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OCCURRENCE, VIRULENCE AND PATHOGENICITY OF *PYTHIUM APHANIDERMATUM* CAUSING TOMATO DAMPING OFF

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Abstract

An intensive survey for the occurrence of tomato damping off (*Pythium aphanidermatum*) was carried out during the year 2018–19 in order to assess the incidence of tomato damping off disease in different districts of Tamil Nadu. The maximum incidence of damping off (pre and post emergence - 38.65 & 60.98 per cent) was recorded at Sivapuri village in Cuddalore district followed by 37.55 and 59.69 per cent at Pollachi in Coimbatore district. Radial growth (mm/day) over 4 day incubation and visual assessment of colony morphology indicated variability among the isolates. Among the twenty isolates, maximum mycelia growth and fast sporulent germination at an early stage of incubation 24 h interval for 4 days (1–4 days after incubation), there were maximum mycelia growth was observed in I_4 (33.56 to 90.00 mm) followed by I_7 recorded 32.25 to 89.00 mm radial growth (mm/day) 1-4 days after incubation. Further, various inoculum levels tested, application of 100g/kg of soil inoculum load of *P. aphanidermatum* registered the maximum incidence of 37.68 per cent pre-emergence damping-off and 69.78 per cent of post-emergence damping-off.

Key words : Tomato, *Pythium*, Incidence, Radial growth, Inoculum level.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops in the world. It is one of the important food and cash crops for many low-income farmers in the tropical countries. (Prior *et al*., 1994). Tomato is a solanaceae family and belongs to the genus *Lycopersicon*, grown for its edible fruits (Jones, 1999). The ripened fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food (Dias, 2012). In India, the tomato-growing in an area of approximately 894 ha of the total vegetable-growing area with total production accounting for approximately 19167 of total vegetable production (Anonymous, 2017).

Among the pathogens that affect the tomato crop, soil-borne fungal pathogens, including genus belonged to *Pythium, Sclerotium*, *Fusarium*, *Rhizoctonia,* and *Verticillium* genera causing the root rot or damping- off and wilt which affect the quality with yield reduction (Mandal *et al.,* 2017). Stanghellini (1974) found that worldwide distribution of *Pythium* spp. cause damping-off of many plants. Martin and Loper (1999) reported that *Pythium* species are ubiquitous soil-borne oomycetes that rank from opportunistic up to highly virulent pathogens on many plant species. Elshahawy *et al.,* (2018) Symptoms observed on affected tomato plants developed symptoms of wilting, dead plant, root rot with crown and stem rot on above ground plant parts. Damping off incited by *Pythium aphanidermatum* causes more than 60 per cent losses in seedlings both in nursery and main field (Manoranjitham *et al.,* 2000). Jukte *et al.,* (2016) proved that in tomato pre- emergence damping off caused seed, any young seedlings to rot before they emerge from the growing in soil. In post-emergence damping off the pathogen cause water soaked soft brown lesion at base of the stem near soil line, pinches off stem causing the seedling to topple over and die. (Elshahawy *et al.,* 2018) reported that *Pythium* isolates recovered from diseased tomato plants were morphologically similar to each other. Based on their morphology, all isolates were identified as *P. aphanidermatum*. Therefore, the objectives of this research were to isolate and identify the *Pythium* species that cause tomato damping off, to characterize their relative occurrence, virulence and pathogenicity.

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Materials and Methods

Survey of tomato damping off from different districts of Tamil Nadu

Soil samples were collected from major tomato growing region of Tamil Nadu pertaining to districts such as Ariyalur, Coimbatore, Cuddalore, Madurai, Nagapattinam, Perambalure, Puthukottai, Thanjavur, Thoothukudi and Virudhunagar (Table 1). The unit of soil samples was 1kg. the separate earthen pots filled with collected samples were 15×30 cm dia. PKM.1 variety approximately 100 seeds were sown uniformly in all the earthen pots. The pots were watered and maintained at 80 per cent water holding capacity using gravimetric method. The pots were maintained in greenhouse at 28± 2°C with 12 h. of light and 12 h. of darkness. After 10 days, seedlings expressing the symptoms from all the pots were collected on a tissue paper separately and the pathogens associated with diseased samples were isolated by tissue segment method on *Pythium* specific medium (Vaartaja and Bumbieris, 1964).

Collection and isolation of pathogens

A total of 20 isolates (I_1 to I_{20}) causing *P. aphanidermatum* was isolated from soils collected from different region of Tamil Nadu. The infected tomato stems, were gently washed under sterile water, cut into small pieces, about 0.55–10.00 mm in length, superficially sterilized by sodium hypochlorite solution (5%) for 2 min, washed 2-3 times with sterile distilled water and dried in sterilized filter paper (Elshahawy *et al*., 2016). The small, superficially sterilized pieces were then placed on the surface of potato dextrose agar (PDA) plates. 4 days after incubation at 25°C (±2°C), the frequency of occurrence (%) and radial growth rate (mm/day) was recorded. These plates were then incubated at 28±2°C in an incubator for 4 days. The emerging fungal growth was transferred on fresh medium of potato dextrose agar in Petri plates. Identification of the pathogen was done by comparing the morphological characters (Elshahawy *et al.,* 2018)

Mass multiplication of *Pythium* species

A total of twenty isolates were multiplied in sand maize medium (Muthusamy, 1972). Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content, filled in polypropylene bags and autoclaved at 20 psi for two h. four days old actively growing mycelial disc (six mm) of *Pythium* species were inoculated into each bag under aseptic condition and the bags were incubated at room temp. (28± 3°C) for ten days and the inoculum thus obtained was used for the subsequent experiments.

Assessing the virulence of *Pythium* isolates

The pot mixture was prepared by thoroughly mixing clay loam soil, sand and farm yard manure at the ratio of 1:1:1. The inoculum of each isolate of *Pythium* species collected from different locations were separately mixed @100g/kg of soil and filled in 15×30 cm dia. earthen pots ten days before sowing (Sankar, 1994). Surface sterilized (using 0.1% $HgCl_2$ for 30 sec. followed by two washings in dist. water) tomato seeds (var. PKM 1.) were sown @100 seeds per pot. The uninoculated soil served as control. In each pot, healthy tomato seeds were sown and replicated thrice.. The pots were maintained in glasshouse with regular, judicious and uniform watering. The pre-emergence damping-off was recorded at seven

Table 1: Isolation and identification of *Pythium* species from major tomato growing tracts of Tamil Nadu.

S.No	Isolate number	Name of the village	District	Name of the species
1.	I_1	Andimadam	Ariyalure	*Pythium graminicola*
2.	I_2	Jayankondam	Ariyalure	*P. aphanidermatum*
3.	I_3	Meensurity	Ariyalure	*P. graminicola*
4.	I_4	Sivapuri	Cuddalore	*P. aphanidermatum*
5.	I_5	Keerapalayam	Cuddalore	*P. ultimum*
6.	I_6	Vriddhachalam	Cuddalore	*P. deliense*
7.	I_7	Pollachi	Coimbatore	*P. aphanidermatum*
8.	I_8	Udumalaipettai	Coimbatore	*P. debaryanum*
9.	I_9	TNAU	Coimbatore	*P. aphanidermatum*
10.	I_{10}	Erure	Perambalure	*P. deliense*
11.	I_{11}	Kunnam	Perambalure	*P. vaxans*
12.	I_{12}	Aruppukottai	Virudhunagar	*P. aphanidermatum*
13.	I_{13}	DPP. AC & RI	Madurai	*P. graminicola*
14.	I_{14}	Melure	Madurai	*P. aphanidermatum*
15.	I_{15}	Kovilpatti	Thoothukudi	*P. debaryanum*
16.	I_{16}	Kudumiyanmalai	Puthukottai	*P. aphanidermatum*
17.	I_{17}	Kollidam	Nagapattinam	*P. aphanidermatum*
18.	I_{18}	Illupure	Puthukottai	*P. ultimum*
19.	I_{19}	Aduthurai	Thanjore	*P. aphanidermatum*
20.	I_{20}	Thiruvaiyaru	Thanjore	*P. aphanidermatum*

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days after sowing and post-emergence damping-off was recorded at 14 days after sowing and per cent disease incidence was calculated using the standard

$$\text{Percent Disease Incidence (Pre Emergence)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Percent Disease Incidence (Post Emergence)} = \frac{\text{Number of seedlings affected}}{\text{Total number of seeds germinated}} \times 100$$

Statistical analysis

The data were statistically analyzed using the Wasp version 2.0 developed by the Indian Council of Agricultural Research, Goa (Gomez and Gomez, 1984) Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels (P<0.05) and means were compared by Duncan's Multiple Range Test (DMRT). Laboratory experiments were laid out in Randomized Block Design (RBD).

Results and Discussion

The soil samples were collected from major tomato growing areas of Tamil Nadu and they were subjected to twenty *Pythium* isolates were isolated and brought into pure culture from different districts *viz.,* Ariyalur, Coimbatore, Cuddalore, Madurai, Nagapattinam, Perambalure, Puthukottai, Thanjavur, Thoothukudi and Virudhunagar pertaining to Tamil Nadu (Table 1). The six different *Pythium* species *viz., Pythium aphanidermatum*, *P. debaryanum, P. deliense, P. graminicola, P. ultimum* and *P. vexans* were identified.

The data illustrated in revealed that the level of pathogenicity varied between the isolates. Among the twenty isolates of *Pythium* species the isolate-I_4 (Sivapuri) recorded the maximum disease incidence of 43.65 per cent pre-emergence damping-off 67.98 per cent post-emergence damping-off. Followed by the isolate-I_7 and I_2 collected from Pollachi and Jayankondam recorded the disease intensity of 40.55 and 64.69 per cent pre and post emergence damping off were found. The isolate-I_{19} (Thiruvaiyaru) recorded the least incidence of pre and post-emergence damping-off recording 12.68 and 22.35 per cent, respectively (Table 2). Similar findings (Robertson *et al.,* 2013; Matthiesen *et al.,* 2016; Rojas *et al.,* 2017a, 2017b) were recorded on *Pythium* populations with four major species (P. *ultimum, P. torulosum, P. paroecandrum, and P. spinosum*), among which *P. torulosum* was the most frequently isolated species in soybean and corn rotation field. Olson *et al.* (2016) similar finding that *Pythium* Species differed in pathogenicity and virulence on seedlings resulting in pre - emergence damping off and some post - emergence damping off. Navi *et al.,* (2019) proven green house condition there was increased in damping off disease incidence among the 10 isolates *P. ultimum* var. *Ultimum* maximum incidence was observed 79.80 per cent.

Table 2: Effect of *Pythium* isolates on the incidence of pre and post-emergence tomato (var. PKM 1) damping-off in pot culture.

S.No	Isolates	Pre-emergence damping-off (%)	Post-emergence damping-off (%)
1.	I_1	33.87^{c} (35.58)	35.25^{j} (34.60)
2.	I_2	37.15^{b} (38.96)	01.25^{e} (51.56)
3.	I_3	23.78^{h} (29.18)	57.00^{d} (49.02)
4.	I_4	38.65^{a} (41.35)	60.98^{a} (55.53)
5.	I_5	19.35^{j} (26.09)	51.87^{f} (46.07)
6.	I_6	25.67^{g} (30.44)	47.00^{g} (43.28)
7.	I_7	37.55^{b} (39.55)	59.69^{b} (53.54)
8.	I_8	28.94^{f} (32.54)	43.56^{h} (41.29)
9.	I_9	16.45^{kl} (23.92)	61.35^{c} (51.67)
10.	I_{10}	31.00^{d} (33.83)	58.00^{d} (49.60)
11.	I_{11}	21.54^{i} (27.65)	38.82^{i} (38.53)
12.	I_{12}	34.88^{c} (36.19)	54.55^{e} (47.61)
13.	I_{13}	20.87^{i} (27.18)	32.66^{k} (34.85)
14.	I_{14}	33.66^{c} (35.46)	27.38^{l} (31.55)
15.	I_{15}	29.66^{ef} (32.99)	40.25^{i} (39.37)
16.	I_{16}	17.15^{k} (24.46)	58.33^{d} (49.80)
17.	I_{17}	25.00^{gh} (30.00)	50.66^{f} (45.37)
18.	I_{18}	15.24^{l} (22.97)	25.00^{m} (30.00)
19.	I_{19}	12.68^{m} (20.86)	22.35^{n} (28.21)
20.	I_{20}	30.54^{de} (33.55)	28.65^{l} (32.36)
21.	Control	1.281	2.751
CD (p=0.05)			

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs.

Values in the parentheses represent arc sine transformed value.

Pythium isolates differ in colony morphology and growth rates

Colony growth rate in diameter (mm/day) and visual assessment of colony morphology indicated variability among the isolates. Among the twenty isolates, maximum mycelia growth and fast sporulent germination at an early stage of incubation 24 h interval for 4 days (1–4 days after incubation), there were maximum mycelia growth was observed in I_4 (33.56 to 90.00 mm) followed by I_7 recorded 32.25 to 89.00 mm radial growth (mm/day) were observed at 1-4 days after incubation (Table 3). Similarly, Elshahawy *et al.,* (2018) reported that morphology and cultural character observed in *P. aphanidermatum*

Table 3: Radial growth rate (mm/day) of Pythium isolates recorded at 24 h interval for 4 days.

S.No.	Isolates	*Pythium* isolates	Radial growth (mm/day) over 4 day incubation			
			1	2	3	4
1.	I_1	*Pythium graminicola*	19.99^{f}	49.65^{f}	70.00^{bcd}	86.78^{abc}
2.	I_2	*P. aphanidermatum*	25.45^{b}	55.67^{b}	75.87^{a}	87.99^{ab}
3.	I_3	*P. graminicola*	20.65^{f}	45.25^{g}	68.67^{cd}	85.55^{bcd}
4.	I_4	*P. aphanidermatum*	33.56^{a}	60.23^{a}	78.55^{a}	90.00^{a}
5.	I_5	*P. ultimum*	18.43^{g}	50.33^{ef}	71.87^{bc}	86.99^{abc}
6.	I_6	*P. deliense*	21.87^{e}	42.90^{g}	69.56^{bcd}	87.45^{abc}
7.	I_7	*P. aphanidermatum*	32.25^{a}	57.23^{b}	76.87^{a}	89.00^{ab}
8.	I_8	*P. debaryanum*	24.98^{bc}	52.67^{de}	63.55^{f}	88.55^{ab}
9.	I_9	*P. aphanidermatum*	25.88^{b}	55.67^{b}	75.87^{a}	88.00^{ab}
10.	I_{10}	*P. deliense*	22.55^{de}	50.55^{ef}	60.21^{g}	87.56^{abc}
11.	I_{11}	*P. ultimum*	20.22^{f}	51.67^{ef}	70.00^{bcd}	83.35^{cde}
12.	I_{12}	*P. aphanidermatum*	25.88^{b}	55.67^{b}	75.87^{a}	87.00^{ab}
13.	I_{13}	*P. graminicola*	15.89^{i}	54.78^{bcd}	65.00^{ef}	80.76^{e}
14.	I_{14}	*P. aphanidermatum*	24.99^{bc}	55.12^{bcd}	67.23^{de}	86.67^{abc}
15.	I_{15}	*P. debaryanum*	16.99^{h}	49.88^{f}	62.11^{fg}	81.98^{de}
16.	I_{16}	*P. aphanidermatum*	25.88^{bc}	61.45^{a}	76.87^{a}	88.56^{ab}
17.	I_{17}	*P. aphanidermatum*	24.78^{c}	50.43^{ef}	68.55^{d}	84.99^{bcd}
18.	I_{18}	*P. ultimum*	18.99^{g}	52.89^{cde}	71.98^{b}	82.32^{de}
19.	I_{19}	*P. aphanidermatum*	25.00^{bc}	55.33^{bc}	75.45^{a}	85.55^{bcd}
20.	I_{20}	*P. aphanidermatum*	23.45^{d}	55.67^{b}	75.87^{a}	86.99^{abc}
CD (p=0.05)			0.982	2.562	3.251	2.983

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRTs.

Values in the parentheses represent arc sine transformed value.

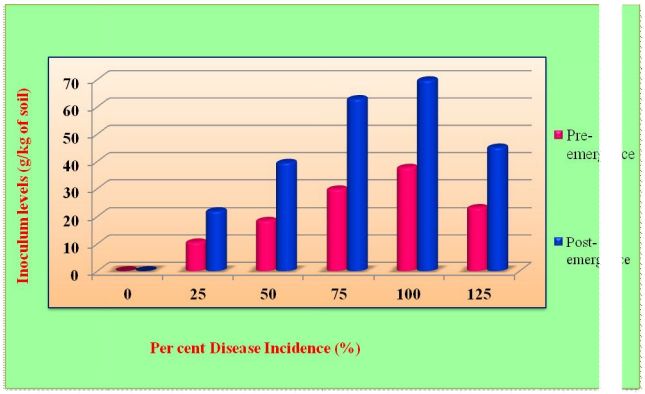

Fig. 1: Effect of different inoculum levels on the incidence of tomato (var. Pkm 1) damping-off in pot culture.

isolation of after 5 days of growth on PDA. Alhussaen (2019) similar finding *P. aphanidermatum* culture was 5 days old grown on potato dextrose agar medium isolated from diseases cucumber plants.

Effect of different inoculum levels on the incidence of tomato damping-off

Among the various inoculum levels tested, application of 10 per cent (100g/kg of soil) inoculum load of *P. aphanidermatum* to 1kg of soil registered the maximum incidence of 37.67 per cent pre-emergence damping-off and 69.78 per cent of post-emergence damping-off, which was followed by the application of 7.5 per cent (75g/kg of soil) inoculum load of *P. aphanidermatum lowest incidence of* 29.76 per cent pre emergence and 62.88 per cent post emergence damping off (Fig. 1). Lamichhane *et al.,* (2017) showed that incidence of damping off causing *Pythium* species may vary from 5 to 80 per cent. Elshahawy *et al.,* (2018) proven isolation and pathogenicity to tomato plants incited damping off found to causes higher percentage of incidence in *Pythium* species. The above results lend support to the present findings.

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