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Effect of organic matter sources on *in vitro* growth and enzyme production by *Pythium indicum* M. S. Balakr., a damping-off pathogen and its fungal antagonist, *Trichoderma viride* Pers.: Fr.

R. NEELAMEGAM
Department of Botany, S. T. Hindu College
Nagercoil 629 002, Tamil Nadu, India
E-mail: rnmsthc @ yahoo. com

ABSTRACT: **All organic matter sources showed inhibitory effect on the *in vitro* growth of *Pythium indicum* M.S. Balakr., while most of them support the growth of *Trichoderma* viride Pers.: Fr. Several organic matter sources increase the production of PTE-ase and Protease enzymes of fungal antagonist. However, the synthesis of Protease enzyme by pathogen was suppressed by all organic matter sources, while PTE-ase enzyme production was inhibited by few organic matter sources. Synthesis of cellulase by *P. indicum* was induced by many organic matter sources, but in case of *T. viride*, the same was inhibited except neem cake and wheat bran. Out of 12 organic matter sources tested, Neem cake and Wheat bran support the antagonist on its *in vitro* growth and synthesis of all the three enzymes as compared to control/ pathogen.**

KEY WORDS: Cellulase, organic matter, pectin transeleminase, protease, *Pythium indicum*, *Trichoderma viride*

INTRODUCTION

Soil amendment with organic matter is one of the promising and frequently used methods of attaining biocontrol of soil born pathogen. The decomposition of organic matter either reduces the disease incidence by suppressing inoculum of pathogen (Papavizas and Lumsden, 1980; Kauraw and Singh, 1982) or increases the disease incidence by multiplying the soil pathogens (Kauraw and Singh, 1982; Martin and Hancock, 1986). Organic matter may also enhance the population of other soil micro-flora including antagonists. Therefore evaluation of cheap and effective soil amendment that supports the antagonist and suppresses the pathogen is necessary. Hence, an attempt was made in this study to evaluate the effect of various organic matter sources on the *in vitro* growth and enzyme production of damping-off pathogen, *Pythium. indicum* M.S. Balakr and its antagonistic fungi, *Trichoderma viride* Pers.: Fr.

MATERIALS AND METHODS

The fungal pathogen, *P. indicum* used in this study, was isolated from tomato seedling affected by damping-off disease, and identified based on the characteristics described by Balakrishnan (1948) and its pathogenicity was established. The fungal antagonistic organism,

T. viride, obtained from Tamil Nadu Agricultural University, Coimbatore, was used in this study.

The twelve organic matter sources selected for this study (Table 1) were collected, dried for 24 h at 80°C, powered, and stored till use. Ethanol extract (80%) of these organic matter sources was used to estimate total phenol (Bray & Thorpe, 1954). The organic matter sources were separately incorporated at 0.5 per cent (w/v) level in 50 ml of Czapeks dox broth and then the medium was sterilized, inoculated with the test fungus, *P. indicum / T. viride*, incubated at room temperature (28±2°C) under static conditions for 10 days. The mycelial mat was filtered in a Buchner Funnel through a previously dried and weighed Whatman No: 1 filter paper. The filter paper with mycelial mat was dried in a hot-air oven at 80°C for 24 h to a constant weight and the dry weight of the fungal mat was recorded.

Various phenolic compounds (Table 2) were separately incorporated by Seitz's filtration into sterilized Czapeks dox broth in the place of sucrose so that 0.001M final concentrations of the respective chemicals were obtained. The final pH of the broth was adjusted to 7. 0. The broth was inoculated with *P. indicum / T. viride*, incubated and the mycilial dry weight was determined as described elsewhere.

The mycelium free culture filtrate of *P. indicum / T. viride*, was centrifuged and used as enzyme source for the assay of cell wall degrading enzymes (Pectin Transeleminase (PTE), Cellulase and Protease) produced by *P. indicum / T. viride*. Control was maintained with the test fungus inoculated medium with out organic matters. PTE-ase (Pectin lyase) activity was measured by estimating the production of TBA (Thiobarbituric Acid) reacting substance as described by Mahadevan and Sridhar (1984). Cellulase (Endo B-(1,4)-Gluconase) enzyme activity was measured by estimating the amount of reducing sugar released (Nelson, 1944) as described by Mahadevan and Sridhar (1984). Protease enzyme activity was determined by measuring the amount of amino acid released with the help of Ninhydrin method (Moore and Stein, 1948) as described by Mahadevan and Sridhar (1984). All the *in vitro* studies were carried out with three replications. The results were analyzed statistically by using one-way ANOVA.

RESULTS AND DISCUSSION

Total phenol content of organic matter sources

All the organic matter sources were tested for its total phenol content and the results (Table 1) indicated that the total phenolic content was high in all the leaf materials and Mahua oil cake, tested. On the other hand it is moderate in neem oil cake, sesame oil cake, FYM and wheat bran, and low in groundnut oil cake and Sawdust.

Organic matter sources on *in vitro* growth of test fungus

The results of present study (Table 1) shows that all the organic matter sources suppressed the growth of *P. indicum*, while most of them, except *Mahua* leaf, *Morinda* leaf, Groundnut cake and Sesame cake, promote *T. viride* growth. The toxic substances present in the plant residues may inhibit the growth of pathogen as suggested by Singh (1983). Several workers observed the inhibitory effect of crop residues on *Pythium* spp. (William and Schmitthenner, 1960; Singh and Pandey, 1965; Sankhla *et al.*, 1969).

Phenolic compounds on *in vitro* growth of test fungus

All phenolic compounds (0.001-M) tested in this study (Table 2) shows inhibitory effect on the *in vitro* growth of *P. indicum*, while most of them support the growth of *T. viride* as compared to *P. indicum* / control. Similar observations were made by Muthusamy *et al.* (1974) on *P. aphanidermatum* growth with phenolic compounds (Catecol and Pyrogallol).

Organic matter sources on *in vitro* enzyme production by test fungus

The test fungus (*P. indicum / T. viride*) produces pectic (PTE-ase), cellulolytic (cellulase) and potease enzymes (Table 3) considerably. As

Table 1. Effect of organic matter on the *in vitro* growth of *P. indicum / T. viride*

Sl.no.	Organic matter	Total phenol content in organic matter @	Fungal growth #	
			P. indicum	*T. viride*
1.	Neem leaf	3.97	105.0 (- 39.8)	177.0 (+ 21.8)
2.	*Mahua* leaf	5.13	80.7 (- 53.7)	107.3 (- 26.2)
3.	*Morinda* leaf	4.12	155.0 (- 11.1)	136.0 (- 06.4)
4.	*Peltoporum* leaf	4.78	79.0 (- 54.7)	147.0 (+ 01.2)
5.	*Thespesia* leaf	3.70	134.3 (- 22.9)	177.0 (+ 21.8)
6.	Groundnut oil cake	2.03	113.3 (- 35.0)	132.0 (- 09.2)
7.	*Mahua* oil cake	4.53	101.7 (- 41.7)	148.3 (+ 02.1)
8.	Neem oil cake	2.50	133.0 (- 23.7)	153.0 (+ 05.3)
9.	Sesame oil cake	2.45	77.3 (- 55.7)	106.0 (- 27.0)
10.	Farm Yard Manure	3.17	107.3 (- 38.4)	148.0 (+ 01.9)
11.	Sawdust	1.90	144.3 (- 17.2)	172.7 (+ 18.9)
12.	Wheatbran	3.23	136.7 (- 21.6)	151.7 (+ 04.4)
13.	CONTROL	00.00	174..3 (00.00)	145.3 (00.00)
	CD (P = 0.05)	0.90	24.9	20.9

- mg / 50ml Czapek's dox broth, amended with organic matters (0.5 % w/v), incubated for 10 days as still culture at RT (28±2°C)
Figures in parentheses are per cent change over control.
@ - mg/ gram on dry weight basis* - Significant at (P=0.05)

Table 2. Effect of various phenolic compounds on *in vitro* growth of *P. indicum / T. viride*

Sl. No.	Phenolic compounds @	Fungal growth #	
		P. indicum	*T. viride*
1.	Phenol	84.7 (-48.3)	18.3 (-83.2)
2.	Catecol	37.0 (-77.4)	86.0 (-21.1)
3.	Hydroquinone	48.3 (-70.5)	112.0 (+02.8)
4.	Pyrogallol	113.7 (-30.5)	138.0 (+26.6)
5.	Anthronillic acid	68.0 (-58.5)	120.7 (+10.7)
6.	Gallic acid	37.3 (-77.2)	114.0 (+04.6)
7.	Cinnamic acid	34.7 (-78.8)	133.0 (+22.0)
8.	Hydroxyquinoline	51.3 (-68.7)	26.7 (-75.5)
9.	Quin hrdrone	23.0 (-85.9)	97.0 (-11.0)
10.	Control	163.7 (00.0)	109.0 (00.0)
	CD (P = 0.05)	10.41	18.18

@ - 0.001-M concentration of phenolic compounds was used to test the fungal growth.
# - mg/ 50-ml of Czapeks dox broth/ 10 days; * - Significant at (P=0.05)
Figures in parentheses are per cent change over control.

Table 3. Effect of organic matter on the *in vitro* production of pectic transeliminase, cellulase and protease enzyme by *P. indicum* / *T. viride*

Sl. no.	Organic matter ‴	PTE-ase #		Cellulase $		Protease ¥	
		P. indicum	*T. viride*	*P. indicum*	*T. viride*	*P. indicum*	*T. viride*
1	Neem leaf	0.562	0.488	1.590	0.877	0.295	0.772
2	*Mahua* leaf	0.580	0.196	0.710	0.634	0.235	0.804
3	*Morinda* leaf	0.672	0.348	1.061	1.462	0.504	0.614
4	*Peltoporum* leaf	0.713	1.419	1.787	0.405	0.472	1.730
5	*Thespesia* leaf	0.843	0.552	1.026	0.021	0.113	1.010
6	Ground nut oil cake	0.190	0.835	1.890	0.929	0.150	1.259
7	*Mahua* oil cake	0.984	1.136	0.556	1.136	0.307	1.273
8	Neem oil cake	0.177	0.694	0.545	2.216	0.308	0.784
9	Sesame oil cake	0.317	1.392	0.739	1.031	0.400	0.937
10	Farm Yard Manure	0.160	1.070	0.191	1.702	0.519	0.456
11	Saw dust	0.381	0.405	0.544	1.143	0.338	1.155
12	Wheat bran	0.288	2.923	0.331	2.417	0.626	0.646
13	CONTROL	0.442	0.286	0.616	2.036	0.763	0.286
	CD (P = 0.05)	0.054	0.082	0.104	0.103	0.060	0.077

- Czapek's dox broth, amended with organic matters (0.5 % level (w/v)), incubated for 10 days as still culture at RT (28±2°C) * - Significant at (P=0.05) # - Unit / h / mg of protein

$ - mg of reducing sugars released /hr/mg of protein ¥ - mg of amino acid released /hr/ mg of protein

compared to pathogen, antagonist produces low PTE-ase, Protease enzymes, and high cellulase enzymes. Several workers observed the synthesis of hydrolytic (pectic and cellulolytic) enzymes by *Pythium* spp. (Elad *et al.*, 1985) and by antagonistic organisms (Baker, 1987) has been reported.

Organic matters (except *Mahua* leaf) generally stimulate the synthesis of PTE-ase by *T. viride*, while some of the organic matters show inhibitory or stimulatory effect on PTE-ase enzyme production (Table 3) in case of *P. indicum* as compared to control. However, most of the organic matters favour the PTE-ase enzyme production by antagonist as compared to pathogen.

Most of the organic matters show stimulatory effect on cellulase production by *P. indicum* while the same was suppressed in case of *T. viride* as compared to control (Table 3). Out of 12 organic matter sources, seven favour the antagonist in cellulase enzyme production when compared to pathogen.

The results (Table 3) of present study reveal that all organic matters tested inhibit the synthesis of protease by *P. indicum*. But, the production of this enzyme in case of *T. viride* was enhanced by all organic matters as compared to control.

The inhibitory effect of organic matter on *in vitro* production of hydrolytic enzymes might be

due to phenolic compounds occurring in organic matters (Table 1). The production of cell wall degrading enzymes by an antagonist would likely be involved simultaneously in parasitism and antibiosis, as suggested by Fravel (1988). Out of 12 organic matter sources tested in this study, neem cake and wheat bran support the antagonist on its *in vitro* growth and enzyme production as compared to control / pathogen.

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