taken up to screen a wide variety of fungi *in vitro* and *in vivo* for identification of potential antagonistic fungi effective against T. *paradoxa* for use in integrated disease management.

MATERIALS AND METHODS

Ten local isolates belonging to *Trichoderma harzianum* and *Gliocladium virens* were isolated from the soils, barks of healthy- and disease affected palms from five locations viz., Kudlu, Kallangai, Neerchal, Uduma and Edaneer in Kasaragod District of Kerala State. Twenty two exotic fungal isolates were obtained from biocontrol laboratories, Tamil Nadu Agricultural University, Coimbatore, Indian

Institute of Spices Research, Kozhikode, G. B. Pant University of Agriculture and Technology, Pantnagar (U. P) and IARI Type Collection Centre, New Delhi. Evaluation of 32 test fungi for their antagonistic effect on a virulent isolate of *T. paradoxa,* (T.p-1 isolate from Kasaragod) was carried out by dual culture and antibiosis tests.

Direct opposition method using simultaneous inoculation of pathogen and opposing fungus as described by Webber and Hedger (1986) was adopted. Growth of opposing fungus on *T. paradoxa* colony if any was recorded. After five days, attempts were made to reisolate *T. paradoxa* (viability test) by transferring discs cut from the interaction sites to fresh malt agar plates and examining the renewed growth. Based on the observations in dual cultures, the type of interaction occurring between *T. paradoxa* and the opposing fungus was identified using the key developed by Webber and Hedger (1986). Antibiosis test was carried out using cellophane paper method described by Dennis and Webster (1971a).

Antibiosis test for production of volatile inhibitory metabolites was carried out by the technique described by Dennis and Webster (1971 b) with modification. Two petri dish bases containing 20 ml of two per cent malt agar medium were sealed together with an adhesive tape. Prior to sealing the test fungus was inoculated on the medium of bottom plate while the upper plate containing medium was allowed to absorb volatiles produced by test fugus, if any. These were incubated at 25±2 ^{0} C up to ten days. The upper malt agar plates were carefully removed after the required exposure with the test fungus and *T. paradoxa* cultured on these plates.

Three replications were used in each case and plates were incubated at 25±2^{0}C. In all cases, radial growth of *T. paradoxa* was recorded after 48 h. and compared with the growth in control plates. Based on the observations, the percentage inhibition of T. *paradoxa* was calculated. Where complete inhibition (100%) of *T. paradoxa* occurred due to diffusible metabolites, the fungicidal/fungistatic effect of these metabolites was determined by transferring *T. paradoxa* inoculum discs to fresh two per cent malt agar plates and examining the renewed growth. Potential antagonistic fungi effective against T. *paradoxa* were identified on the basis of percentage inhibition, observed in dual culture/ antibiosis tests. The selected antagonistic fungi were further evaluated on two other isolates of *T. paradoxa* viz., T.p-2 (Adoor isolate) and T.p -3 (Kasaragod-II isolate).

Dual culture studies were carried out to find out any mutual inhibitory interactions occurring among the selected antagonistic fungi. This would help in identifying the compatible antagonistic fungi which could be mixed together in the biocontrol studies.

Efficacy of the selected antagonistic fungi was further evaluated *in vivo* using detached petiole inoculation method described by Usman (1988). Fresh healthy coconut leaf petioles from the lower whorl of WCT palms were cut into bits of 30 cm length and washed with sterile water. Two boreholes (0.5 x 0.5 cm) at a distance of 3.5 cm from each other were made on the center of the frond bit on the dorsal surface with a sterilized cork borer. Seven day old *T. paradoxa* and antagonistic fungi grown on coconut rachis were inserted in these holes separately and covered with wet cotton pads.

Controls were maintained by inoculating with *T. paradoxa alone.* Five replications were used in each case and the inoculated petiole bits were covered with polythene bags and incubated at 25 +2^{0}C for15 days. After 15 days, the petiole bits were split opened and the lesion size (lesion area in cm) was recorded and the lesion area of *T. paradoxa* inoculated along with antagonist was compared with control. The percentage reduction in the lesion area, if any, was calculated.

Table 1. Antagonistic effect of Trichoderma sp. and *G. virens* on *T. paradoxa*

Antagonistic fungi and its origin	Dual culture test Per cent Inhibition	Over growth	Antibiosis test Per cent Inhibition
T. harzianum			
Kallangai *	69.8	52.3	78.6
Kudlu*	66.0	25.3	70.6
Neerchal*	65.5	25.0	70.0
Uduma-Bark*	63.6	22.0	62.7
Ednaeer-Bark*	62.3	21.0	64.3
Kallangai-Bark*	61.7	10.7	64.3
Calicut	64.7	25.5	59.2
Coimbatore	64.2	25.3	63.1
Pantnagar	60.4	25.5	55.6
Delhi	59.8	21.7	43.7
Mean	**63.8**	**22.7**	**63.2**
T. viride			
Calicut	67.9	17.0	73.5
Coimbatore	65.5	15.0	63.1
Pantnagar	61.7	16.0	61.9
Delhi	47.2	9.0	40.5
Mean	**60.6**	**14.3**	**59.7**
T. hamatum			
Calicut	62.3	34.0	62.3
Delhi	69.8	35.0	64.2
Mean	**66.0**	**34.5**	**63.3**
T. koningii - Delhi	56.0	22.0	42.0
T. aureoeviriede			
Delhi	54.7	24.3	43.2
T. pesudokoningii			
Delhi	53.4	22.7	40.1
T. longibrachiatum			
Delhi	49.6	20.7	39.6
Mean	**53.5**	**22.4**	**41.2**
G. virens			
Uduma*	78.7	17.0	100.0
Kallangai*	78.1	7.0	100.0
Kudlu-Bark*	74.9	14.3	100.0
Neerchai-Bar*	71.7	16.0	100.0
Calicut	69.8	14.7	100.0
Pantnaga	67.9	17.0	72.6
Delhi	67.9	16.0	100.0
Mean	**72.7**	**14.6**	**96.1**
C.D. at P = 0.05:	2.46	0.89	5.14

* Local isolates

RESULTS AND DISCUSSION

In the dual culture test, isolates belonging to *Trichoderma* sp. and *G. virens* showed inhibitory effect on *T. paradoxa* (Table 1). Among the test isolates studied, *G.virens, T. harzianum, T. hamatum* and *T.viride* were identified as potential antagonists based on their inhibitory effect on *T. paradoxa.* On the other hand, mutual inhibitions were observed between *T. paradoxa* and other species like Chaetomium *globosum, Myrothecium verrucaria, Fusrium lateritium, Trichothecium roseum* and Cladosporium *cladosporiodes* (Table 2) and hence these species were found unsuitable against *T. paradoxa*, as an antogonist. Among the test isolates collected from different places, *G. virens* (Uduma), *T. harzianum* (Kallangai), *T. viride* (Calicut) and *T. hamatum* (Delhi) showed maximum inhibition on *T. paradoxa.* Inhibitory effect of *T. harzianum* and *T. viride* used in the present study was comparable with the earlier report (Gowda, 1987).

Growth of *T. paradoxa* towards *Trichoderma* sp./*G. virens* stopped completely within 48 h and thereafter these antagonists grew over the *T. paradoxa* colonies up to 7.0 to 35.0 mm. This indicates the existence of overgrowth category of interaction between *T. paradoxa* and *Trichoderma* sp./ *G. virens.* Webber and Hedger (1986) reported overgrowth category of interaction between *Caratocystis ulmi* and *G. roseum, T. viride* and *T. polysporum.*

Table 2 . *In vitro* antagonistic effect of the isolates of other fungi on *T. paradoxa*

Test isolate and its origin	Dual culture test Per cent Inhibition	Over growth	Antibiosis test Per cent Inhibition
C. globusum - Delhi	77.4	25.0	85.0
M. verrucaria - "	57.9	5.0	23.5
F. lateritium - "	51.5	25.0	15.1
Tr. roseum - "	*40.0*	*25.4*	2.4
Cl. Cladosporioides-"	18.9	57.5	0.0
Mean	47.9	27.6	25.5
C.D.at P= 0.05:	2.46	3.62	5.14

Table 3: Antagonistic effect of four selected fungi on three isolates of *T. paradoxa*

Test fungus	Percentage of inhibition of *T. paradoxa* isolate							
	Dual culture test				Antibiosis test			
	T.p-1	T.p-2	T.p-3	Mean	T.p-1	T.p-2	T.p -3	Mean
G.virens Uduma	78.68	43.00	79.67	67.11	100.0	100.0	100.0	100.0
T. harazianum Kallangai	69.81	46.67	66.00	60.82	78.57	58.67	68.33	68.52
T. viride Culicut	67.92	38.00	57.67	54.53	73.45	55.33	60.33	63.04
T. hamatum Delhi	69.81	43.33	60.33	57.82	64.19	52.33	59.67	58.73
Mean	71.56	42.75	65.91		79.05	66.58	72.08	-
C.D. at P = 0.05:								
Antagonists		3.49				2.99		
T.p. isolates		3.03				2.60		
Antagonists Vs T.p.isolate		6.06				5.19		

From five day old cultures, *T. paradox* was not reisolated from the interaction sites indicating the loss of viability of T. *paradoxa* due to the antagonistic activity of *G.virens* (Uduma/ Kallangai isolate). However, T. *paradoxa* was reisolated from the interaction sites between *T. paradoxa* and other isolates of *G. virens* and *Trichoderma* spp. Dennis and Webster (1971a) and Webber and Hedger (1986) reported production of diffusible inhibitory metabolites by *Trichoderma* sp. and *Gliocladium* sp. against several pathogenic fungi. In the present study, growth of T. *paradoxa* was inhibited by all 32 isolates, expect *Cl. cladosporioides* (Tables 1and 2). This may be attributed to the production of diffusible metabilites which in turn inhibited the growth of T. *paradoxa.*

Test isolates of *G. virens* except the Pantnagar isolate, registered complete inhibition over T. *paradoxa.* Metabolites produced by *G. virens* (Uduma and *Kallangai*) were found to be fungicidal *while* the others had fungistatic effect on T. *paradoxa.* Similar reports are available on antifungal activity of *T. harzianum*, (Dennis and Webster 1971a; Ghisalberti and Rowland, 1993), *T. viride* (Mathur and Bhatnagar 1994) and *T. hamatum* (Dennis and Webster, 1971b) on several pathogenic fungi.

Growth of T. *paradoxa on* the plates exposed for ten days to the 32 test fungi indicated that non-production of volatile compounds. If at all produced by the test fungi, they were ineffective in inhibiting the growth of *T. paradoxa. Trichoderma* spp. was reported to produce volatile compounds inhibitory to several plant pathogenic fungi (Dennis and Webster, 1971a; Webber and Hedger, 1986; Mathur and Bhatnagar, 1994). However, this phenomenon was not pronounced in the present study.

Among the four antagonistic fungi tested, G. *virens* (Uduma) showed higher mean inhibition on *T. paradoxa* isolates followed by T. *harzianum*, *T. viride* and *T. hamatum* (Table 3). *T. paradoxa* isolate T.p-1 (KSD - I) which is a virulent isolate on coconut (Ramanujam and Nambiar, 1996) was inhibited severely by all the four antagonists. Mutual inhibitions were observed among the four selected antagonists and hence, their combinations could not be used in the field for biocontrol. However, they can be used individually to control the pathogen of stem bleeding disease of coconut.

G. virens (Uduma), *T. harzianum* (Kallangai), and *T. viride* (Calicut) showed significantly higher rate of inhibition on *T.*

Table 4. Antagonistic effect of selected fungi on *T. paradoxa* of coconut petioles

Test isolate and its origin	Lesion area (cm)	Per cent reduction
G. virens Uduma	28.70	69.96
T. harizanum Kallangai	31.66	66.86
T. viride Calicut	34.98	63.38
T. hamatum Delhi	40.46	57.65
T. paradoxa KSD	95.54	-
Mean	46.27	64.46
C.D. at P = 0.05:	11.47	5.25

paradoxa lesion development than the Delhi isolate *T. hamatum* (Table 4). Since these four antagonists showed significant reduction in the T. *paradoxa* lesion development on coconut petioles, the same were selected for further use in field trials.

REFERENCES

Dennis, C. and Webster, J. 1971 a. Antagonistic properties of species group of *Trichoderma* I. Production of non-volatile antibiotics. *Transaction of British Mycological Society* **57**: 25-39.

Dennis, C. and Webster, J. 1971 b. Antagonistic properties of species group of *Trichoderma* II. Production of volatile antibiotics. *Transaction of British Mycological Society* **57**: 41-48.

Ghisalberti, E.L. and Rowland, G.Y. 1993. Antifungal metabolites from *Trichoderma harzianum*. *Journal of Natural Products* **56**: 1799-1804.

Gowda, P.V. 1987. In vitro *studies on the interactions of antagonistic mycoflora with* Thielaviopsis paradoxa, *the causal agent of coconut stem bleeding disease.* M.Phil. dissertation, Mangalore University, Mangalore, Karnataka.

Mathur, K. and Bhatnagar, K. 1994. Effect of volatile and non-volatile substances produced by *Trichoderma viride* on stem blight pathogen of cowpea. *Journal of Biological control* **8: 64.**

Radhakrishnan, T.C. 1990. Control of stem bleeding disease of coconut. *Indian Coconut Journal* **20**:13-14.

Ramanujam, B. and Nambiar, K.K.N. 1996. Studies on virulence of different isolates of *Thielaviopsis paradoxa. Journal of Plantation Crops* **24**(suppl.): 149-152

Ramanujam, B., Nambiar, K.K.N. and Anil Kumar 1993. Chemical control of stem bleeding disease of coconut. pp. 615-616. In : *Advances in Coconut Research and Development.* Eds. Nair, M. K., Khan, H.H., Gopalasundaram, P. and Rao, E. V.V B. Oxford and IBH Publishing Company Private Limited, New Delhi.

Usman, N.M. 1988. *Studies on stem bleeding disease on coconut. 1.survival of Thielaviopsis paradoxa in soil. 2.In vivo interaction of T. paradoxa with antagonistic fungi.* M. Phil dissertation, Mangalore University, Mangalore, Karnataka.

Webber, J.E. and Hedger, J.N.1986. Comparison of interactions between *Ceratocystis ulmi* and Elm bark saprobes *in vitro* and *in vivo. Transation of British Mycological Society 86*: 93-101.