International Journal of Advanced Biochemistry Research 2024; SP-8(10): 1141-1146

International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(10): 1141-1146
www.biochemjournal.com
Received: 18-09-2024
Accepted: 23-10-2024

C Gunaseeli
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

P Renganathan
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

A Muthukumar
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

T Suthin Raj
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

Corresponding Author:
C Gunaseeli
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

Isolation, cultural, morphological characterisation and pathogenicity of tomato damping-off caused by *Pythium aphanidermatum*

C Gunaseeli, P Renganathan, A Muthukumar and T Suthin Raj

DOI: https://doi.org/10.33545/26174693.2024.v8.i10Sn.2671

Abstract
Tomato is one of the most important vegetable crop that grown all over the world. The cultivation of tomatoes holds immense significance in the agriculture industry, yet it grapples with various challenges, including the emergence of diseases caused by fungi. One such disease is damping-off, primarily caused by *Pythium aphanidermatum*, leading to substantial declines in crop yields. A comprehensive survey was conducted across the key tomato cultivation districts of Tamil Nadu: Dharmapuri, Cuddalore, and Krishnagiri. The study focused on gathering 10 distinct isolates of *Pythium* sp., designated as Pa_1-Pa_{10}, for subsequent examination. Through a meticulous analysis of their morphological characters and growth patterns, these isolates were successfully identified as *Pythium aphanidermatum*. The investigation revealed significant diversity and variations in both morphological features and cultural behaviours among the isolates. Pathogenicity assessments unveiled that isolate Pa_9 displayed the highest virulence within the group, with a disease incidence rate of 34.88%. In contrast, the lowest disease incidence rate of 9.28% was recorded in Pa_6 Keerapalayam village of Cuddalore district.

Keywords: Damping-off, tomato, *Pythium aphanidermatum*

Introduction
One of the most often grown crops in India and throughout the world is the tomato (*Solanum lycopersicum* L.) (Maham *et al* 2020) [1]. Tomato is one of the most economically attractive vegetable crops due to its high yield with the highest economic value (Pastor *et al* 2016) [2]. The tomato that is grown for human consumption belongs to the family *Solanaceae* and genus *Solanum*. Tobacco, chili, pepper, potato, eggplant, and other noteworthy cultivated plants are members of the *Solanaceae* family, generally referred to as the nightshade family. The ripened fruits are a very good source of vitamin A, B and C, minerals (Gondal *et al* 2012) [3], vitamins, essential amino acids, sugars and fibers (Naika *et al* 2005) [4] and high concentrations of iron (Fe) and phosphorus (P) (Pastor *et al* 2014) [5], which add wide varieties of colours and flavour to the food.

Tomato produces an antioxidant called ‘lycopene’ which helps in slowing down the growth of cancerous cells (Bhowmik *et al* 2012) [6]. It is widely recommended by nutritionists and dietician in weight control programme and reducing the weight (Keswani 2015) [7]. The benefits of tomato includes low cholesterol, reduce heart diseases, low blood pressure, protection from cell damage, regulates blood sugar, counteract acidosis, reduce migraines, boost immunity, strengthen bones, fights against cancer, combating strokes, protection against eye disorders, wound repair and helps in curing diabetes (Bhowmik *et al* 2012) [6]. The primary tomato-producing nations globally include China, India, the USA, Turkey, Egypt, Iran, Italy, Spain, and Brazil. Among that China ranks first in the cultivated area of 12.33 lakh hectares with the production of 56.8 million tonnes per year.

Tomato crop is highly susceptible to plant pathogens *viz*., bacteria, fungi and viruses which deteriorate the yield and quality of tomato fruit (Pattnaik *et al* 2012) [8]. Among the various fungal plant pathogenic agents, several soil-borne plant pathogens such as *Rhizoctonia solani, Fusarium solani, Phytophthora capsici, Sclerotium rolfsii* and *Fusarium oxysporum* are reported to attack tomato plants, causing damping-off and root rot diseases (Mohamed *et al* 2015; Hamza *et al* 2016; Shenashen *et al* 2017) [9-11].

Pythium aphanidermatum (Edson) Fitzpatrick, a significant pathogen within the *Pythium* genus, is recognized globally for causing damping-off, root rot, and wilt in tomato plants, posing a substantial threat to tomato cultivation (Jayaraj *et al* 2005; Christy Jeyaseelan *et al* 2012; Kipngeno *et al* 2015) [12-14].

Materials and Methods

Isolation, identification and purification of the pathogen (*P. aphanidermatum*)

The pathogen was isolated from the damping-off infected tomato seedlings collected from different localities in Tamil Nadu by the tissue segment method (Rangaswami 1958) [15] on Potato Dextrose Agar medium (PDA medium). The collected samples were gently rinsed with running tap water to get rid off the debris and sand and cut the samples into small pieces (0.55-10.00 mm) by using sterilized scalpel. The minutely dissected samples were surface sterilized with 1% Sodium hypochlorite (NaOCl) solution for 2 minutes followed by rinsing with three changes of sterile distilled water and dried in sterilized filter paper (Elshahawy *et al* 2016) [16]. Finally four pieces were plated at equal distance in a sterilized Petri plate containing solidified PDA medium (Ainsworth 1961) [17] impregnated with streptomycin. The plates were incubated at room temperature (28±2°C) for 3 days and examined for the fungal mycelial growth. Auxenic culture of the pathogen was maintained aseptically by transferring a single hyphal tip of the fungus to the PDA slants by using hyphal tip technique (Rangaswami 1972) [18]. The pure culture was stored in refrigerator at 5°C for further studies and was sub cultured periodically. Totally 10 isolates were maintained and labelled as Pa_1 to Pa_{10}. The pathogen was identified as *Pythium aphanidermatum* by comparing the characters described by Middleton (1943) [19]; Balakrishnan (1948) [20].

Cultural and morphological characteristics of various isolates of *P. aphanidermatum*

Nine mm mycelial disc from a 5 days old culture of the pathogen was taken with sterilized inoculation loop and inoculated at the centre of sterilized Petri dish containing 15 ml of solidified PDA medium and incubated for 5-7 days. The growth and morphological characteristics of the isolates, including colony morphology, mycelial growth rate, colony color, and septation, were meticulously observed, with measurements taken under a microscope after calibration with ocular and stage micrometer.

Pathogenicity test

The pot mixture was prepared by thoroughly mixing clay loam soil, sand and farm yard manure at the ratio of 1:1:1. The inoculums of each isolate was separately mixed @ 10 g/kg of soil and filled in 15x30 cm diameter earthen pots 10 days before sowing. The tomato seeds (Var. PKM 1) were surface sterilized with 1% Sodium hypochlorite for 30 sec followed by two washings in distilled water and sown 100 seeds/pot. The pots were maintained in glass house with regular, judicious and uniform watering and the growth was observed. The pre and post- emergence damping-off incidence of each isolate was recorded at 7 and 14 DAS correspondingly by using the formula

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

After 15 days, the plant showing the shrinkage at the collar region were pulled out and the pathogen was re-isolated from the infected portion and the organism was maintained on PDA slants by sub culturing periodically. The culture thus obtained was compared with that of the original culture and pathogenicity (Koch postulates) was proved (Usharani and Satheesh 2007; Karmel Reetha and Muthukumar 2019) [21, 22].

Among the ten isolates, *P. aphanidermatum* (Pa_9) is the most virulent isolate which is collected from Chenathur at Krishnagiri district based on the disease incidence and this isolate (Pa_9) will be used for further studies.

Results and Discussion

Survey on the incidence of damping-off of tomato from major tomato growing areas in Tamil Nadu

The survey was conducted during 2020-2021 in major tomato growing areas of Dharmapuri (Pulikkarai, Papparapatti, Nallampalli, Andipatti), Cuddalore (Sivapuri, Keerapalayam, Virudhachalam) and Krishnagiri (Irumathur, Chenathur, Potchampalli) districts of Tamil Nadu to assess the damping-off disease incidence of tomato plants from different soils *viz*., red sandy soil, black clay loam soil, sandy clay soil, sandy coastal alluviam, red loam soil, clay soil, sandy loam and the data were presented in Table 1. The results of the survey revealed that the maximum disease incidence was recorded in sandy loam soil in Chenathur (34.88%) of Krishnagiri district followed by sandy clay soil in Sivapuri (31.18%) of Cuddalore district, red sandy soil in Papparapatti (29.97%) of Dharmapuri district and the minimum incidence was recorded in sandy coastal alluviam soil in Keerapalayam (9.28%) of Cuddalore district. Karmel Reetha and Muthukumar (2019) [22] surveyed different districts of Tamil Nadu for the occurrence of tomato damping-off (*Pythium aphanidermatum*) and reported that the maximum incidence of damping-off (pre and post-emergence, 38.65% and 60.98%) was recorded at Sivapuri village in Cuddalore district and the least incidence was recorded (pre and post-emergence, 12.68% and 22.35%) at Aduthurai village in Thanjore district. Similarly, Jayalakshmi *et al* (2021) [23] surveyed various parts of Tamil Nadu for the occurrence of damping-off and wilt pathogens of tomato and stated that the maximum damping-off incidence was 96.67%.

Collection and isolation of samples from different tomato growing areas of Tamil Nadu

A survey was conducted in major tomato growing areas in Tamil Nadu during the year 2020-2021 which revealed the occurrence of damping-off disease. The maximum disease incidence was recorded in Chenathur (34.88%) of Krishnagiri district followed by Sivapuri (31.18%) of Cuddalore district, Papparapatti (29.97%) of Dharmapuri district and the minimum incidence was recorded in Keerapalayam (9.28%) of Cuddalore district (Table 2). Karmel Reetha and Muthukumar (2019) [22] surveyed different districts of Tamil Nadu for the occurrence of tomato damping-off (*Pythium aphanidermatum*) and reported that the maximum incidence of damping-off (pre and post-emergence 38.65% and 60.98%) was recorded at Sivapuri village in Cuddalore district and the least incidence was recorded (pre and post-emergence, 12.68% and 22.35%) at Aduthurai village in Thanjore district. Similarly, Kumar *et al* (2018) [24] reported that the tomato damping-off incidence was ranged from 41.3% to 85.00%. Similar findings are reported by Kamali *et al* (2020) [25] surveyed major vegetable growing tracts of southern districts of Tamil Nadu namely Madurai (Poonchuthi TNAU-AC&RI, Kallikudi, Maikudi) and Tenkasi (Panpozhi) and reported the highest incidence of damping-off (85.33%) in Maikudi, located in the Tirumangalam block of Madurai district, whereas Panpozhi in the Shenkottai taluk of Tenkasi district recorded the lowest incidence (25.33%) (Fig 02 and 03).

Cultural and morphological characteristics of various isolates of *P. aphanidermatum*

The ten infected tomato plant samples were collected from different locations of Dharmapuri, Cuddalore, and krishnagiri districts of Tamil Nadu. All the ten isolates were identified as *Pythium aphanidermatum*, based on their cultural characters through a microscope and by analysing the SEM image (Fig 04 and 05). And these isolates were designated as Pa_1 to Pa_{10}. All the isolates of *P. aphanidermatum* were grown on Potato dextrose agar medium showed variation in their colony characteristics are presented in Table 2.

Colony colour

On the basis of colony colour, the test isolates Pa_2, Pa_4, Pa_5, Pa_6, Pa_8, Pa_9 and Pa_{10} showed white colour whereas Pa_1, Pa_3 and Pa_7 showed creamy white colour colonies.

Colony appearance and shape

The isolates were identified on the basis of colony appearance and shape. The test isolates Pa_1, Pa_3, Pa_8 and Pa_{10} produced cottony appearance and the isolates Pa_2, Pa_4, Pa_5, Pa_6, Pa_7 and Pa_9 produced fluffy growth appearance. Out of the ten isolates, six isolates (Pa_1, Pa_3, Pa_4, Pa_6, Pa_7 and Pa_{10}) showed circular margin, the remaining isolates (Pa_2, Pa_5, Pa_8 and Pa_9) showed irregular margin.

Mycelial growth

The results revealed that all the isolates exhibited variability with respect to mycelial growth. Among them, the maximum mycelial growth was recorded by Pa_9 (90.00mm) followed by Pa_5 (89.89mm), Pa_2 (88.85mm), Pa_3 (87.92mm), Pa_8 (86.45mm), Pa_{10} (85.54mm), Pa_7 (84.59mm), Pa_4 (83.18mm) and Pa_1 (82.55mm) in the decreasing order of merit. The minimum growth was recorded by Pa_6 (79.95mm).

Subharathinam *et al* (2020) [26] isolated *Pythium* spp. in 21 different locations of Tamil Nadu, the cultural characters like colony colour and colony pattern revealed that, all the isolates produced white colour with cottony growth.

The findings are similar with Kamali *et al* (2020) [25], worked on five isolates of *Pythium aphanidermatum* collected from tomato growing areas of Madurai and Thenkasi district of Tamil Nadu. Jayalakshmi *et al* (2021) [23] collected infected samples of tomato from different parts of Tamil Nadu. Isolated and characterized the pathogen based on their morphological characters and they confirmed the isolates as *Pythium aphanidermatum* by observing the hyaline, non- septate mycelium, oospore and lobed sporangial formation.

Pathogenicity

A pot culture experiment was conducted to test the virulence of isolates collected from different locations of tomato growing areas of Tamil Nadu. Among the ten isolates collected, the isolate Pa_9 collected from Chenathur village of Krishnagiri district was found to be more virulent, it recorded the maximum disease incidence of 35.68% of pre-emergence and 67.59% of post- emergence damping-off under artificial inoculation (Table 3). Similar findings are reported by Karmel Reetha and Muthukumar (2019) [22], who reported that 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. Hassanisaadi (2021) [27] experimented the pathogenicity test and observed the infected tomato seedlings roots, which became necrotic. The pathogen-seedling interaction revealed that *Pythium aphanidermatum* was pathogenic on *Lycopersicon esculentum* (Fig 06 and 07).

Table 1: Survey on the incidence of damping-off of tomato from major tomato growing areas in Tamil Nadu

S. No.	Isolates	Location	District	Isolate identification code	Soil type	Variety	Disease Incidence (%)
1.	*Pythium aphanidermatum*	Pulikkarai	Dharmapuri	Pa_1	Red sandy soil	CO 1	12.48^h (20.68)
2.	*P. aphanidermatum*	Papparapatti		Pa_2	Red sandy soil	Local	29.97^b (33.19)
3.	*P. aphanidermatum*	Nallampalli		Pa_3	Black clay loam soil	PKM 1	26.73^c (31.13)
4.	*P. aphanidermatum*	Andipatti		Pa_4	Red sandy soil	PKM 1	15.23^g (22.97)
5.	*P. aphanidermatum*	Sivapuri	Cuddalore	Pa_5	Sandy clay soil	CO 1	31.18^b (33.94)
6.	*P. aphanidermatum*	Keerapalayam		Pa_6	Sandy coastal alluviam	COTH 1	9.28^i (17.73)
7.	*P. aphanidermatum*	Virudhachalam		Pa_7	Red loam soil	PKM 1	18.09^f (25.17)
8.	*P. aphanidermatum*	Irumathur	Krishnagiri	Pa_8	Clay soil	CO 1	24.42^d (29.61)
9.	*P. aphanidermatum*	Chenathur		Pa_9	Sandy loam	PKM 1	34.88^a (36.19)
10.	*P. aphanidermatum*	Potchampalli		Pa_{10}	Sandy loam	Local	20.25^e (26.74)

Pa* - *Pythium aphanidermatum* isolates

*Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

Table 2: Isolation and cultural characters of different isolates of *Pythium aphanidermatum*

S. No.	Isolates	Colony characters			
		Colour	Appearance	Shape	Mycelial growth (mm) (3 DAI)
1.	Pa_1	Creamy white	Cottony	Circular	82.55^{f}
2.	Pa_2	White	Fluffy	Irregular	88.85^{ab}
3.	Pa_3	Creamy white	Cottony	Circular	87.92^{abc}
4.	Pa_4	White	Fluffy	Circular	83.18^{ef}
5.	Pa_5	White	Fluffy	Irregular	89.89^{a}
6.	Pa_6	White	Fluffy	Circular	79.95^{g}
7.	Pa_7	Creamy white	Fluffy	Circular	84.59^{def}
8.	Pa_8	White	Cottony	Irregular	86.45^{bcd}
9.	Pa_9	White	Fluffy	Irregular	90.00^{a}
10.	Pa_{10}	White	Cottony	Circular	85.54^{cde}

*Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

Table 3: Pathogenicity of *P. aphanidermatum* isolates under pot culture

S. No.	Isolates	Pre-emergence damping-off (%)	Post-emergence damping-off (%)
1.	Pa_1	18.24^{i} (25.28)	39.45^{h} (38.90)
2.	Pa_2	29.51^{c} (32.90)	60.18^{c} (50.87)
3.	Pa_3	28.24^{d} (32.10)	56.28^{d} (48.60)
4.	Pa_4	20.12^{h} (26.65)	42.15^{h} (40.48)
5.	Pa_5	33.21^{b} (35.18)	64.13^{b} (53.20)
6.	Pa_6	15.54^{j} (23.21)	35.72^{i} (36.70)
7.	Pa_7	22.19^{g} (28.10)	45.75^{g} (42.56)
8.	Pa_8	25.78^{e} (30.51)	52.17^{e} (46.24)
9.	Pa_9	35.68^{a} (36.67)	67.59^{a} (55.29)
10.	Pa_{10}	24.78^{f} (29.85)	48.75^{f} (44.28)

*Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

Fig 1: Symptoms of Tomato damping-off disease

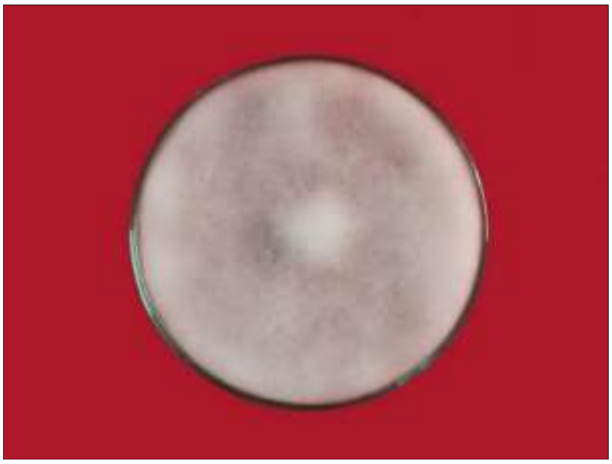

Fig 2: Auxenic culture of *Pythium aphanidermatum*

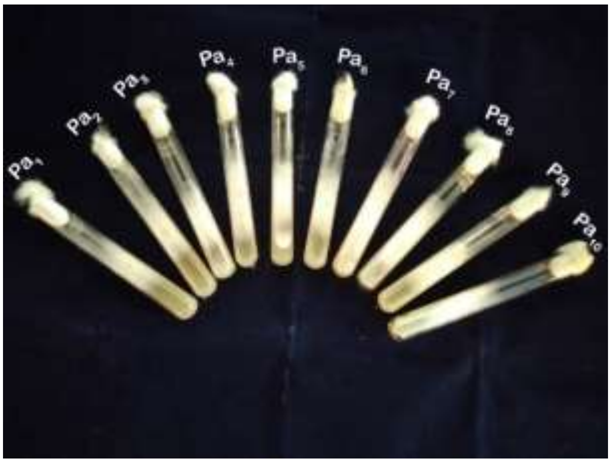

Fig 3: Different isolates of *P. aphanidermatum*

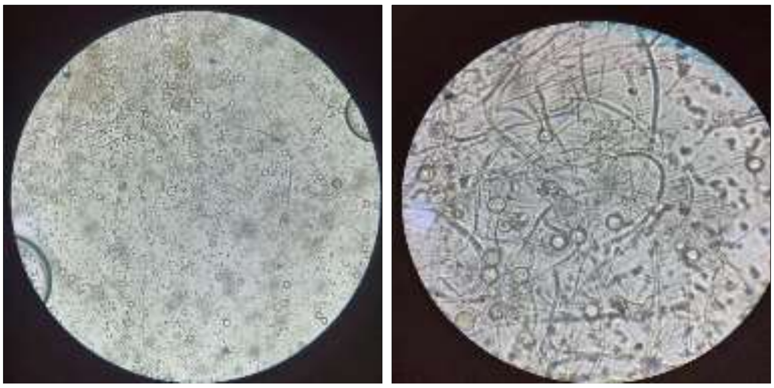

Fig 4: Microscopic view of *P. aphanidermatum*

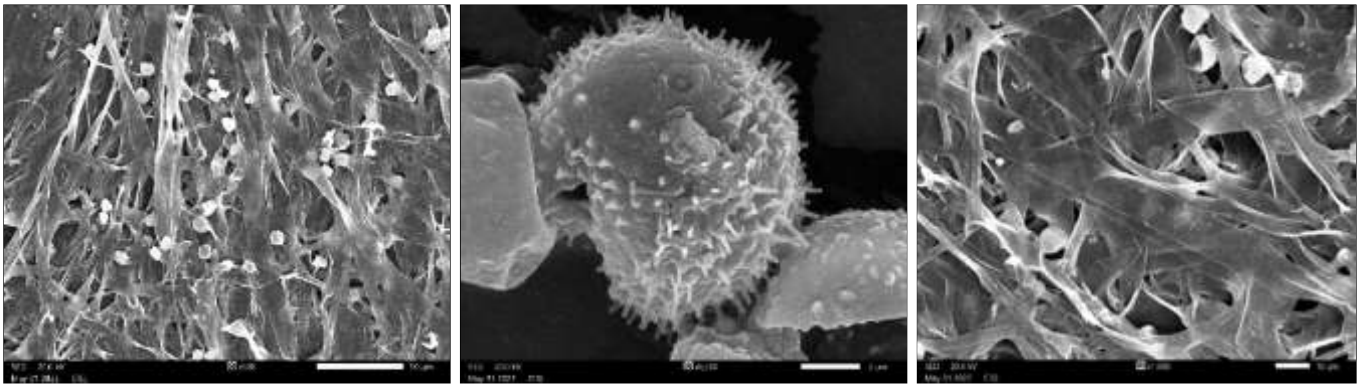

Fig 5: SEM observation of *P. aphanidermatum* (Pa$_9$)

Fig 6: Mass multiplication of *P. aphanidermatum*

Fig 7: Pathogenicity of *P. aphanidermatum*

Conclusion

The study focused on the isolation, cultural and morphological characterisation, and pathogenicity of tomato damping-off caused by *Pythium aphanidermatum*. The isolates displayed significant variation and diversity in their morphological and cultural characteristics, indicating the existence of genetic diversity among the pathogen population. Pathogenicity studies revealed that isolate Pa$_9$ was the most virulent among the isolates, with a high disease incidence rate of 34.88%, while the lowest disease incidence rate of 9.28% was observed in Keerapalayam village of Cuddalore district. These findings provide valuable insights into the pathogenicity and diversity of *Pythium aphanidermatum*, which could be useful in developing effective strategies to manage and control tomato damping-off. Further research is needed to better understand the factors contributing to the virulence and genetic diversity of this pathogen, which could ultimately help to reduce crop losses and support sustainable agriculture practices.

References

1. Maham SG, Rahimi A, Subramanian S, Smith DL. The environmental impacts of organic greenhouse tomato production based on the nitrogen-fixing plant (Azolla). Journal of Cleaner Production. 2020;245:118679.
2. Pastor N, Masciarelli O, Fischer S, Luna V, Rovera M. Potential of *Pseudomonas putida* PCI2 for the protection of tomato plants against fungal pathogens. Current Microbiology. 2016;73:346-353.
3. Gondal AS, Ijaj M, Riaz K, Khan AR. Effect of different doses of fungicide (Mancozeb) against *Alternaria* leaf blight of tomato in tunnel. Journal of Plant Pathology & Microbiology. 2012;3:125.

4. Naika S, van Lidt de Jeude J, de Goffau M, Hilmi M, van Dam B. Cultivation of tomato: production, processing and marketing. Agromisa Foundation and CTA. Wageningen, Netherlands; 2005.
5. Pastor N, Rosas S, Luna V, Rovera M. Inoculation with *Pseudomonas putida* PCI2, a phosphate solubilizing rhizobacterium, stimulates the growth of tomato plants. Symbiosis. 2014;62:157-167.
6. Bhowmik D, Kumar KS, Paswan S, Srivastava S. Tomato—a natural medicine and its health benefits. Journal of Pharmacognosy and Phytochemistry. 2012;1(1):33-43.
7. Keswani C. Proteomics studies of thermotolerant strain of *Trichoderma* spp. [Ph.D. thesis]. Varanasi: Banaras Hindu University; 2015.
8. Pattnaik MM, Kar M, Sahu RK. Bioefficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicum esculentum*. Asian Journal of Plant Science. 2012;2:129-142.
9. Mohamed HA, Nasr NF, Abdelkreem KI. Management of tomato damping-off disease caused by *Fusarium oxysporum* and *Rhizoctonia solani* using chemical and biological degradable olive mill wastewater. Egyptian Journal of Biological Pest Control. 2015;25(2):367-377.
10. Hamza A, Mohamed A, Derbalah A. Unconventional alternatives for control of tomato root rot caused by *Rhizoctonia solani* under greenhouse conditions. Journal of Plant Protection Research. 2016;56(3):298-305.
11. Shenashen M, Derbalah A, Hamza A, Mohamed A, El Safty S. Recent trend in controlling root rot disease of tomato caused by *Fusarium solani* using aluminasilica nanoparticles. International Journal of Advanced Research in Biological Sciences. 2017;4(6):105-119.
12. Jayaraj J, Radhakrishnan NV, Kannan R, Suganya D, Venkatesan S, Sakthivel K, *et al* Development of new formulations of *Bacillus subtilis* for management of tomato damping-off caused by *Pythium aphanidermatum*. Biocontrol Science and Technology. 2005;15(1):55-65.
13. Jeyaseelan EC, Tharmila S, Niranjan K. Antagonistic activity of *Trichoderma* spp. and *Bacillus* spp. against *Pythium aphanidermatum* isolated from tomato damping-off. Archives of Applied Science Research. 2012;4(4):1623-1627.
14. Kipngeno P, Losenge T, Maina N, Kahangi E, Juma P. Efficacy of *Bacillus subtilis* and *Trichoderma asperellum* against *Pythium aphanidermatum* in tomatoes. Biological Control. 2015;90:92-95.
15. Rangaswami G. An agar block technique for isolating soil microorganism with special reference to pythiaceous fungi. Science and Culture. 1958;24:85.
16. Elshahawy IE, Karima HH, Abd-El-Khair H. Compatibility of *Trichoderma* spp. with seven chemical fungicides used in the control of soil borne plant pathogens. Research Journal of Pharmacy and Technology. 2016;7(1):1772-1785.
17. Ainsworth GC. Dictionary of fungi. Kew Burrey, England: Common Wealth Mycological Institute; 1961. p. 547.
18. Rangaswami G. Diseases of crop plants in India. New Delhi: Prentice Hall of India Pvt. Ltd.; 1972. p. 520.
19. Middleton JT. The taxonomy, host range and geographic distribution of the genus *Pythium*. Memoirs of the Torrey Botanical Club. 1943;20(1):1-171.
20. Balakrishnan MS. South Indian phycomycetes-I. Proceedings of the Indian Academy of Sciences - Section B. 1948;27(6):161-173.
21. Usharani S, Satheesh G. Management of damping-off of tomato caused by *Pythium aphanidermatum* (Edson.) Fitzp. through biocontrol agents, organic amendments and soil solarization. Plant Disease Research. 2007;22:27-29.
22. Reetha KA, Muthukumar A. Occurrence, virulence and pathogenicity of *Pythium aphanidermatum* causing tomato damping-off. Plant Archives. 2019;19(2):2476-2480.
23. Jayalakshmi R, Mehetre ST, Kannan R, Paramasivam M, Santhnakrishnan VP, Kumar KK, *et al* Isolation and characterization of damping-off and wilt pathogens of tomato. The Pharma Innovation Journal. 2021;10(5):233-239.
24. Kumar R, Gupta SK, Gupta M. Investigation on etiology and epidemiology of damping-off of tomato seedlings in mid hills of Himachal Pradesh. Indian Phytopathology. 2018;71(4):513-518.
25. Kamali P, Akila R, Thiruvudainambi S, Vellaikumar S, Theradimani M. Antimycotic efficacy of organic amendments against *Pythium aphanidermatum* (Edson) Fitzp., the incitant of tomato (*Solanum lycopersicum* L.) damping-off. International Journal of Current Microbiology and Applied Sciences. 2020;9(11):3582-3589.
26. Subharathinam M, Sanjeevkumar K, Balabaskar P, Kumar S. Isolation, identification, and molecular characterization of *Pythium* species from brinjal growing tracts of Erode and Cuddalore district. Plant Archives. 2020;20(1):3411-3416.
27. Hassanisaadi M, Bonjar GHS, Hosseinipour A, Abdolshahi R, Barka EA, Saadoun I. Biological control of *Pythium aphanidermatum*, the causal agent of tomato root rot by two *Streptomyces* root symbionts. Agronomy. 2021;11:846.