Biological Forum – An International Journal **15(5): 283-288(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Exploration of Potent Biocontrol Agent against the Damping off Disease of Tomato (*Pythium aphanidematum)* and its *in-vitro* Management

Sultana A.S.[1*], Thiruvudainambi S.[2], Masurkar P.[3] and Kumar A.[4]
[1]PG Scholar, Department of Plant Pathology,
Tamil Nadu Agricultural University (Tamil Nadu), India.
[2]Professor, Department of Plant Pathology,
Tamil Nadu Agricultural University (Tamil Nadu), India.
[3]Assistant Professor, Department of Plant Pathology,
Lovely Professional University (Tamil Nadu), India.
[4]Assistant Professor, Department of Entomology,
Bihar Agricultural University, Sabour, Bhagalpur (Bihar), India.

(Corresponding author: Sultana A.S.)*
(Received: 03 March 2023; Revised: 06 April 2023; Accepted: 20 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: The identification of potential biocontrol agents from the large-scale screening conducted in surveyed regions, as well as evaluating their efficacy, was a very cumbersome task. A total number of twenty isolates of two different biocontrol agents ten each of *Trichoderma* sp. and *Pseudomonas* sp. were isolated from soils of Tirunelveli Tamil Nadu, India, and were evaluated for their ability to inhibit plant pathogenic *Pythium aphanidematum in vitro*. We evaluated the bio-control agent from the same place as the disease. It was found that the isolates had the pathogenic inhibitory ability and also control *Pythium aphanidematum* damping-off in tomatoes in interested field soils. Both the isolates tested significantly reduced damping-off severity in tomatoes. A difference in their effect on plant disease severity, percentage of dead plants, and plant biomass in the pathogen's presence was also observed among both fungal and bacterial isolates. In vitro, the efficacy of the screened potential bio-control agents i.e. four *Trichoderma* and three *Pseudomonas* species was analyzed. 4 fungal (TNAUF2, TNAUF6, TNAUF9, TNAUF12) and 3 bacterial (TNAUB3, TNAUB6, and TNAUB10) isolates showed significant inhibition against *Pythium aphanidematum* over control. The highest percent inhibition was found in TNAUF6 (*T. viride*) isolate and the minimum percent inhibition was found in TNAUB10 (*Pseudomonas fluorescens*). This study is important because native bio-control agents are expected to work more efficiently.

Keywords: Biological Control, Damping off, Tomato, *Trichoderma*, *Pseudomonas*, *Pythium.*

INTRODUCTION

Tomato is one of the most favored vegetable crops in terms of culinary and food preservation purposes. With a total area of 83,000 hectares and a yield of 7,90,000 tonnes, it is one of the most commercially significant vegetable crops in India. Vitamin C, lycopene, and beta-carotene, three antioxidants that support healthy health are abundant in tomatoes. The carotenoid, potassium, vitamin C, and vitamin A content of tomatoes largely determines their nutritional value. Ripe tomatoes have significant quantities of carotenoids, of which carotenes make up between 90 and 95% (Guil-Guerrero and Rebolloso-Fuentes 2009). Propagated through the seed the tomato crop is affected by a number of fungal, bacterial and viral pathogens. Damping off, septorial leaf spot, bacterial stem, fruit canker, early blight, bacterial Leaf spot, bacterial wilt leaf curl, and mosaic are some diseases that cause economic losses to crops
(Kumar *et al.*, 2020). In India, the earliest record of damping off was made in Pusa (Butler, 1918), and yield losses of up to 50% were noted in Tamil Nadu (Lukyanenko, 1991). A detailed accounting of the damages caused by tomato damping-off making seedlings is challenging, but the financial losses caused by damping-off are reflected by a direct cost, owing to seed or seedlings, as well as an indirect cost made up of an increased replanting fee and reduced yields as a result of the later planting dates (Babadoost and Islam 2003; Bacharis *et al.*, 2010; Horst 2013). In India and around the world, damping-off is a common occurrence. From the time of planting until the fourth to sixth week after seeding, damping-off signs might be seen (Horst, 2013). Depending on when they first appeared, the disease symptoms can be split into two periods. First is pre-emergence damping off and second is post-emergence damping off. Tomato damping-off caused by *Pythium aphanidematum* is a complex soil-borne pathogen from class oomycetes having a wide host range (Pravin and Sharma 2015). Pathogen infects plants via motile zoospores and needs high humidity to spread in the plants. Several strategies must be used to effectively manage damping-off, including treating seeds to increase germination and seedling vigor, using resistant or tolerant cultivars, adopting best cropping practices, and timely treating seedlings with potent products like some systemic fungicides as well as

biopesticides or biocontrol agents. Some biocontrol agents like *Trichoderma sp.* having the quality not only to arrest the spread of the pathogen but also to invade the surface of its colony and sporulate over the colony (Elshahawy and El-Mohamedy 2019). *Pseudomonas* sp. is also one of the potential living bacteria which it's the damping-off pathogen by synthesis of primary and secondary metabolites in the surrounding environment (Sharma *et al.,* 2007). Several studies on the in-vitro efficacy of *Trichoderma* were checked against *Pythium aphanidermatum* in tomatoes (Madhumita *et al.,* 2022; Liu *et al.,* 2022) while *Pseudomonas* was also found to inhibit the *Pythium* by production of secondary metabolites (Soesanto *et al.,* 2022; Stouvenakers *et al.,* 2022; Haque and Khan 2022). The bio-control agent's like *Tricoderma* spp and *Pseudomonas* spp efficacy also depend upon the soil ecology (Shahriar *et al.,* 2022; Chin *et al.,* 2022). If the habitat of disease collection and bio-control agent were the same then there were higher chances that the native bio-control agent will control the soil-borne diseases more effectively (Win *et al.,* 2021; Al-Hussini *et al.,* 2019; Al-Daghari, 2020; Haghi *et al.,* 2023). In these regards, efforts has been made to identify potential native biocontrol agent against the *Pytium aphanidermatum.* This study aimed to identify a potential native biocontrol agent for controlling *Pythium aphanidematum* and develop an effective in-vitro management strategy for tomato damping off. Several potential biocontrol agents were screened for their antagonistic activity against the pathogen. The antagonistic activity was evaluated through in-vitro dual-culture assays, where the growth inhibition of *Pythium aphanidematum* was assessed.

MATERIALS AND METHODS

Isolation and purification and maintenance of *Pythium aphanidematum*. The sick tomato plants were transported to the lab, where 1 cm sections of the contaminated stem were taken and washed with tap water for three minutes before being superficially cleaned with 1 percent sodium hypochlorite (NaOCl) for one to two minutes. As a result, they were dried for 5 minutes before being put on PDA-containing petri plates and incubated at 20 to 25 °C. To create pure cultures, mycelial discs with a 4 mm diameter were placed in petri plates containing PDA from the borders of colonies that had developed within 4-5 days. After that, these isolates were put into test tubes containing PDA and kept at +4°C for subsequent examination. Fungal isolates were identified at the species level using morphological keys and spore identification.

Isolation and purification and maintenance of *trichoderma species.* Rhizospheric soil (10 g) was combined with 250 ml of phosphate-buffered saline, streptomycin (100 mg/l), and penicillin (100 mg/l), which were added to the mixture to inhibit the growth of native bacteria. The mixture was then incubated at 28 ±2 °C for two hours on a rotary shaker at a speed of 150 rpm. The inoculation plates were incubated for 7 days at a temperature of 28 ±2 °C, and fungal growth was periodically visually monitored. The probable fungus was isolated onto potato dextrose agar (PDA) slants at the conclusion of the incubation period. Growing the fungi on PDA at 28 ±2 °C for 5-7 days allowed us to distinguish the different fungal isolates based on their morphological characteristics. The colour, texture, border, and sporulation of the fungi colonies were all visually assessed. According to the procedure recommended by Rifai, 1969 and Leahy et al. 1990 individual fungal isolates were identified at the species level using morphological keys and species descriptions (conidiophores, form, size, arrangement, and phialides arrangements were evaluated microscopically).

Isolation and purification and maintenance of *Pseudomonas* sp. Soil was used to isolate the bacteria pseudomonas sp. The material was serially diluted and then bulk seeded on a sterile King's B agar plate, accordingly. Appropriate colonies were chosen, purified, sub-cultured, preserved for further study, and exposed to identification tests after incubation under aerobic conditions at 37°C. *Pseudomonas* species isolation from a soil sample performed for uses of it as a biocontrol agent. According to Bergey's Manual of Systematic Bacteriology, the isolates of the chosen strains were identified using cultural, morphological, and biochemical tests such Gram staining.

Evaluation of Antagonistic Potential of Different Bio-Agents against *Pythium aphanidematum*

Dual Culture Assay of Bio-Controls against the *Pythium aphanidematum*

By *Trichoderma* sp. Using the dual-culture approach, it was determined if each strain or isolate had an antagonistic impact on the pathogens *Pythium aphanidematum*. On PDA medium, the reported *Trichoderma* isolates were grown for six days at 20°C. Each isolate's 5-mm-diameter discs were infected on one side of PDA medium on a Petri dish, and the pathogen disc was inoculated on the other. As a control, plates with only the pathogen were used. Data on growth zones and colony diameters were logged after incubation of five and seven days periods at 25.2°C with alternating light and darkness.

The following calculation was used to compute the percentage of the pathogen's growth that was inhibited:

The formula percent Inhibition of Radial Growth (PIRG) = (R1 - R2)/R1*100 was used to determine the percent growth inhibition against pathogen growth. Where R1, pathogen mycelium radius on the control plate, and R2, pathogen mycelium radius in the dual culture plate (*Trichoderma* and pathogen).

By *Pseudomonas* Sp. We utilized a 5 mm hole punch to extract the plugs containing mycelia from the phytopathogenic fungi that had been grown for 7 days and placed each plug in the middle of PDA culture media. Then, we streaked 2.5 cm from one side of the plug using a sterile loop that had been dipped into the overnight culture of ST-TJ4 bacterial solution. The length of the inhibitory zone between the bacterial colony and the fungus pathogen was assessed after 5-7 days. As a control, fungi that weren't exposed to bacteria were used.

Inhibition rate (percent) = (Cd - Td) 100 percent /Cd, where Cd is the radial mycelia growth in the control

and Td is the radial growth of the fungal pathogens in the treatment, was used to determine the plant pathogenic fungus inhibition rate (dual culture).

RESULTS

Collection of biocontrol agents from surveyed fields and their growth pattern and morphological features. The soil sample collected during survey and processed by serial dilution method for isolation of bioagents. Soil samples were collected from different locations and from each location it was collected from three differently cultivated fields i.e. vegetable, cereal and pulse fields to obtain diverse nature of fungus and bacterium. From the collected samples total 10 fungal samples and 10 bacterial were processed for further identification on the basis of morphology of bioagents.

The distinct *Trichoderma* colonies and bacterial colony with varied colony identified and transferred in PDA and NA for further purification. The fungal isolates were given abbreviation of TNAUF and numbered as TNAUF1, TNAUF2 and so on up to TNAUF10 while the bacterial isolates were abbreviated as TNAUB and numbered as TNAUB1, TNAUB2 and so on up to TNAUB10. The morphological and cultural identification were done for both the fungal and bacterial isolates.

Morphological and cultural characteristics of *Trichoderma* isolates and their identification. The Trichodema isolates were checked for colony colour, conidiophores, phialides and conidia for species level identification. The isolates TNAUF1, TNAUF3, TNAUF6 and TNAUF9 showed dark green colony. These isolates conidiophores and phialides were branched irregularly, slightly hook like and its base found constricted. These isolates conidia were found spherical to subglubose. On the basis of these characteristics these isolates was identified as *T. viride*.

The isolates TNAUF10 colony colour i.e. whitish grey, conidiophores and phialides were ampuliform, base consticted, swollen but peak slender and conidia were subglobose to ellipsoidal and smooth walled. All these isolates were grouped as *T. harzianum* on the basis of morphology and conidial characters.

Isolate number TNAUF2, TNAUF4, TNAUF5, TNAUF7 and TNAUF8 identified as *T. atroviride* as their morphological features were matched. The colony colour were found yellow green, condiophores centered were less swollen with single phialides. The conidia were found ellipsoidal to subglobose in shape (Table 1, Fig. 1).

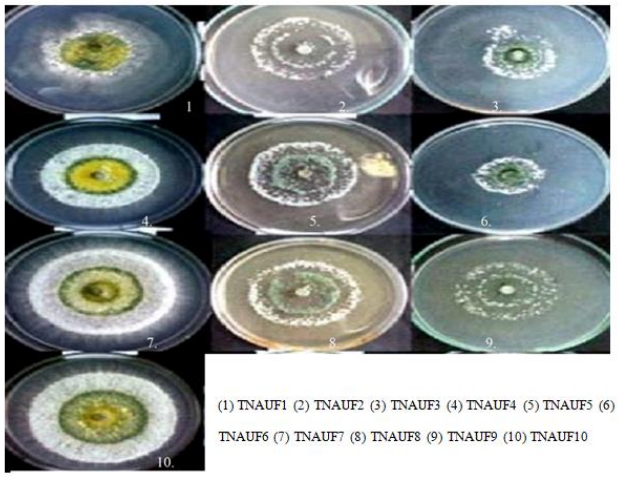
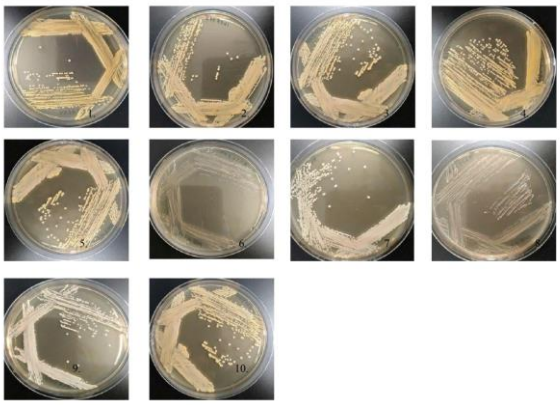


Fig. 1. Cultural and Morphological Variability of *Trichoderma* Isolates.

Table 1: Morphological and growth pattern of features of fungal isolates.

Sr. No.	Isolate	Colony colour	Conidiophores and phialides	Conidia	Identified as
1.	TNAUF1	Dark green	Branched Irregularly, slightly hook like, base constricted	Sperical or subglubose	*Trichoderma viride*
2.	TNAUF2	Yellow green	Less swollen centre, single phialides	Ellipsoidal to subglobose	*Trichoderma atroviride*
3.	TNAUF3	Dark green	Branched Irregularly, slightly hook like, base constricted	Sperical or subglubose	*Trichoderma viride*
4.	TNAUF4	Yellow green	Less swollen centre, single phialides	Ellipsoidal to subglobose	*Trichoderma atroviride*
5.	TNAUF5	Yellow green	Less swollen centre, single phialides	Ellipsoidal to subglobose	*Trichoderma atroviride*
6.	TNAUF6	Dark green	Branched Irregularly, slightly hook like, base constricted	Sperical or subglubose	*Trichoderma viride*
7.	TNAUF7	Yellow green	Less swollen centre, single phialides	Ellipsoidal to subglobose	*Trichoderma atroviride*
8.	TNAUF8	Yellow green	Less swollen centre, single phialides	Ellipsoidal to subglobose	*Trichoderma atroviride*
9.	TNAUF9	Dark green	Branched Irregularly, slightly hook like, base constricted	Sperical or subglubose	*Trichoderma viride*
10.	TNAUF10	Whitish to greyish	Ampuliform, base consticted, Swollen, peak slender	Subglobose to ellipsoidal, Smooth walled	*Trichoderma harzianum*

Morphological and cultural characteristics of *Pseudomonas* isolates and their identification. All the isolated bacteria were appeared as round raised and smooth shiny colony in morphology but difference in their colony color were observed. The isolates TNAUB1, TNAUB2, TNAUB3, TNAUB4, TNAUB5 and TNAUB10 were found light yellow in colour and showed fluorescence when grown on Kings B media as fluorescent green. All these bacterial isolates were identified as Pseudomonas fluorescence. While the bacterial isolates TNAUB6, TNAUB7, TNAUB8 and TNAUB9 showed off white colony colour and not flourish any colour when grown on the Kings-B media. These bacterial isolates were identified as *P. aeruginosa* (Table 2, Fig. 2).



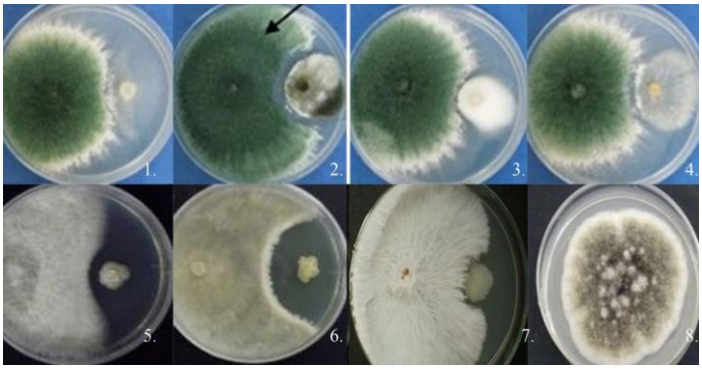
(1) TNAUB1 (2) TNAUB2 (3) TNAUB3 (4) TNAUB4 (5) TNAUB5 (6) TNAUB6 (7) TNAUB7 (8) TNAUB8 (9) TNAUB9 (10) TNAUB10

Fig. 2. Cultural and Morphological Variability of *Pseudomonas* Isolates.

Table 2: Morphology and growth pattern of bacterial isolates

Sr. No.	Isolate	Colony Morphological	Colour	Pigmentation on Kings B	Identified as
1.	TNAUB1	Round raised smooth shiney	Light yellow	Fluorescent green	*Pseudomonas fluorescens*
2.	TNAUB2	Round raised smooth shiney	Light yellow	Fluorescent green	*Pseudomonas fluorescens*
3.	TNAUB3	Round raised smooth shiney	Light yellow	Fluorescent green	*Pseudomonas fluorescens*
4.	TNAUB4	Round raised smooth shiney	Light yellow	Fluorescent green	*Pseudomonas fluorescens*
5.	TNAUB5	Round raised smooth shiney	Light yellow	Fluorescent green	*Pseudomonas fluorescens*
6.	TNAUB6	Round raised smooth shiney	Off-white	-	*Pseudomonas aeruginosa*
7.	TNAUB7	Round raised smooth shiney	Off-white	-	*Pseudomonas aeruginosa*
8.	TNAUB8	Round raised smooth shiney	Off-white	-	*Pseudomonas aeruginosa*
9.	TNAUB9	Round raised smooth shiney	Off-white	-	*Pseudomonas aeruginosa*
10.	TNAUB10	Round raised smooth shiney	Light yellow	Fluorescent green	*Pseudomonas fluorescens*

Antagonistic activity of collected fungal and bacterial isolates on *Pythium aphanidematum.* Out of total number of 10 *Trichoderma* sp. and 10 *Psedomonas* sp. isolates recovered from soil samples of different type of surveyed fields were taken for screening. Out of these 10 fungal and 10 bacterial isolates 4 fungal (TNAUF2, TNAUF6, TNAUF9, TNAUF12) and 3 bacterial (TNAUB3, TNAUB6 and TNAUB10) isolates showed significance inhibition against *Pythium aphanidematum* over control (Table 3). The highest percent inhibition was found in TNAUF6 (*T. viride*) isolate i.e. 82.49 % followed by TNAUF9 (*T. viride*) isolate i.e. 78.32 %. The minimum percent inhibition was found in TNAUB10 (*Pseudomonas fluorescens*) i.e. 33.64% followed by TNAUB6 (*Pseudomonas fluorescens*) with 48.33 percent inhibition (Table 3, Fig. 3). These studies suggest the higher percent of inhibition by the native isolated *T. viride* and more effective for the disease.



1. *Trichoderma viride* (TNAUF2) 2. *T. viride* (TNAUF6) 3. *T. viride* (TNAUF9) 4. *T. harzianum* (TNAUf12) 5. *Pseudomonas fluorescens* (TNAUB6) 6. *Pseudomonas fluorescens* (TNAUB6) 7. *Pseudomonas fluorescens* (TNAUB18) 8. Control

Fig. 3. Dual culture activity of the bio-agents against *A. solani*.

Table 3: Antagonistic activity of isolated microbes.

Sr. No.	Isolate name	Biocontrol agents	Percent inhibition of *Pythium aphanidematum*
1.	TNAUF2	*Trichoderma atroviride*	72.84±1.14
2.	TNAUF6	*Trichoderma viride*	82.49±0.21
3.	TNAUF9	*Trichoderma viride*	73.32±1.87
4.	TNAUF10	*Trichoderma harzianum*	70.27±0.03
5.	TNAUB3	*Pseudomonas fluorescens*	53.12±0.46
6.	TNAUB6	*Pseudomonas fluorescens*	48.35±0.47
7.	TNAUB10	*Pseudomonas fluorescens*	33.64±0.80
	Control	-	24.33±0.88
		SEM	0.91
		CD	3.75
		CV	2.35

DISCUSSION

The morphological and cultural identification were done for both the fungal and bacterial isolates. The distinct *Trichoderma* colonies and bacterial colony with varied colony identified and transferred in PDA and NA for further purification. Kumar *et al.* (2012) collected *Trichroderma* isolates from several South Andaman locales and twelve *Trichoderma* spp. isolates were evaluated for their cultural, morphological, and antagonistic activity against soil-borne and foliar-borne pathogens. The collection identification of biocontrol agents from different soil samples were also studied by Singh (2017). In their collection they recovered the 25 *Trichoderma* and 25 bacterial isolates. Verma *et al*. (2015) also isolated the beneficial bacterial isolates i.e. PGPR from the Northern hill zone of the Himalaya and evaluated the biocontrol ability on *Fusarium graminearum* and *Rhizoctonia solani*. The same criteria were adopted by El-Sobky *et al.* (2019) to identify the *Trichoderma* species. In their experiment they have checked colony colour, mycelia growth and their phialides. Shah *et al*. (2012) classified *Trichoderma* species in to three species i.e. *T. harzianum, T. viride and T. pseudokoningii* on the basis of cultural and morphological variability basis. Each of the three species could be distinguished from the others based on traits such mycelial growth rate, colony appearance, form of conidia and conidiophores, and phialide branching pattern.

İnceoğlu *et al.* (2012) also assessed the bacterial community of potato rhizosphere for the identification of beneficial bacterial strains. Vacheron *et al*. (2016) identified few fluorescent species of the *Pseudomonas.* Lauritsen et al. (2021) identified the *Pseudomonas* species on the basis of conventional method during the isolation later they have evaluated the species on the basis of 16s r-RNA sequencing.

In vitro efficacy of the screened potential bio-control agents i.e. four *Trichoderma* and three *Pseudomonas* species was analysed. 4 fungal (TNAUF2, TNAUF6, TNAUF9, TNAUF12) and 3 bacterial (TNAUB3, TNAUB6 and TNAUB10) isolates showed significance inhibition against *Pythium aphanidematum* over control. The highest percent inhibition was found in TNAUF6 (*T. viride*) isolate and minimum percent inhibition was found in TNAUB10 (*Pseudomonas fluorescens*). Arunachalam, and Sharma (2012) found also found that the *Trichoderma* controls the *Pythium aphanidematum* effectively. *Pythium aphanidematum* pathogens control measures also studied by Muthukam *et al.* (2010) and they found that the *T. virid+P. fluorescens + Zimmu leaf extact* reduces the pre and post emergence damping-off incidence. By using the dual culture method, the effectiveness of the biocontrol agents *Trichoderma viride, Trichoderma harzianum, Trichoderma virens, Pseudomonas fluorescens, Bacillus subtilis,* and *Pseudomonas putida* was examined for its antagonism against *P. aphanidermatum* by Priya and Patel, 2016 and they found *Trichoderma viride* (54.22%) and *Pseudomonas peutala* (53.33) were at par with each other. Mythukumar, 2009 also used *T. viride* and *P. fluorescens* formulations against the *Pythium aphanidematum.* In screening native potential *Trichoderma spp* and *Pseudomonas* spp was evaluated and because of the location specificity they showed more inhibition and acted more effectively on soil borne pathogen *Pythium aphinidermatumn* which having the same location. The native bio-control agent would control the soil borne diseases more successfully if the habitat of disease collection and the bio-control agent were the same habitat.

CONCLUSION AND FUTURE SCOPE

The *in vitro* management strategies provide valuable insights for optimizing the application of the biocontrol agent in a controlled environment. Further research is warranted to evaluate the efficacy of the biocontrol agent under field conditions and assess its compatibility with existing integrated pest management practices.

REFERENCES

Al-Daghari, D. S. S., Al-Abri, S. A., Al-Mahmooli, I. H., Al-Sadi, A. M. and Velazhahan, R. (2020). Efficacy of native antagonistic rhizobacteria in the biological control of *Pythium aphanidermatum*-induced damping-off of cucumber in Oman. *Journal of Plant Pathology, 102,* 305-310.

Al-Hussini, H. S., Al-Rawahi, A. Y., Al-Marhoon, A. A., Al-Abri, S. A., Al-Mahmooli, I. H., Al-Sadi, A. M. and Velazhahan, R. (2019). Biological control of damping-off of tomato caused by *Pythium aphanidermatum* by using native antagonistic rhizobacteria isolated from Omani soil. *Journal of Plant Pathology, 101*, 315-322.

Arunachalam, M. K. and Sharma, P. (2012). Confrontation assay for Trichoderma as a potential biocontrol agent against *Pythium aphanidermatum* and *Sclerotinia*

sclerotiorum. *Pest Management in Horticultural Ecosystems*, *18*(1), 74-77.

Babadoost, M. and Islam, S. Z. (2003). Fungicide seed treatment effects on seedling damping-off of pumpkin caused by *Phytophthora capsici*. *Plant disease*, *87*(1), 63-68.

Bacharis, C., Gouziotis, A., Kalogeropoulou, P., Koutita, O., Tzavella-Klonari, K. and Karaoglanidis, G. S. (2010). Characterization of *Rhizoctonia* spp. isolates associated with damping-off disease in cotton and tobacco seedlings in Greece. *Plant disease*, *94*(11), 1314-1322.

Butler, E. J. (1918). Fungi and Diseases in Plants. Thaker Spinck and Co. Calcutta pp 547.

Chin, J. M., Lim, Y. Y. and Ting, A. S. Y. (2022). Biopriming Pseudomonas fluorescens to vegetable seeds with biopolymers to promote coating efficacy, seed germination and disease suppression. *Journal of the Saudi Society of Agricultural Sciences*, *21*(8), 493-505.

Elshahawy, I. E. and El-Mohamedy, R. S. (2019). Biological control of Pythium damping-off and root-rot diseases of tomato using Trichoderma isolates employed alone or in combination. *Journal of Plant Pathology*, *101*(3), 597-608.

Guil-Guerrero, J. L. and Rebolloso-Fuentes, M. M. (2009). Nutrient composition and antioxidant activity of eight tomato (*Lycopersicon esculentum*) varieties. *Journal of Food Composition and analysis*, *22*(2), 123-129.

Haghi, Z., Mostowfizadeh-Ghalamfarsa, R. and Steinberg, C. (2023). The efficacy of Iranian Pythium oligandrum isolates in biocontrol of soil-borne fungal pathogens of tomato. *Journal of Plant Pathology*, *105*(1), 185-196.

Haque, Z. and Khan, M. R. (2022). Host resistance and bio-management of tobacco root-rot caused by *Pythium aphanidermatum*. *Indian Phytopathology*, *75*(3), 703-712.

Horst, R. K. (Ed.). (2013). *Westcott's plant disease handbook*. Springer Science & Business Media.

Kumar, A., Kumar, V., Gull, A. and Nayik, G. A. (2020). Tomato (*Solanum Lycopersicon*). In *Antioxidants in Vegetables and Nuts-Properties and Health Benefits* (pp. 191-207). Springer, Singapore.

Liu, Y., He, P., He, P., Munir, S., Ahmed, A., Wu, Y. and He, Y. (2022). Potential biocontrol efficiency of Trichoderma species against oomycete pathogens. Frontiers in Microbiology, 13.

Lukyanenko, A. N. (1991). Disease resistance in tomato. In *Genetic improvement of tomato* (pp. 99-119). Springer, Berlin, Heidelberg.

Madhumitha, B., Gnanaprakash, S., Jayapradha, C., Thankappan, S., Rathikannu, S. and Priyanga, T. (2022). In vitro bio-efficacy of biocontrol agents and oil cakes against *Pythium aphanidermatum* from tomato. *Journal of Environmental Biology*, *43*(6), 864-872.

Muthukumar, A., Eswaran, A., Nakkeeran, S. and Sangeetha, G. (2010). Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. *Crop Protection*, *29*(12), 1483-1488.

Mythukumar, A. (2009). Trichoderma viride and Pseudomonas fluorescens formulations against *Pythium aphanidermatum* in vitro. *Indian Journal of Plant Protection*, *37*(1/2), 204-206.

Parveen, T. and Sharma, K. (2015). Pythium diseases, control and management strategies: a review. *International Journal of Plant, Animal and Environmental Sciences*, *5*(1), 244-257.

Parveen, T., Meena, M., Jain, T., Rathore, K., Mehta, S. and Sharma, K. (2020). *Pythium aphanidermatum* and its control measures. In *Pythium* (pp. 299-313). CRC Press.

Priya, S. H. E. T. H., and Patel, S. J. (2016). Efficacy of Bio-agents against *Pythium aphanidermatum* in vitro. *Advances in Life Sciences*, *5*(5), 1716-1721.

Shahriar, S. A., Islam, M. N., Chun, C. N., Kaur, P., Rahim, M. A., Islam, M. M., Uddain, J. and Siddiquee, S. (2022). Microbial metabolomics interaction and ecological challenges of Trichoderma species as biocontrol inoculant in crop rhizosphere. *Agronomy*, *12*(4), 900.

Sharma, A., Jansen, R., Nimtz, M., Johri, B. N. and Wray, V. (2007). Rhamnolipids from the rhizosphere bacterium *Pseudomonas* sp. GRP3 that reduces damping-off disease in chilli and tomato nurseries. *Journal of natural products*, *70*(6), 941-947.

Soesanto, L., Latifah, S., Manan, A., Mugiastuti, E. and Suharti, W. S. (2022). Various pH Media Influence Production of Pseudomonas fluorescens P20 Raw Secondary Metabolites for Controlling Damping-off (*Pythium* sp.) in Cucumber Seedlings. *AGRIVITA, Journal of Agricultural Science*, *44*(1), 178-186.

Stouvenakers, G., Massart, S. and Jijakli, M. H. (2022). First Study Case of Microbial Biocontrol Agents Isolated from Aquaponics through the Mining of High-Throughput Sequencing Data to Control *Pythium aphanidermatum* on Lettuce. *Microbial Ecology*, 1-13.

Win, T. T., Bo, B., Malec, P., Khan, S. and Fu, P. (2021). Newly isolated strain of *Trichoderma asperellum* from disease suppressive soil is a potential bio-control agent to suppress Fusarium soil borne fungal phytopathogens. *Journal of Plant Pathology*, *103*, 549-561.

How to cite this article: Sultana A.S., Thiruvudainambi S., Masurkar P. and Kumar A. (2023). Exploration of Potent Biocontrol Agent Against the Damping off Disease of Tomato (*Pythium aphanidematum)* and its *in-vitro* Management. *Biological Forum – An International Journal*, *15*(5): 283-288.