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Antagonistic potential of some soil fungi on *Phytophthora colocasiae* Racib.

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Soils from different parts of West Bengal, India, were screened to isolate potential antagonists of *Phytophthora colocasiae* Racib., the incitant of leaf blight and corm rot of Taro (*Colocasia esculenta* (L.) Schot.). Out of 58 microbial isolates (40 fungi, 8 bacteria and 10 actinomycetes) screened, only 10 fungal isolates showed antagonistic potential in dual culture plate technique *in vitro*. Out of these 10 isolates 5 were identified as *Trichoderma viride, while 3 of T. harzianum*, one each as *Gliocladium virens* and an unidentified sterile fungal culture. The mycoparasitic/hyperparasitic activities of these isolates on *P. colocasiae* were brought about through several morphological changes like coiling of hyphae, formation of haustoria like structures, disorganisation of host cell contents and penetration into host hyphae.

Key words : Biological control, *Phytophthora colocasiae, Trichoderma harzianum, T. viride, Gliocladium virens,* mycoparasite, antagonists

INTRODUCTION

Since the discovery that *Trichoderma lignorum (Gliocladium virens)* has great potentiality for biocontrol the mycoparasitic/hyperparasitic effects of *Trichoderma* spp. and *Gliocladium* spp. on several fungi have been established (Panchenari and Dix, 1980; Chet *et al.*, 1981; Elad *et al.*, 1982, 1983; Papavizas, 1985). But the possibility of control of *Phytophthora colocasiae* through these antegonistic microorganisms has not been adequately explored inspite of the destructiveness of the pathogen on host, *Colocasia esculenta.*

Microorganisms like fungi, bacteria and actinomycetes were isolated from soils of different regions with a view to explore their antagonistic as well as biological control possibilities of the disease (*Phytophthora* blight of *Colocasia)* and the hyperparasitic activities of the probable antagonistic microorganisms were explored by dual culture plate method (Dennis and Webster, 1971) and mechanism of hyphal interactions by Chet *et al.*, (1981)

MATERIALS AND METHODS

Screening of soils for antagonists

Fresh soil samples were collected from different locations upto plough depth discarding the surface soil, processed and preserved (Johnson and Curl, 1972) for further use.

The microorganisms were isolated by serial dilution plating (Parkinson *et al.*, 1971) using respective media for each group of microorganism [Peptone - Dextrose - Rose Bengal Agar (Martin, 1950), Soil Extract Agar, (Allen, 1957), Soybean meal - Glucose - agar (Tsao *et al.*, 1960) respectively].

Some of the dilution plates after three days of incubation were used for direct screening of antagonistic properties by sandwich plate method. The potentialities of the probable antagonists were further screened through dual culture plate method (Dennis and Webster, 1971). The

mycoparasites were rated for their antagonistic property following Bell's test (Bell *et al.*, 1982).

The paired cultures were observed for a period of 9 days before being discarded. An isolate of the mycoparasite was considered antagonistic to the pathogen when the mean score for a given comparison (rounded to the nearest whole class number) was ≤ 2. but not highly antagonistic if the number was ≥ 3. Selected cultures from pairing of mycoparasite X pathogen resulting in the different antagonism classes were observed microscopically to determine the approximate state of the pathogen mycelium after 9 days.

Hyphal interaction between the test hyperparasite and the pathogen

Observations on the mechanisms of hyphal interactions were carried out following method of Chet *et al.* (1981). The sterilised glass slide coated with a thin layer of oat-agar medium was placed inside a pair of sterilised moist petriplate. Agar disks covered with mycelium of host fungi (*P. colocasiae)* were placed on one end of the agar coated glass slide and disks with mycoparasitic organisms were placed on the other end. In each case the mycoparasite and host fungus grew toward each other and the hyphae intermingled on the glass slide. All plates were incubated in a humid chamber at 25±1°C. The coated slides were observed for host-parasite interactions under Zeiss Phase contrast microscope and Leitz Nomarski differential Interference-contrast light microscope.

RESULTS AND DISCUSSIONS

Screening and rating of antagonists

From the results (Table 1) it was observed that out of 58 isolates (40 fungi, 8 bacteria and 10 actinomycetes) screened *in vitro,* 10 isolates of fungi were rated as class 1 mycoparasites. Those were SW_1, SW_2, S_1, S_2, S_3, S_7, TH_1, TH_2, TH_3 and 4F and they were identified as *Trichoderma viride,* sterile culture, *T. viride, T. viride, T. harzianum, T. viride, T. viride, T. harzianum, T. harzianum, Gliocladium virens* and *T. viride* in order of sequences by Indian Agriculture Research Institute (I.A.R.I.), New Delhi - 110 012, India.

Further, the results (Table 2) showed that among ten selected class 1 mycoparasites, isolate 4F (04; *T. viride)* exhibited highest (6.86) mycoparasitism over *P.colocasiae* followed by SW_1 (02; *T. viride),* S_7 (03; *T.viride)* and S_3 (08; *T. harzianum)* respectively.

Hyphal interaction between the test hyperparasites and the pathogen

The nature of physical interactions between the test hyperparasites and the pathogen was examined by light microscopy with the following mode of hyphal interactions.

SW_1 : Pathogen Interaction : The mycoparasite SW_1 coiled around and formed appressoria over hyphae of the host pathogen, followed by considerale shrinkage of protoplast of *P. colocasiae.* Granulations of protoplast and formation of oil drops in protoplast of the test pathogen were also noted within 4 to 5 days of interaction with mycoparasite.

SW_2 : Pathogen interaction : The hyphae of the mycoparasite (S_2) were highly coiled around the host hyphae (*P. colocasiae).* Majority of the growing hyphal branches of hyperparasite were directed towards the mycelium of the test pathogen indicating chemotactic response and attraction.

S_3 : Pathogen Interaction : The host hyphae (*P. colocasiae)* were highly entangled by hyphae of the mycoparasite. Hyphae of *P. colocasiae* became thinner and finally collapsed.

Table 1. Rating of mycoparasites in dual culture plate by Bell's method

Serial No.	Organism/ isolate	Mean[a,b,c] scores	Serial No.	Organism/ isolate	Mean[a,b,c] scores
1	SH_1	4.07(4.00)	31	TH(Patna)	1.21*
2	S_2	1.11*(1.00)	32	*Penicillium* spp. P_1	4.00 (4.00)
3	TH(BCKV)	4.00(4.00)	33	P_2	4.12(4.00)
4	S_{12}	4.10(4.00)	34	P_3	3.21(4.00)
5	BIS	4.13(4.00)	35	P_4	4.81(5.00)
6	GV_1	4.12(4.00)	36	P_5	4.17(4.00)
7	SS_1	4.00(4.00)	37	*Aspergillus* sp - A_1	3.61(4.00)
8	GV_2	4.31(4.00)	38	A_2	3.17(3.00)
9	2F	4.40(4.00)	39	A_3	3.51(4.00)
10	S_7	1.31*(1.00)	40	A_4	4.01(4.00)
11	BAG	3.82(4.00)	41	*Streptover ticillium*	No inhinition
12	MAN-TH	4.20(4.00)	42	*Bacillus subtills*-B_1	-
13	TH_3	1.05*(1.00)	43	B_2	-
14	4F	1.01*(1.00)	44	B_3	
15	S_1	1.20*(1.00)	45	B_4	
16	S_3	1.03*(1.00)	46	*Pseudomonas* sp.-PS_1	-
17	TH(pant)	1.21*(1.00)	47	PS_2	-
18	TH_4	2.11(2.00)	48	PS_3	-
19	GV_3	4.11(4.00)	49	*Actinomycetes*-A_1	-
20	GV(ME)	3.90(4.00)	50	A_2	-
21	SH_2	3.05(3.00)	51	A_3	-
22	BAL-DIG	4.00(4.00)	52	A_4	-
23	SWN	4.02(4.00)	53	A_5	-
24	GV_4	4.61(5.00)	54	A_6	-
25	SH_1-TH	4.07(4.00)	55	A_7	
26	GV_6	4.21(4.00)	56	A_8	-
27	PEDK	3.87(4.00)	57	A_9	-
28	J	3.02(3.00)	58	A_{10}	
29	TSW_1	1.00*(1.00)	59	Control -1 (*P.colocasiae)*	-
30	SW_2	1.00*(1.00)	60	Control-II (Blank)	-

a - Mean of three replicates,
b - data recorded after 8 days of growth
c - scale, of classes described by Bell *et al.*(1982) was followed.
Figure in parenthesis indicates the whole class.
'-' Signifies no inhibition of growth
* Indicates class 1 category

S_7 : Pathogen Interaction : The hyphal branches of the mycoparasite (S_7) grew parallel to the host hyphae and subsequently penetrated and grew inside the host cell. The contents of the host fungus (*P. colocasiae)* gradually exhausted and the hyphae appeared empty and dead.

4F : Pathogen Interaction : The hyphae of mycoparasite (4F) twined around the host hyphae and the protoplast of the host in some places became granular and finally disintegrated.

TH_1 : Pathogen Interaction : Granulations and shrinkage of the protoplast of the host hyphae were found in about 4 days of interaction. The host hyphae gradually vacuolated, less granular, shrunken and finally collapsed.

TH_3 : Pathogen Interaction : The hyphae of mycoparasite grew rapidly over the hyphae of *P. colocasiae* and coiled around host hyphae. Granulations and shrinkage of the protoplast of the host hyphae were evident in about 4 days of interactions.

SW_2 : Pathogen interaction : Interactions between the hyperparasite (SW_2) and host fungus stimulated rapid branching and formation of granular structures in hyphae of *P. colocasiae*. The branches of the host fungus appeared moniliform and swollen with intermittent lysis of hyphal wall.

Table 2. Comparative mycoparasitic activity of different isolates of hyperparasites showing class I activity against *P. colocasiae*

Mycoparasites Code No.	Identity of mycoparasites	Growth of mycoparasite over *Phytophthora colocasiae* (cm) at 24 h interval[a]			
		24	48	72	96
SW_2(01)	Sterile culture	0.63	1.20	1.83	2.47
SW_1(02)	*Trichoderma viride*	1.06	1.97	3.67	5.50
S_7(03)	*T. viride*	0.63	1.60	3.66	5.13
4F (04)	*T. viride*	2.20	4.03	5.80	6.86
S_1 (05)	*T. harzianum*	0.61	1.73	2.78	4.74
S_2 (06)	*T. viride*	0.50	1.53	2.66	4.63
TH_1 (07)	*T. harzianum*	0.87	1.73	2.20	2.71
S_3(08)	*T. harzianum*	1.00	2.20	3.00	5.12
TH_2(09)	*T. viride*	0.93	1.96	2.73	3.03
TH_3 (10)	*Gliocladium virens*	1.03	2.17	3.20	4.30
S.Em ± 0.1094					
C.D.(P 0.05) 0.328					

[a] Each insertion is an average of five replications

The suggested mechanisms for hyperparasitic/mycoparasitic activity by two genera *Trichoderma* and *Gliocladium,* are antibiosis, lysis, and competition including mycoparasitism (Papavizas and Lumsden, 1980; Ayers and Adams, 1981; Cook and Baker, 1983).

Researchers dealing with *Trichoderma* and *Gliocladium* observed that the hyphae of the antagonists parasitized hyphae of other fungi *in vitro* and brought about several morphological changes like coiling, haustoria formation, disorganisation of host cell contents and penetration into the host (Chet *et al.,* 1981; Papavizas, 1985). The results of the present investigation on the mode of hyphal interactions between different mycoparasites and host fungus (*P. colocasiae)* revealed the evidences like : (i) growth of hyphal branches of mycoparasite towards the hyphae of *P. colocasiae* indicating a chemostactic attraction, (ii) twinning and /or coiling of hyphae of mycoparasite(s) around the hyphae of *P. colocasiae,* (iii) formation of appressoria like structures over the hyphae of host fungus, (iv) penetration of hyphae of mycoparasite inside the host cell, and (v) appearance of granulation and development of vacuoles in the cell protoplasm of host fungus followed by lysis and disintegration of hyphae of host fungus. All these characters noted in the present experiments clearly agreed with severals of the previous observations (Durrell, 19681; Chet *et al.,* 1981). Moreover, much restricted growth followed by stimulated branching and formation of knots in hyphae of *P. colocasiae* were observed as a result of interactions with an unidentified sterile hyphae. Similar

morphological abnormalities in host cells (*P. ultimum)* due to antagonistic effect of *T. viride* on dual culture have been oserved by Dennis and Webster (1971).

The phenomenon of physical contact followed by disorganisation of host cells and concomitant changes in the cells (may be morphological or cytological or both) of the parasite have been equivocally established for *Trichoderma* (Elad *et al.,* 1982;1983) and *Gliocladium (*Tu, 1980; Howell, 1982).

The present experiment clearly established the antagonistic potential of several of Indian isolates of *Trichoderma* and *Gliocladium* on *P. colocasiae,* thus opening a new avenue for biological control possibilities of this dreaded pathogen through these antagonistic microorganisms.

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