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Fungal antagonists of *Phytophthora parasitica* var. *piperina*

SWAPAN KUMAR GHOSH
Department of Botany, R.K.M.V.C. College, Rahara, North 24-Parganas, West Bengal

Soils from different parts of West Bengal for isolating potential fungal antagonists of *Phytophthora parasitica* var. *piperina*, the causal organism of leaf rot and foot rot of *Piper betle*. Out of thirty (30) fungal isolates screened, only nine (9) showed class 1 antagonistic potentiality in dual culture plate technique *in vitro*. Out of these nine, five were indentified as *Trichoderma viride*, isolate three as *T. harzianum* and one as *Gliocladium virens*. The mycoparasitic activities of these isolates on *P. parasitica* var. *piperina* were accompanied by several morphological changes like coiling of hyphae, formation of haustoria like structures, disorganisation of host cell contents and penetration into host hyphae.

Key word : Biological control, *P. parasitica* var. *piperina, T. viride, T. harzianum, G. virens, antagonists, mycoparasites*

INTRODUCTION

Leaf rot and foot rot of *Piper betle* is a serious disease occuring in almost all the betel (Pan) growing regions of India and the world and causes enormous loss of the crop (Mehrotra, 1986). The causal organism, *Phytophthora parasitica* var. *piperina* to this disease was well established by several workers (Choudhury, 1944; Asthana, 1947). The disease can to some extent be controlled by spraying fungicides (Vyas and Chaurasia, 1976). Since the leaves of the crop is directly used as a masticatory its biological control is an alternative and more attractive effort than chemical control. Hence, in the present study, an attempt has been employed to isolate some mycoparasites from soil and their antagonistic effects were rated *in vitro* against *P. parasitica* var. *piperina.*

MATERIALS AND METHODS

Screening of soils for antagonists

Fresh soil samples were collected from different locations upto plough depth discarding the surface soil (Johnson and Curl, 1972). The microorganisms (fungi) were isolated by serial dilution plating (Parkinson *et al.*, 1971) using Peptone-Dextrose-Rose Bengal Agar (Martin,1950) medium. Different soil dilutions were used and colonies developed were isolated separately on PDA slants.

Rating of mycoparasites by Dual Culture plate method

The mycoparasites were rated for their antagonistic property following Bell's test (Bell *et al.*, 1982). Mycelial discs (5 mm diam.) from the margin of actively growing colony of *P. parasitica* var. *piperina* and that of antagonists were inoculated simultaneously at opposite ends of a petri plate containing sterilized OA + YE [oat meal (5%) agar + Yeast extract (5%), Ghosh, 1991] medium. The plates were incubated at 28 $\pm$ 1^{o}C for 8 days in a B.O.D. incubator and were subsequently scored for degree of antagonism on a 1-5 scale of classes (Bell *et al.*, 1982).

An isolate of mycoparasite was considered antagonistic to the pathogen when the mean score was $\leq$ 2 but not highly antagonistic if number was $\geq$ 2.

Hyphal interaction between the test hyperparasites and the pathogen

Observations of hyphal interactions were carried out following Chet *et al.* (1981).

In this method, twenty percent strength Oat meal agar was poured into sterilized petri plates, each

containing a glass slide. The glass slide coated by a thin layer of agar medium was inoculated with *P. parasitica* var. *piperina* on one end and mycoparasite on the other end. These sets were incubated in a humid chamber at 25 ± 1°C for seven days. The coated slides were then removed from the agar and observed under Zeiss phase contrast microscope and Leitz Normarski differential interference contrast light microscope (Germany).

RESULTS AND DISCUSSION

Screening and rating of antagonists

The results (Table 1) showed that out of thirty (30) fungal isolates, only nine yielded class 1 mycoparasitism against *P. parasitica* var. *piperina*. These isolates were S_1, S_2, S_3, S_7, SW_1, TH_1, TH_2, TH_3 and 4F ; and they were identified as *Trichoderma harzianum, T. viride, T. harzianum, T. viride, T. viride, T. harzianum, T. viride, Gliocladium virens* and *T. viride* respectively by I.A.R.I., New Delhi.

The data (Table 2) indicated that *T. viride* (S_2) exhibited maximum growth or mycoparasitism (6.50 cm) over *P. parasitica* var. *piperina* followed in descending order by *T. harzianum* (TH_1), *T. harzianum* (S_3) and *Gliocladium virens* (TH_3).

Table 1 : Rating of some mycoparasites in dual culture after 8 days of incubation following scale of classes of Bell *et al.* (1982)

Isolate/ fungi	Mean Score	Isolate/ fungi	Mean Score
SH_1	3.40 (3.01)	TH_6	4.40 (4.0)
S_1	1.35 (1.0)*	4F	1.21 (1.0)*
S_2	1.00 (1.0)*	GV_1	3.07 (3.0)
S_3	1.30 (1.0)*	GV_2	4.70 (5.0)
S_4	3.10 (3.0)	GV_3	3.55 (3.0)
S_5	4.10 (4.0)	GV_4	3.68 (4.0)
S_6	5.00 (5.0)	GV_5	3.00 (3.0)
S_7	1.05 (1.0)*	*Aspergillus*(A_1)	5.00 (5.0)
SW_1	1.01 (1.0)*	A_2	4.01 (4.0)
SW_2	4.05 (1.0)	A_3	3.65 (4.0)
TH_1	1.11 (1.0)*	A_4	4.11 (4.0)
TH_2	1.09 (1.0)*	*Penicillium*(P_1)	3.90 (4.0)
TH_3	1.00 (1.0)*	P_2	4.77 (5.0)
TH_4	4.10 (4.0)	P_3	3.12 (3.0)
TH_5	5.00 (5.0)	P_4	4.00 (4.0)

I = Mean of three replications.
Figures in paranthesis indicates of whole class.
* = Class-1 mycoparasite.

Hyphal interaction between the test hyperparasites and the pathogen

In this experiment only *T. viride* (S_2), *T. harzianum* (TH_1) and *G. virens* were tested against the pathogen under microscope and their mode of hyphal interaction were presented as follows:

The isolate S_2 formed appressoria like structure over the hyphae of the pathogen. Its hyphae also formed coiling and twinning around the pathogen. Shrinkage of protoplast of the pathogen was conspicuous.

Majority of the hyphal branches of the isolate TH_1 showed directed growth towards the mycelium of the pathogen indicating chemotactic response and attraction.

Hyphal branches of *G. virens* (TH_3) penetrated into the cells of hyphae of the pathogen and grew inside. The cell contents of the pathogen became exhausted and the hyphae subsequently appeared empty and dead.

High mycoparasitic activity of *Trichoderma viride* and *T. harzianum* and *Gliocladium virens* on other fungi has been previously established (Barnet and Lilly, 1962; Ayers and Adams, 1981; Cook and Baker, 1983). However, information of mycoparasitism of these antagonists on *Phytophthora qenus* is insufficiant (Ghosh, 1991). The present finding (Tables 1 and 2) is in agreement with that of Ghosh (1991).

Table 2 : Comparative mycoparasitic potentiality of some isolates of mycoparasite (having class I activity) over *P. parasitica* var. *piperina*

Our Code	IARI Harb No.	Identity of mycoparasite	Growth of mycoparasite (cm) over *P. parasitica* var. *piperina*
SW_1 (02)	108	*Trichoderma viride*	3.02
S_7 (03)	109	*T. viride*	3.31
4F (04)	110	*T. viride*	4.73
S_1 (05)	111	*T. harzianum*	4.50
S_2 (06)	112	*T. viride*	6.50 (1)
TH_1 (07)	113	*T. harzianum*	5.95 (2)
S_3 (08)	114	*T. harzianum*	5.66 (3)
TH_2 (09)	115	*T. viride*	4.61 (5)
TH_3 (10)	116	*Gliocladium virens*	5.21 (4)
SEm ±			0.1072
C.D. ($P < 0.05$)			0.311

Several workers dealing with *Trichoderma* and *Gliocladium* reported hyperparasitic activity of their hyphae on other fungi through several morphological and cytological changes, haustoria formation penetration and disorganisation of host cell contents (Chet *et al.*, 1981 ; Howell, 1982 ; Elad *et al.*, 1982; Papavizas, 1985).

Similar morphological abnormalities in host cells of *P. ultimum* due to antagonistic effect of *T. viride* was also noted by Dennis and Webster (1971).

The present observations of the antagonistic potential of Indian isolates of *Trichoderma* and *Gliocladium* on *Phytophthora parasitica* var. *piperina* were in conformity with the previous reports and are of significant importance in the field of biological control of the pathogens.

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