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Bio–efficacy of culture filtrate of *Serratia marcescens* and oil cakes against on the growth of *Pythium aphanidermatum* (Edson) Fitz. Causing damping-off of brinjal

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Abstract
Damping-off disease of brinjal caused by *Pythium aphanidermatum* is considered as one of the major constraints to brinjal production. To overcome these problems the present study, we investigated the *in vitro* biological control of *Pythium aphanidermatum* by using new biocontrol agent like *Serratia marcescens* and locally available oil cakes [Groundnut (*Arachis hypogaea*), Sesame (*Sesamum indicum*), Coconut (*Cocos nucifera*), Neem (*Azadirachta indica*), and Elluppai (*Mahua longifolia*), Pungam (*Pongamia glabra*)]. The culture filtrate of *S. marcescens* at 40 and 50 % conc. completely inhibited the mycelial growth and minimum mycelial dry weight of *P. aphanidermatum.* Among the locally available six oil cakes evaluated *Mahua longifolia* oil cake water extract @ 50 % conc. was found to be highly inhibitory to the mycelial growth and minimum mycelial dry weight of *P. aphanidermatum.*

Keywords: Culture filtrate, *Pythium aphanidermatum*, *Serratia marcescens*, mycelial growth, oil cakes, mycelia dry weight

Introduction
The brinjal (*Solanum melongena* L.) is one of the most popular and principal vegetable crop grown in India and other parts of the world. The crop belongs the family Solanaceae and genus *Solanum.* The cultivated brinjal is presumed to be of Indian origin with China as secondary centre of origin. It has been cultivated for many centuries. The leading brinjal producing countries in the order of importance are China, India, Japan, Italy and Spain. Themajor brinjal producing states in India are Andhra Pradesh, Maharashtra, Karnataka, Orissa, Madhya Pradesh, Tamil Nadu and West Bengal (Das *et al.*, 2000) [3]. Such a potential crop is known to suffer from several fungal, bacterial and phytoplasmal diseases. Among these damping-off of brinjal seedlings caused by several species of *Pythium* is very common all over the world. Besides, it occurs both in tropical and temperate climates and in almost every glass house and green house conditions (Klean *et al.*, 2003) [6].
In the light present day constraints of plant disease management practices, especially those imposed for the use of pesticides. Biological management in increasingly captaining the imagination of many plant pathologist and gaining importance as a possible practical agricultural method for the management of the soil borne pathogens (Papavizas and Lumsden, 1980) [10]. *S. marcescens* a gram negative soil bacterium produces chitinolytic enzymes and certain antifungal low molecular weight molecules which cause degradation of the fungal cell walls (Someya *et al*., 2000) [13]. Therefore in the present study we investigated the effect culture filtrate of *S. marcescens* and oil cakes of *in vitro* studies in damping-off (*Pythium aphanidermatum*).

Materials and methods
Preparation of culture filtrate of *S. marcescens*
Erlenmeyer flasks of 250 ml containing 50 ml chitin luria broth were sterilized and *S. marcescens* were inoculated. The flasks were incubated at room temperature (28 ± 2°C) for two days. After incubation the whole content was filtered aseptically through bacteriological filter and suspension was centrifuge at 12,000 rpm to remove the spores. The filtrate was used for testing their antagonistic ability over the pathogen.

Effect of culture filtrates of *S. marcescens* on the mycelial growth of *P. aphanidermatum*
The culture filtrate of *S. marcescens* was separately incorporated into sterilized PDA medium at 10, 20, 30, 40 and 50 per cent by adding the calculated quantity of the culture filtrate to the

medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @15 ml and allowed to solidify. Each plate was inoculated at the centre with seven days old (9 mm) PDA culture disc of *P. aphanidermatum*. Metalaxyl @ 0.1 per cent conc. was used for comparison. The diameter of the mycelial growth (in mm) of *P. aphanidermatum* was measured when the mycelial growth fully covered the control plates.

Effect of culture filtrates of *S. marcescens* on the mycelial dry weight of *P. aphanidermatum*

The culture filtrates of *S. marcescens* isolate were incorporated into sterilized PDA broth at 10, 20, 30, 40 and 50 per cent by adding the calculated quantity of the culture filtrate to the broth by means of a sterile pipette. The PDA broth without the culture filtrate served as control. Metalaxyl @ 0.1 per cent conc. was used for comparison. The flasks were inoculated with nine mm mycelial disc of *P. aphanidermatum* collected from the periphery of seven days old culture. The flasks were incubated for 15 days at room temperature at 28 ± 2°C and filtered thereafter through filter paper Whatman No. 42 under vacuum. The mycelial mat was dried in hot air oven at 60°C until constant weight obtained. The mycelial dry weight was recorded.

Effect of water extracts of various oil cakes on the mycelial growth *P. aphanidermatum*

The procedure given by (Jha *et al*., 2007) [5] was adopted for this experiment. Six locally available oilcakes *viz.*, Groundnut (*Arachis hypogaea*), sesame (*Sesamum indicum*), Coconut (*Cocos nucifera*), Neem (*Azadirachta indica*) and Elluppai (*Mahua longifolia*), Pungam (*Pongamia glabra*) were evaluated *in vitro* against *P. aphanidermatum.* All the oil cakes were powdered and soaked overnight in sterile distilled water at the rate of one g. per ml of water. It was then ground and filtered through cheese cloth. This formed the standard extract solution (100 %). The extract of the organic amendments were separately incorporated in to sterile PDA medium at 10, 20, 30, 40 and 50 per cent levels by adding calculated quantity of the extracts to the medium by means of a sterile pipette. The PDA medium without extract served as control. These extract impregnated media were transferred to sterile Petri dishes separately @ 15 ml per plate and allowed to solidify. After solidification a disc of (9 mm) pathogen was placed at the centre of the Petri plate and incubated at room temp. 28+20 C. After 7days of incubation period the mycelial growth of the pathogen was recorded.

Effect of water extracts of various oil cakes on the mycelial dry weight of *P. aphanidermatum*

Fifty ml of PDA broth was taken in 250 ml Erlenmeyer flask sterilized and amended with different conc. of organic amendments [Groundnut (*Arachis hypogaea*), Sesame (*Sesamum indicum*), Coconut (*Cocos nucifera*), Neem (*Azadirachta indica*), and Elluppai (*Mahua longifolia*), Pungam (*Pongamia glabra*)] filtrates *viz.*, 10, 20, 30, 40 and 50 per cent conc. and inoculated with the 9mm mycelial disc of *P. aphanidermatum* collected from the periphery of seven days old culture. The flasks were incubated for 15 days at room temp. 28+20 C and after the incubation period the contents were filtered through previously weighed filter paper, Whatman No. 42. Then mycelial mat was dried in hot air oven at 600 C until attaining constant weight and then the weight was recorded.

Results and Discussions

Effect of culture filtrate of *S. marcescens* on the mycelial growth and mycelial dry weight of *P. aphanidermatum*

The results of the *in vitro* studies conducted to find out the effect of culture filtrate of *S. marcescens* on the mycelial growth and mycelial dry weight of *P. aphanidermatum* are summarized in table 1. The mycelial growth of *P. aphanidermatum* was found to be reduced with an increase in the conc. of culture filtrates of *S. marcescens* and the reduction was significantly the maximum in the case of *S. marcescens* with 38.62, 29.48, 15.96, 0.00 and 0.00 mm at 10, 20, 30, 40 and 50 per cent conc. of the culture filtrate respectively as against the maximum growth of 90 mm in the control. The same trend was maintained in the case of liquid medium assay. The minimum mycelial dry weight (1.21 mg) of *P. aphanidermatum* was recorded in 50 per cent conc. of the culture filtrate of *S. marcescens* which was at par with *S. marcescens* @ 40% and metalaxyl @ 0.1 per cent (1.25 and 1.32 mg respectively). The *S. marcescens* was inhibited of mycelial growth of several pathogens was well established by other worker (Stefan kurze *et al.,* 2001) [15]. The cell free extracts of *S. marcescens* effectively inhibited the growth of *R. solani* (Strit *et al*., 1993) [16]. Someya *et al.* (2000) [13] reported that *S. marcescens* completely inhibited the radial growth of *R. solani* and *F. oxysporum* f.sp. *cyclaminis*. The antifungal metabolites produced by *S. marcescens* might be attributed as the reason for the reduction in the growth of the test pathogen.

In liquid media, the biomass production of *P. aphanidermatum* was strongly inhibited by *S. marcescens* at 40 per cent conc. (Table 1). Perusal of literature revealed that the inhibitory effect of *S. marcescens* against various fungal pathogens (Stefan kurze, *et al.,* 2001; Bruton *et al.,* 2003) [15, 2]. Sanjeevkumar, (2008) [11] reported that the biomass production of *F. oxysporum* f.sp. *cubense* was strongly inhibited by *S. marcescens*. Several antifungal compounds from *S. marcescens* have been purified and characterized, such as chitinase, prodigiosin, and protease. These compounds attack the cell walls of phytopathogenic fungi to cause cell lysis and subsequent death. Interestingly, each strain of *S. marcescens* may have their own set of fungicides that allow them to fight against different spectra of fungi. The two chitinolytic enzymes and prodigiosin from *S. marcescens* strain B2 inhibit spore germination of grey mold pathogen *Botrytis cinerea* (Someya *et al*., 2001; Giri *et al*., 2004) [14, 4] while the chitinase from *S. marcescens* MO-1 inhibits the development of a number of phytopathogens such as *Alternaria citri, Fusarium oxysporum, Trichoderma harzianum, Aspergillus niger and Rhizopus oryzae* (Okay *et al.,* 2013) [9]. These reports are in line and add support to the present findings.

Effect of water extracts of various oil cakes on the mycelial growth and dry weight of *P. aphanidermatum*

Among the organic amendments tested, mahua oilcake @ 50 per cent recorded the maximum inhibition (12.50 mm) of mycelial growth of *P. aphanidermatum* which was followed by pungam oilcake and coconut oilcake with 19.33 and 28.52 mm of mycelial growth respectively (Table 2). Similar results as in solid medium were observed when the experiment was conducted using liquid medium. The flasks inoculated with *P. aphanidermatum* alone recorded the maximum mycelial dry weight of 309.88 mg. whereas, the flasks inoculated with *P. aphanidermatum* and amended with mahua oilcake extract, recorded a significant reduction in mycelial dry weight with

115.25, 94.75, 60.12, 52.25 and 30.25 mg in 10, 20, 30, 40 and 50 per cent conc. respectively. This was followed by the extracts of pungam oilcake, coconut oilcake, neem oilcake, and sesame oilcake. The least inhibitory effect was observed with groundnut oilcake (Table 3). All the organic sources tested inhibited the mycelial growth of the test pathogen. However, the water extract of 'mahua' and 'pungam' oilcakes showed maximum reduction in biomass production. The effectiveness of organic sources for managing fungal pathogens have earlier been reported (Sudhasha, 2001; Sanjeevkumar, 2008) [17, 11]. The extract of elluppai and coconut oilcake significantly reduced the mycelial growth of *F. oxysporum* affecting tuberose crop (Muthukumar, 2002) [8]. Similarly, Anitha (2006) [1] observed that many oilcake extracts were found effective in inhibiting the mycelial growth of *F. oxysporum* f.sp. *lycopersici.* Neem cake effectively inhibited the growth and population of *F. oxysporum* f.sp. *cubense* and vascular discoloration in banana suckers (Saravanan *et al.,* 2004) [12]. Manikandan (2017) [7] suggested that application of mahua oil cake highly reduced the damping-off incidence in chilli. These above reports are in conformity with the present findings.

Table 1: Effect of culture filtrate of *S. marcescens* on the mycelial growth and mycelial dry weight of *P. aphanidermatum*

Tr. No.	Conc. of the culture filtrate (%)	Mycelial growth (mm)	Per cent inhibition over control	Mycelial dry weight (mg)	Per cent inhibition over control
1	10	38.62	57.08	197.45	36.91
2	20	29.48	67.24	114.23	63.50
3	30	15.96	82.26	53.82	82.80
4	40	NG	100.00	1.25	99.60
5	50	NG	100.00	1.21	99.61
6	Metalaxyl @ 0.1%	NG	100.00	1.32	99.57
7	Control	90.00	-	312.98	-
	SEd CD (P=0.05)	0.74 1.62	-	0.41 0.97	-

NG - Nil growth

Table 2: Effect of water extracts of various oil cakes on the mycelial growth of *P. Aphanidermatum*

Tr. No.	Source	Mycelial growth (mm) Concentration (%)					Per cent inhibition over control				
		10	20	30	40	50	10	20	30	40	50
1.	Pungam oilcake	70.58	48.93	28.67	21.39	19.33	21.57	45.63	68.14	76.23	78.52
2.	Sesame oilcake	86.43	78.97	69.91	59.12	46.99	3.96	12.25	22.32	34.31	47.78
3.	Neem oilcake	86.52	77.82	65.66	56.93	42.29	3.86	13.53	27.04	36.74	53.01
4.	Coconut oilcake	85.30	60.30	44.86	37.49	28.52	5.22	33.00	50.15	58.34	68.31
5.	Mahua oilcake	60.25	31.35	27.18	20.50	12.50	33.05	65.16	69.80	77.22	86.11
6.	Groundnut oilcake	87.50	82.75	73.05	62.13	49.18	2.77	8.05	18.83	30.96	45.35
7.	Control	90.00	-	-	-	-	-	-	-	-	-
	SEd CD (p=0.05)	0.01 0.03	0.41 0.92	0.38 0.84	0.25 0.63	0.51 1.31	-	-	-	-	-

Table 3: Effect of water extracts of various oil cakes on the mycelial dry weight of *P. aphanidermatum*

Tr. No.	Source	Mycelial dry weight (mg) Concentration (%)					Per cent inhibition over control				
		10	20	30	40	50	10	20	30	40	50
1.	Pungam oilcake	129.93	101.87	84.00	78.93	52.19	58.07	67.12	72.89	74.52	83.15
2.	Sesame oilcake	210.74	195.52	180.59	170.13	165.50	31.99	36.90	41.72	45.09	46.59
3.	Neem oilcake	190.54	173.26	160.20	150.99	130.65	38.51	44.08	48.30	51.27	57.83
4.	Coconut oilcake	200.50	185.90	173.48	150.15	71.63	35.29	40.00	44.01	51.54	76.88
5.	Mahua oilcake	115.25	94.75	60.12	52.25	30..25	62.80	69.42	80.59	83.13	90.23
6.	Groundnut oilcake	290.00	220.33	210.33	200.15	190.54	06.41	28.89	32.12	35.41	38.51
7.	Control	309.88	-	-	-	-	-	-	-	-	-
	SEd CD (p=0.05)	0.31 0.75	0.48 1.01	0.61 1.72	0.87 1.93	-	-	-	-	-	-

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