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Antimycotic Efficacy of Organic Amendments against *Pythium aphanidermatum* (Edson) Fitzp the Incitant of Tomato (*Solanum lycopersicum* L.) Damping-off

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A B S T R A C T

Damping-off of tomato incited by *Pythium aphanidermatum* (Edson) Fitzp. is one of the devastating diseases of tomato with substantial yield losses primarily in the nurseries than in main fields of our nation. Agrochemical application plays a significant role in the management of vegetable crops damping-off but its consecutive application causes detrimental consequences to the kingdom of flora and also leads to the development of fungicidal resistance among the pathogenic populations. With these circumstances, the present study was conducted to exploit the antifungal potential of ten different organic amendments over *Pythium aphanidermatum* under *in vitro* conditions. Out of the ten organic amendments tested, mahua cake extract (10%) exhibited the highest mycelial reduction of 79.44% with the least mycelia growth of 1.85 cm over control (9cm). This was succeeded by neem cake extract (10%) (68.89% mycelial inhibition).

Introduction

Tomato (*Solanum lycopersicum* L.) popularly known as "king of vegetables" belongs to the nightshade family crop originated from Peruvian and Mexican regions. India is the second largest producer of tomato next to China. In India, tomato is widely cultivated in 781' 000 ha with the total production of 19007' 000 MT and the productivity is 24 MT ha^{-1} during 2018-19. In Tamil Nadu, the total tomato production is 814'000 tonnes which accounts for about 4.36 % of total country's production in 2018-19. Among the solanaceous crops, tomatoes are the rich source of vitamins (A, B, and C), sucrose, fructose, lipid, protein, minerals (phosphorus, calcium, potassium, and magnesium) with pigments like lycopene and β-carotene (Peixoto *et al.*, 2017). The solanaceous crops are being used very much in our daily life to satisfy the per capita requirement of the people. Besides these, the productivity of tomato is meagre due to the infectious and non- infectious agents (Jain *et al.*, 2014). Among these agents, pre and post emergence

damping-off provoked by *Pythium* species is responsible for greater than 60 % death of germinated seedlings in the nurseries as well as in the main fields (Jadhav and Ambadkar, 2007). Under the phylum Oomycota *Pythium* is one among the largest genera. Several species of *Pythium* are facultative parasites (Bhalerao *et al.,* 2020).

Damping-off disease prefers the immature host tissue and rapidly kills huge numbers of seedlings in the nursery beds (Thakur and Tripathi, 2015). *Pythium* spp. generally taint the seeds and radicle causing seed rot and damping-off. They affect the already emerged seedlings near the soil line, make them collapse or topple down resulted in post-emergence damping-off (Lamichhane *et al.,* 2017).

Cellulolytic and pectinolytic enzymes produced by *Pythium* spp. are responsible for the tissue disintegration as well as maceration which lead to successful infection. The pre and post emergence damping off of solanaceous crops are mainly incited by various *Pythium* spp. namely *P. aphanidermatum, P. irregulare, P. debaryanum* and *P. ultimum*.

Farmers face major constraints in the management of *Pythium* diseases due to its soil borne nature with prolonged survival of propagules. In the natural farming system soil fertility status could be improved by the application of organic amendments. The physical, chemical and biological properties of the soil could be altered during the decomposition of organic amendments thereby improving the soil texture, structure and biotic conditions of soil (Dhingani and Solanky, 2016). Organic manure is very effective as well as available at low cost with excellent source of nitrogen and phosphorus responsible for the yield maximization in sustainable crop production.

Materials and Methods

Collection and Isolation of the pathogen

An expedition was undertaken to study the prevalence of tomato damping-off in the major vegetable growing tracts of southern districts of Tamil Nadu namely Madurai (Poonchuthi TNAU-AC&RI, Kallikudi, Maikudi) and Tenkasi (Panpozhi). Three weeks old tomato (*Solanum lycopersicum* L.) seedlings expressing the typical damping-off symptoms produced by *Pythium* spp. were gathered and brought to the aseptic laboratory conditions. The gathered samples were rinsed in a tap water to get rid off the debris and sand. The pathogen was isolated from the diseased tomato seedlings via tissue segment method (Kavitha *et al.,* 2005). The minutely dissected samples (0.5cm) were surface sterilized with sodium hypochlorite (1%) for 30 seconds followed by rinsing thrice in sterile water. The moisture free dissected samples were placed equally in four places on the Petri dishes holding the sterilized PDA media and incubated for three days. Auxenic culture of the pathogen was maintained on the PDA slants for further experiments. The pathogen was identified as *P. aphanidermatum* based on the microscopic observations of hyaline coenocytic mycelium, lobed sporangia and apleurotic oospore (Parveen and Sharma, 2015).

Pathogenicity assay

Koch's postulates were used to identify the aggressive, virulent isolate of the pathogen. The five different isolates of *Pythium* species were augmented on sand-maize medium (Ashwathi *et al.,* 2017). The ratio of sand-maize medium is 19:1(1900 g of sand and 100 g of pulverized maize) were deliberately mixed with sterile water for the judicious maintenance of 50% moisture content then put it in a poly bag and autoclaved at1.4 kg

cm^{-2} pressure for 2 consecutive hours for a couple of days. Three days of actively growing *Pythium* mycelial plugs (9 mm) of 2-3 numbers were taken with heat sterilized cork borer and inoculated into the poly bags separately and incubated at room temperature for three weeks. The mass multiplied pathogen at the rate of 5 % (w/w) was incorporated into the earthen pots with sterilized potting mixture (red earth: sand: FYM) in 3:1:1 ratio. After 10 days, surface sterilized PKM 1seeds (25 seeds / pot) were sown with five replications for the five isolates in a completely randomized block design (CRD). The control pots are maintained without adding the pathogenic inoculum. The earthen pots were being maintained under the screen house conditions with proper watering and monitored for the expression of damping-off symptoms. The pre and post emergence damping-off incidence was recorded at 7 and 14 DAS for each isolate correspondingly (Muthukumar *et al.*, 2010).

Antimycotic efficacy of organic amendments against *Pythium aphanidermatum* under *in-vitro*

Preparation of organic amendments extracts (cold water extracts)

Ten various organic amendments *viz*., neem cake, pungam cake, mahua cake, groundnut cake, sesame cake, castor cake, cotton cake, coconut cake, vermi compost and FYM were tested. Organic amendments were pulverized, 100 gram of each amendment was separately soaked in sterile distilled water (1g/1.25ml) and kept 12 hours throughout the night then filtered twice through a cheese cloth and the aqueous filtrate was gathered and centrifugation was done at 10000 rpm for 15 minutes. The aqueous filtrate of each organic amendment was extracted and served as standard solution (100%) (Dhingani *et al.*, 2013). The aqueous extracts were sterilized at 1.4 kg/cm^2 pressure for 15 mins before mixing. The extracts at the rate of 2.5 ml and 5.0 ml were added with 47.5 ml and 45 ml of the medium respectively to attain 5% and 10% concentrations.

Efficacy of organic amendments against the growth of *Pythium aphanidermatum*

The efficacy of ten different organic amendments was screened in opposition to *P. aphanidermatum* by poisoned food technique (Meena *et al.*, 2014). Ten to 15 ml of autoclaved PDA medium amended with organic amendments at 5 and 10% concentrations were poured in the sterilized Petri dishes individually and allowed them to solidify properly. With the help of heat sterilized cork borer a 9 mm mycelial plug was taken and gently located at the mid of the Petri dishes and incubated at 28 $\pm$ 2°C for 3 to 5 days. Three replications were maintained for each and every treatment in a completely randomized design. The control plate was maintained without adding the extract of organic amendments. The radial growth of the mycelium and Percentage inhibition was reckoned when the control plate attained its utmost full growth.

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Where, PI = Percent Inhibition

Dc=Average diameter of the pathogen's mycelial growth in control plate (cm)

Dt=Average diameter of the pathogen's mycelial growth in the treatment plates (cm)

Statistical analysis

Experimental data were analyzed using analysis of variance (ANOVA) in the AGRES. The treatmental means were

separated at 5% significance level using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Collection and isolation of *P. aphanidermatum*

Tomato seedlings with prominent damping-off symptoms incited by *P. aphanidermatum* were gathered from five different tomato cultivating regions of southern districts of Tamil Nadu (The isolates of *Pythium* species were named as IS (PCI)-1, IS (PLI)-2, IS (MDU)-3, IS (KDI)-4 and IS (MAI)-5 in Table 1). The utmost incidence of damping-off (85.33%) was noticed in Maikudi situated in the Tirumangalam block of Madurai district whereas Panpozhi in the Shenkottai taluk of Tenkasi district recorded the least damping-off incidence (25.33%) (Table 1). The pathogens were confirmed as *Pythium aphanidermatum* on the basis of colony morphology (van der Plaats-Niterink, 1981), lobed sporangium and apleurotic oopsore (Schroeder *et al.*, 2013) (Figure 1).

Table.1 Collection of tomato damping-off samples from Southern districts of Tamil Nadu

S.No	Crop	Isolate	Location	District	Latitude	Longitude	Altitude(m)	Percent Disease Incidence (%)*
1	Tomato	IS(PCI)-1	Poonchuthi	Madurai	12.29°N	78.07°E	121	64.00 (53.13)
2	Tomato	IS(PLI)-2	Panpozhi	Tenkasi	9.02°N	77.25°E	181	25.33 (30.22)
3	Tomato	IS(MDU)-3	TNAU-AC&RI	Madurai	12.04°N	78.48°E	141	55.33 (48.06)
4	Tomato	IS(KDI)-4	Kallikudi	Madurai	9.91°N	78.10°E	109	44.67 (41.94)
5	Tomato	IS(MAI)-5	Maikudi	Madurai	9.78°N	78.02°E	132	85.33 (67.48)
CD(P=0.05)								2.60

*Mean of thirty seedlings
Data in parenthesis are arc sine transformed values

Table.2 Pathogenicity of *P. aphanidermatum* isolates inciting damping-off disease in tomato

S.No	Crop	Isolate codes	Location	Disease incidence (%)*	
				Pre emergence	Post emergence
1	Tomato	IS(PCI)-1	Poonchuthi	27.20 (31.44)	81.60 (64.60)
2	Tomato	IS(PLI)-2	Panpozhi	9.60 (18.05)	44.80 (42.02)
3	Tomato	IS(MDU)-3	TNAU-AC & RI Madurai	17.60 (24.80)	76.80 (61.21)
4	Tomato	IS(KDI)-4	Kallikudi	13.60 (21.64)	50.40 (45.23)
5	Tomato	IS(MAI)-5	Maikudi	30.40 (33.46)	88.80 (70.45)
CD(P=0.05)				2.79	2.59

*Mean of five replications
Data in parenthesis are arc sine transformed values

Table.3 Efficacy of organic amendments against the mycelial growth of *P. aphanidermatum* under *in vitro*

S. No.	Organic amendments	Concentration (%)			
		5%		10%	
		Mycelial growth (cm)*	Percent growth reduction over control	Mycelial growth (cm)*	Percent growth reduction over control
1	Neem cake	5.20^{c}(13.18)	42.22	2.80^{b}(09.63)	68.89
2	Groundnut cake	6.75^{g}(15.06)	25.00	4.07^{d}(11.64)	54.78
3	Cotton cake	6.37^{f}(14.62)	29.22	4.75^{e}(12.59)	47.22
4	FYM	8.07^{j}(16.50)	10.33	6.89^{h}(15.22)	23.44
5	Vermi compost	7.58^{i}(15.98)	15.78	5.85^{g}(14.00)	35.00
6	Castor cake	7.07^{h}(15.42)	21.44	5.10^{f}(13.05)	43.33
7	Pungam cake	5.57^{d}(13.65)	38.11	3.88^{c}(11.36)	56.89
8	Mahua cake	3.85^{a}(11.32)	57.22	1.85^{a}(07.82)	79.44
9	Sesame cake	6.17^{e}(14.38)	31.44	4.12^{d}(11.71)	54.22
10	Coconut	5.07^{b}(13.01)	43.67	3.80^{c}(11.24)	57.78
C	Control	9.00^{k}	-	9.00^{i}	-
CD (P=0.05)		0.11	-	0.13	-

*Mean of three replications
Data in parenthesis are arc sine transformed values
Mean in a column followed by same superscript are not significantly different (P=0.05)

Fig.1 Morphological characters of *Pythium* sp.

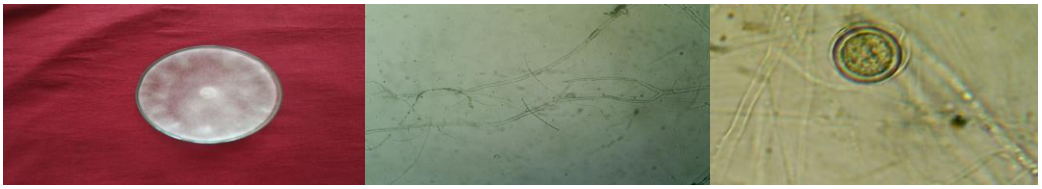

Fig a: Mycelial growth of *Pythium* sp.

Fig b: Microscopic view of mycelial growth of *Pythium* sp.

Fig c: Microscopic view of oospore

Pathogenicity assay

All the five isolates of the pathogen was proved to be pathogenic under pot culture conditions using Koch's postulates. Artificially inoculated seedlings exhibited the characteristic damping-off symptoms which are in accordance with the nursery symptoms. Of these five isolates, Maikudi isolate IS (MAI)-5 was highly virulent showing 30.40 and 88.80 percentage of pre and post-emergence damping-off respectively whereas IS (PLI)-2 isolate was the least virulent with 9.60 and 44.80 percentage of pre and post-emergence damping-off correspondingly (Table 2).

In- vitro efficacy of organic amendments against *P. aphanidermatum*

Ten different organic amendments were evaluated over the most virulent isolate IS

(MAI)-5 of *P. aphanidermatum*. The data depicts that 10% concentration of mahua cake extract recorded the maximum (79.44 per cent) growth reduction over control which was succeeded by neem cake, coconut cake, pungam cake, groundnut cake, sesame cake, cotton cake, castor cake and vermi compost recording 68.89, 57.78, 56.89, 54.78, 54.22, 47.22, 43.33 and 35.00 per cent correspondingly (Table 3). The results obtained are analogous to the experimental results of Alice *et al.*, (1998) who reported that the antifungal principles in mahua cake extract (10%) was responsible for the effective management of jasmine wilt. Farm yard manure is least effective against the pathogen.

The obtained results are supported by the findings of Anitha *et al.*, (2019) who have confirmed that mahua oil cake extract (10%) recorded the highest mycelial reduction (80.11%). Subharathinam *et al.*, (2019) stated that *Madhuca longifolia* oil cake gained the paramount significances with the maximum inhibition (86.11%) of *P. aphanidermatum*. Manikandan (2017) analysed and found out that the incorporation of mahua oil cake extremely declined the incidence of damping-off in *Capsicum annuum.* Renganathan *et al.*, (2020) reported that mahua oil cake exhibited the least mycelial growth (1.57cm) of *M. phaseolina* the incitant of *Sesamum* root rot.

In the present study, FYM (10%) afforded the maximum mycelial growth of 6.89cm with the least disease reduction of 23.44 per cent over control. Similar results was reported by Senjaliya and Nathawat (2015) who reported that except FYM all the screened oilcake extracts *viz*., mustard, peanut, neem and castor showed the significant inhibition of *S.rolfsii* under *in vitro*. Kumar and Kumar (2018) depicted that FYM recorded the minimum mycelial reduction (15%, 20.01%) of *Rhizoctonia solani* at 10 and 20 g/kg soil/pot correspondingly. Rahul *et al.*, (2014) noticed that FYM at higher concentrations promoted the growth of *Rhizoctonia solani* responsible for stem canker and black scurf of *Solanum tuberosum* with minimum inhibition over control.

Organic amendments play a major role in the management of soil borne phytopathogens viz., *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Sclerotium rolfsii*, *Verticillium dahliae*, *Rhizoctonia solani* and *Thielaviopsis basicola* (Shafique *et al.*, 2016). Soil borne diseases are effectively managed by organic amendments through various mechanisms. They alter the soil suppressiveness (Bonanomi *et al.*, 2018), stimulate the activities of beneficial microbiota such as *Rhizobacteria*, *Trichoderma* and *Pseudomonas* species which create competition with the infectious agents during decomposition (Panth *et al.*, 2020).

Lenka and Pun (2014) stated that the extracts of mustard oilcake significantly inhibited the mycelial growth of *Thanatephorus cucumeris* under *in vitro*. Lalitha and Venkatraman (1991) reported that the saponins extracted from mahua oil cake had a inhibitory action over *Penicillium expansum*, *Cephalosporium acrimonium* and *Helminthosporium oryze*.

In conclusion the organic amendments exhibiting antifungal activity against a wide range of soil borne plant pathogens. In the current scenario of organic agriculture the usage of fungicides will be alternated with the application of organic amendments.

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