

**Original Research**

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***In vitro* bio-efficacy of biocontrol agents and oil cakes against *Pythium aphanidermatum* from tomato**

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**Abstract**

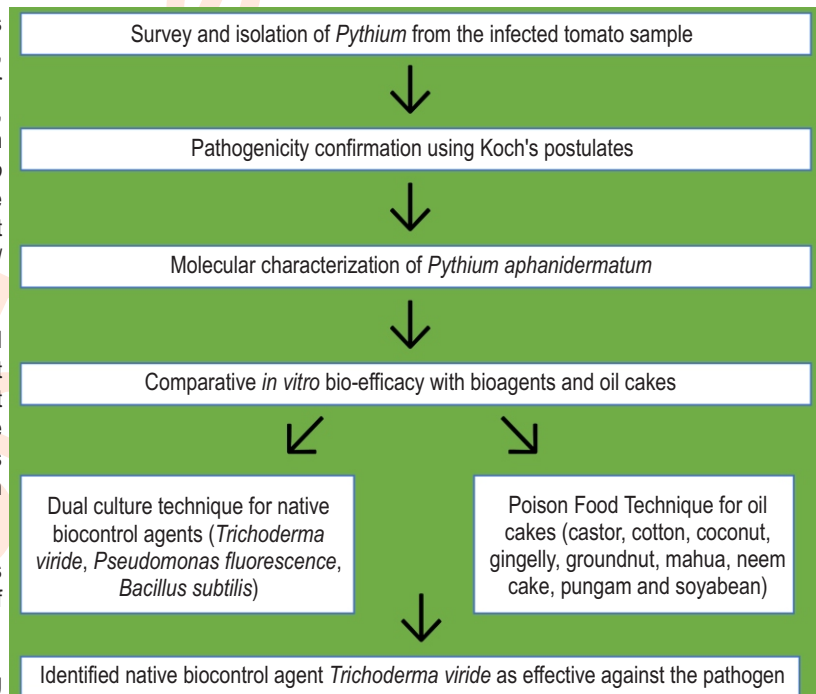
**Aim:** To compare the bio-efficacy of biocontrol agents and oil cakes against *Pythium aphanidermatum* causing damping off disease in tomato under *in vitro* condition.

**Methodology:** Bioefficacy of native biocontrol agents viz., *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* and nine different oil cakes viz., castor cake, cotton cake, coconut cake, gingelly cake, groundnut cake, mahua cake, neem cake, pungam cake and soyabean cake were screened *in vitro* against damping off pathogen through dual culture technique and Poison food technique. Data on percent inhibition was calculated and efficient biocontrol agent / oil cake was identified.

**Results:** Results of dual culture technique revealed that under *in vitro* conditions, the biocontrol agent *Trichoderma viride* (TV4) showed maximum per cent inhibition of 80.15% against *P. aphanidermatum* the Poison Food Technique showed that neem cake was more effective at 5% and 10% with maximum inhibition percentage of 52.44% and 66.20%, respectively.

**Interpretation:** Fungal biocontrol agent *T. viride* was found more efficient against tomato damping off pathogen when compared to with oil cakes.

**Key words:** Biocontrol agents, Bio-efficacy, Damping off, Oil cakes, *Pythium aphanidermatum*, Tomato



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## Introduction

Tomato (*Lycopersicon esculentum* Mill.), member of family Solanaceae is one of the most important vegetables in India. The crop is cultivated under an area of 8.09 million ha with a production of 19.70 million tonnes in India (Ashwathappa et al., 2020) and on a worldwide, the yearly production of fresh tomatoes amounts to approximately 182 million tonnes (FAOSTAT, 2019). Tomato encompasses a wide range of health-promoting amalgams and is easily combined as a nourishing share of a stable diet (Martí et al., 2016). Interestingly consumption of tomatoes is concentrated in China, India, North Africa, the Middle East, the US, and Brazil with per capita ranging from 61.9 to 198.9 kg (FAOSTAT, 2019). The nutritional constituents of tomatoes contain compounds, like antioxidants, vitamins, carotenoids, phenolic compounds, sucrose, hexoses, citrate, malate, and ascorbic acid (Raiola et al., 2014; Liu et al., 2016; Li et al., 2018). Irrespective of several benefits, the successful cultivation of tomatoes is challenged due to water scarcity, soil deficiency, abiotic stress, pest and diseases.

Primarily, the crop is susceptible to a wide range of diseases caused by fungi, bacteria and viruses throughout its growth stages. The common diseases that infect tomatoes include anthracnose, damping off, fusarium blight, leaf curl, bacterial spot, bacterial blight, bacterial canker, buckeye rot, leaf mosaic, early blight, stem rot, powdery mildew, rots and septoria leaf blight (Solanki et al., 2021). Among them, the damping-off disease caused by *Pythium aphanidermatum* has been reported be a serious one. The pathogen is soil-borne in nature and causes pre- or post-emergence symptoms both at nursery stage and field condition. In the case of post-emergence damping-off before infection, seedlings look healthy, however, after infection the seedlings collapse near the soil line, thereby causing huge economic yield losses. Generally, fungicides are used to check soil-borne diseases, but recurrent and indiscriminate use of agrochemicals results in serious environmental hazard (Panth et al., 2020; Sabarwal et al., 2018; Wightwick et al., 2010). On another note, development of resistant races of fungus also leads resistance against chemicals (Delp, 1980; Spotts and Cervantes, 1986).

The fungicide metalaxyl has been reported to be effective in controlling Oomycetes damping-off and lowering disease incidence (Agrios, 1988). Nonetheless, *Pythium* and allied oomycetes spp. have been found to be resistant to metalaxyl, metalaxyl-M, and Mefenoxam (Lookabaugh et al., 2018; Pérez et al., 2009; Utkhede et al., 1988), hence, biological control is the safest and eco-friendly strategy against plant disease management. The mechanism of biological control is, however, slow but it can be long-lasting, cheaper and harmless to the beneficial organisms and living ecosystem, on its mode of action, it either removes the pathogen from the site of infection or eradicates the disease (Ramanathan et al., 2002). Biological control can of disease suppression through antibiosis, competition, mycoparasitism, cell wall degradation and induced

resistance, plant growth promotion and rhizosphere colonization capability (Junaid et al., 2013). So far, biocontrol agent, *Trichoderma* have been efficiently used to manage damping off in tomato (Elshahawy and El-Mohamed 2019). *Trichoderma* species also a biocontrol agent has shown efficacy against diseases caused by soil borne pathogens by including resistance and plant defense reaction or by direct confrontation through mycoparasitism and competition or by producing antibiotics and secondary metabolites (Kucuk and Kivanc, 2003; Odeode et al., 2006; Sivasithamparam and Ghisalberti, 2002; Sridharan et al., 2021). Plant growth-promoting rhizobacteria (PGPR) a potential biocontrol agent, exhibits a wide range of favourable plant-microbe interactions and impacts. These interactions and impacts include both direct and indirect promotion of plant development via biological control of plant diseases (Labuschagne et al., 2010).

Addition to these organic amendments against soil borne diseases is an effective strategy, Organic amendments frequently inherit a high concentration of biochemical components, particularly fungitoxic macromolecules, that were previously present in oil-producing seeds, reducing disease severity and improving antagonistic action, as well as soil fertility (Karmelreetha and Muthukumar, 2020). The conventional application of organic amendments has garnered considerable attention in recent years as a way to improve numerous aspects of soil and manage soilborne diseases (Ruano-Rosa and Mercado-Blanco, 2015). More recently, sustainable organic farming has been regarded for attaining nutritional security and reducing chemical residues in soil and plant systems. Therefore, exploring new organic amendments and novel biocontrol agents has attracted scientific community across globe. Considering the above facts, the present study aimed to evaluate the *in vitro* bio efficacy of organic amendments (oil cakes - castor cake, cotton cake, coconut cake, gingelly cake, groundnut cake, mahua cake, neem cake, pungam cake and soyabean cake) and biocontrol agents (*Trichoderma*, PGPR – *Pseudomonas* and *Bacillus*) against the management of *P. aphanidermatum*.

## Materials and Methods

**Plant pathogen:** Infected plants exhibiting characteristic symptoms of damping off, were collected along with the rhizosphere soil from various tomato growing areas of Coimbatore district, Tamil Nadu. The pathogen was isolated using tissue segment method and purified in sterilised potato dextrose agar media using single hyphal tip transfer. Under a 40x microscopic image analyser, the microscopic characteristics such as mycelium and oospores were recorded. The pathogenicity of *Pythium* isolates was tested, and the plants displaying typical symptoms were selected for re-isolation, confirming Koch's hypothesis. The molecular characterization was carried out by DNA isolation and PCR amplification using universal ITS (Internal Transcribed Sequence) region primers (White et al., 1990). The amplified product was sequenced (Eurofins, Karnataka) for further validation of species. The obtained sequences were aligned using BLAST (Basic Local

Alignment Search Tool) for nucleotide identity searches, then submitted to the Gene bank at the National Centre for Biotechnology Information (NCBI) and given an accession number.

#### Isolation of *Trichoderma*, *Pseudomonas* and *Bacillus* strains:

The native biocontrol agents viz., *Trichoderma*, *Pseudomonas* and *Bacillus* spp. were originally isolated from healthy tomato rhizosphere soil collected from different localities namely Madampatti, Devarayapuram, Karadimadai, Nachipalayam, Arisipalayam, Vadasithur, Arasampalayam, Sencherimalai, Sakkampalayam and Thekkampatti of Coimbatore district, Tamil Nadu. The isolation was done using soil serial dilution plating technique with *Trichoderma* selective medium (Elad and Chet, 1983) for *Trichoderma* spp., King's B medium (King et al., 1954) for *Pseudomonas* spp. and nutritional agar media for *Bacillus* spp. Purification of the obtained isolates were done by single hyphal tip method for fungi and single colony method for bacteria and the plates were maintained for subsequent studies. All the isolates were subjected for identification based on the key to species suggested by Domsch et al. (1993) and Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). The identified isolates were designated as *Trichoderma viride* (TV), *Pseudomonas fluorescens* (PF) and *Bacillus Subtilis* (BS).

#### Efficacy of biocontrol agents under *in-vitro* conditions against *P. aphanidermatum*:

The antagonistic activity was tested by dual culture technique as suggested by Dennis and Webster (1971). In dual culture, 9 mm disc of *P. aphanidermatum* and test biocontrol agent *Trichoderma viride* were positioned opposite to each other near the periphery of the Petri plate. Similarly, the bacterial isolates were streaked (Vidhyasekaran et al., 1997) on one side of the Petri dish (40 mm away from the centre) opposite to test pathogen. Three replications were maintained and a control was maintained by inoculating *P. aphanidermatum* alone at the end of Petri dish onto the medium. The plates were maintained at room temperature, i.e.,  $28\pm 2^\circ\text{C}$  for 5 days. The mycelial growth (in mm) of the pathogen was measured. The effective antagonists were selected based on the per cent inhibition of mycelial growth of the pathogen. Per cent inhibition (PI) was calculated by the formula given by Pandey et al. (2000).

**Preparation of oilcake extracts:** Nine oil cakes viz., castor cake, cotton cake, coconut cake, gingelly cake, groundnut cake, mahua cake, neem cake, pungam cake and soyabean cake were tested against *P. aphanidermatum* and those showing >50% inhibition of mycelial growth were assessed as effective products. Cold water extract of the above-mentioned oil cakes was prepared as follows, the required quantity of oil cake was taken and made into powder. It was soaked in sterile distilled water at the rate of one g ml<sup>-1</sup> and kept overnight. The material was then processed with a mixer and filtered in Whatman No. 1 filter paper and passed through Millipore filters (0.2 µm) using vacuum pump assembly under aseptic conditions to free it from bacterial contaminants. This formed the standard oil cake extract solution (aqueous extract 100 %). This extract was then diluted to the desired quantities using

sterilised distilled water and utilised for future research. Cold water extract of the above-mentioned oil cakes was prepared as per the protocol suggested by Dubey (2002).

#### Efficacy of different oil cakes under *in-vitro* conditions against *P. aphanidermatum*:

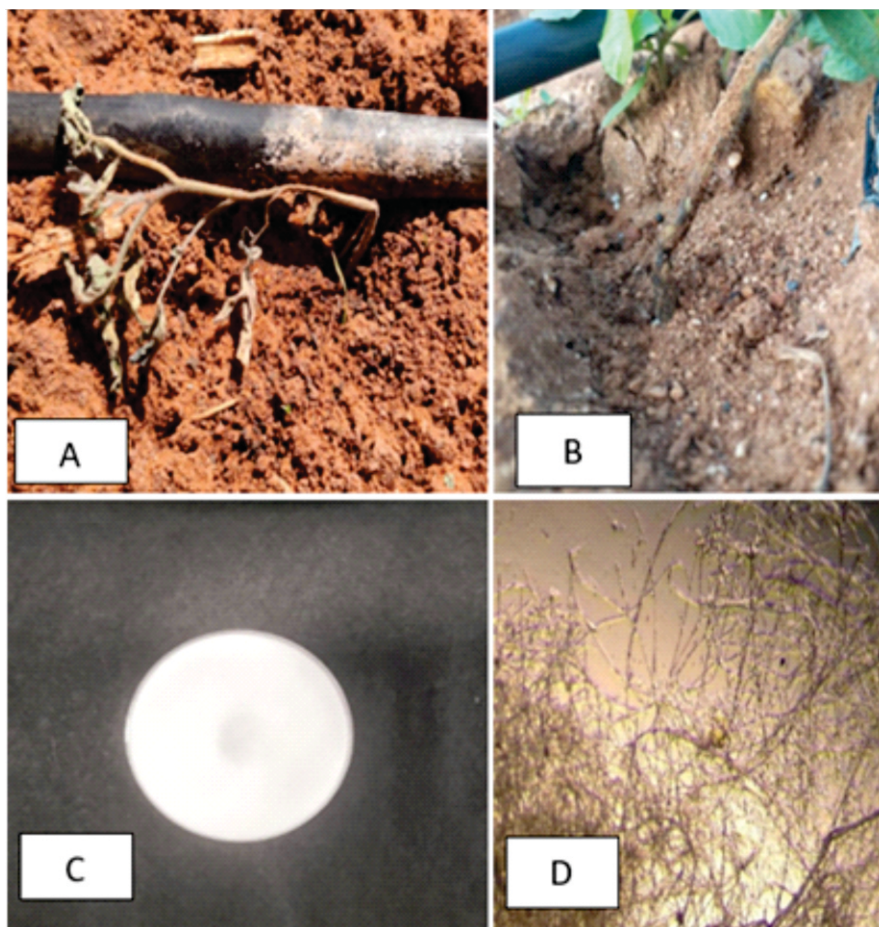
The extracts of nine oil cakes were prepared at 5 % and 10% concentration and their efficacy on the growth of *P. aphanidermatum* was studied by poison food technique (Schmitz, 1930). Under aseptic conditions, the respective oil cakes were added individually to the molten PDA medium to achieve the requisite concentrations of 5% and 10%. An actively growing 9 mm culture disc of *P. aphanidermatum* was placed at the centre of the medium. PDA medium without incorporating the oil cakes served as control. Three replications were maintained for each treatment. The plates were incubated for 5 days at room temperature ( $28\pm 2^\circ\text{C}$ ). The linear mycelial growth of pathogen was measured after 5 days.

**Statistical analyses:** The data obtained from all the experiments were statistically analysed and the treatment means were compared by Duncan' package used for analysis (WASP 2.0) developed by the ICAR Central Coastal Agricultural Research Institute – GOA, India.

## Results and Discussion

Chemical treatments can be used to combat damping off and other soil-borne diseases in vegetable crops. These compounds, on the other hand, have a harmful influence on the environment and individuals. As a result, the use of ecologically friendly chemical alternatives is a global requirement for long-term agricultural pest and disease control. *Pythium aphanidermatum* is a soil-borne phytopathogen that causes damping-off disease in a wide range of host plants that majorly includes tomato, chilli, cucumber, soyabean and hot pepper. Particularly, the pathogen causes significant harm to tomato plants across the world (Kipngeno et al., 2015). In the present research, an intensive field survey for the identification of damping off symptoms around different parts of Coimbatore district, Tamil Nadu revealed that the incidence severity changes from place to place (10% to 20%). The infected plants showed post emergence damping off symptoms as clear toppling over of the emerged seedling (Fig. 1).

The fields located at Madampatti showed highest disease incidence of 18.36%, followed by Karadimadai (18.10%), Senjerimalai (15.77%), Vadasithur (15.72%), Devarayapuram (15.31%), Arisipalayam (12.94%), Sakkampalayam (12.89%), Nachipalayam (11.29%), Thekkampatti (10.28%) and Arasampalayam (10.11%). Similarly, 12-65% incidence of damping off disease was reported in Tamil Nadu by Reetha and Muthukumar (2019). Agrios (1988) found variation in association of fungi with soil borne diseases at different places due to soil ecological and weather parameters. Pathogenicity studies for *Pythium* fulfilled all Koch's postulates (Fig. 2) and the symptomatic plants showed clear toppling over symptoms. In



**Fig. 1:** *Pythium aphanidermatum* causing damping off in Tomato (A) Toppling over symptoms; (B) brownish color water-soaked lesions with mycelia on the collar region; (C) Morphology of 7-day-old colonies of *P. aphanidermatum* grown on potato dextrose agar at  $25\pm 1$  °C in the dark; (D) Aseptate hyaline mycelium with oospores 10 x magnification.

*vivo*, testing showed the maximum disease incidence in Madampatti isolate (54.20%) followed by Karadimadai isolate (45.21%) whereas the minimum infection was observed in Arasampalayam isolate (24.21%) (Table 1). The pathogen was re-isolated and the pathogenicity was confirmed through *in vitro* studies. The findings are in accordance with the previous reports that the re-isolated *Pythium* isolates showed increased damping off disease incidence during pot culture studies (Navi *et al.*, 2019; Reetha and Muthukumar, 2019). *Pythium* isolates on PDA produced a dense, dull whitish to pure whitish cottony mycelial growth with various topographies (Table 1, Fig.1). Microscopic observations revealed the production of aseptate, hyaline mycelium, globose and smooth, thick-walled golden-brown oospores of *Pythium* (Fig. 1). Traditionally, *Pythium* species were identified and classified based on the morphology of asexual and sexual reproductive structures (Van der Plaats-Niterink, 1981). But morphological characters of *Pythium viz.*, structures of sporangia and oospore vary between species (Schroeder *et al.*, 2013).

The genus *Pythium* is complex and consists more than 100 species, among them *P. aphanidermatum*, *P. debaryanum*, *P. ultimum*, *P. irregulare*, *P. vexans* are able to cause damping off disease on various crops (Middleton, 1943; Pearson and Hall, 1973; Rajendraprasad *et al.*, 2017; CABI, 2021). It is difficult and time consuming to distinguish these species using traditional morphological characters (Prabhu *et al.*, 2015). In the present study, PCR amplification of isolates with oomycetes specific primers showed a unique band of around 800 bp in gel electrophoresis (Fig. 3). One virulent isolate among the ten, which fulfilled Koch postulates were sent for sequencing and the sequences were obtained. The obtained sequences were confirmed as *P. aphanidermatum* through NCBI blast and were deposited (Acc No: MZ914651). The sequence showed 100% similarity with *P. aphanidermatum* isolate from Thailand (Lc176487).

Similarly, Nzungize *et al.* (2011) found 96 isolates from a total of 231 *Pythium* isolates, resulting in 800-bp product. Prabhu



Fig. 2: Pathogenicity test for *Pythium aphanidermatum*.

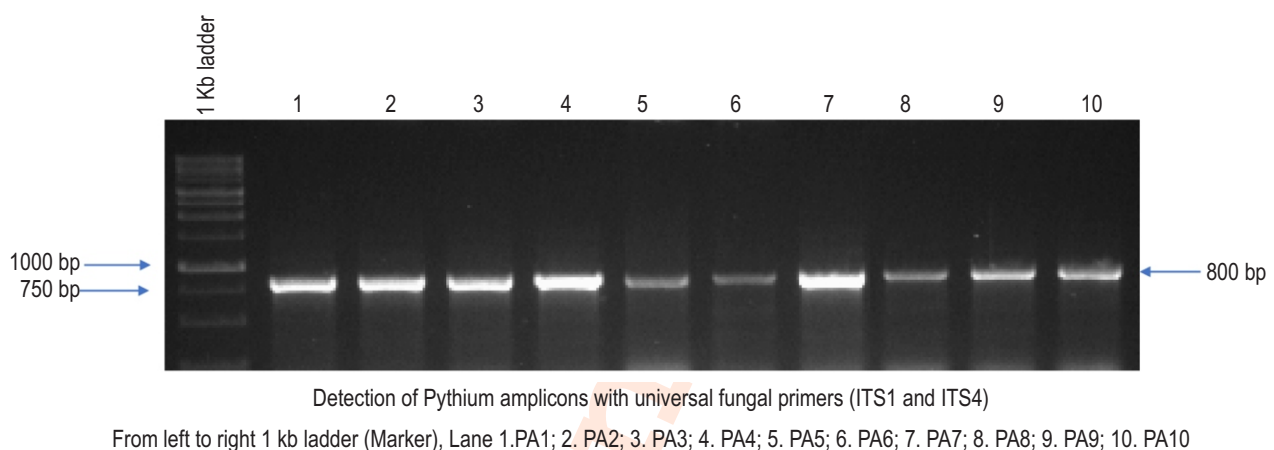


Fig. 3: PCR amplification of ITS region (Oomycetes) of *Pythium* isolates.

*et al.* (2015) observed the product size of 800 bp for the *Pythium* spp. collected from rhizome rot of turmeric. Comparable results were also reported by researchers who observed that ITS region varied from 750 to 1050 bp (Allain Boule *et al.*, 2004; Levesque and De Cock, 2004; Mahuku *et al.*, 2007; Hashem Al-sheikh, 2010). Many bacterial genera (PGPR), including *Agrobacterium*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Klebsiella*, *Micrococcous*, *Rhizobium*, *Pseudomonas*, *Serratia* and different *Trichoderma* species have been reported as a potential biocontrol agents against various fungal pathogens (Ahemad and Kibret, 2014; Bastakoti *et al.*, 2017) which particularly includes, *Trichoderma viride* (Jayaseelan *et al.*, 2012), *Trichoderma harzianum* (Jayaraj *et al.*, 2006), *Trichoderma asperellum* (Manjunath *et al.*, 2017), *Pseudomonas fluorescens* (Prabhukarthikeyan and Raguchander, 2016) and *Bacillus subtilis* (Jayaraj *et al.*, 2005) as effective in controlling damping off in various crops. In the present study, the data on radial mycelial growth and per cent inhibition over control showed maximum per cent inhibition of *P. aphanidermatum*

(80.15%) with the isolate TV4 collected from Nachipalayam (Table 2). The mycelial growth of the pathogen was 17.67 mm after 5 days of inoculation followed by Vadasithur isolate (TV6) with 70.42% inhibition and mycelial growth of 26.33 mm. The least inhibition of 40.08% was recorded in Sökkampalayam isolate (TV9) with radial mycelial growth of 53.33 mm. On evaluating *P. fluorescens* the isolate from Nachipalayam (PF4) registered maximum per cent inhibition over control (62.55%) against *P. aphanidermatum* followed by Arisipalayam (PF5) (Table 2). Compared to other biocontrol agents, the *B. subtilis* isolate from Devarayapuram (BS2) showed maximum per cent inhibition (61.66%) whereas minimum inhibition (36.06%) was observed in from Madampatti (BS1) isolate (Table 2).

The perusal of data revealed that all three biocontrol agents were beneficial and controlled at least 50% of the infection. Interestingly, amongst all, the biocontrol agent *T. viride* was promising one followed by *P. fluorescens*. The results are in agreement with the previous findings of Manoranjitham *et al.*

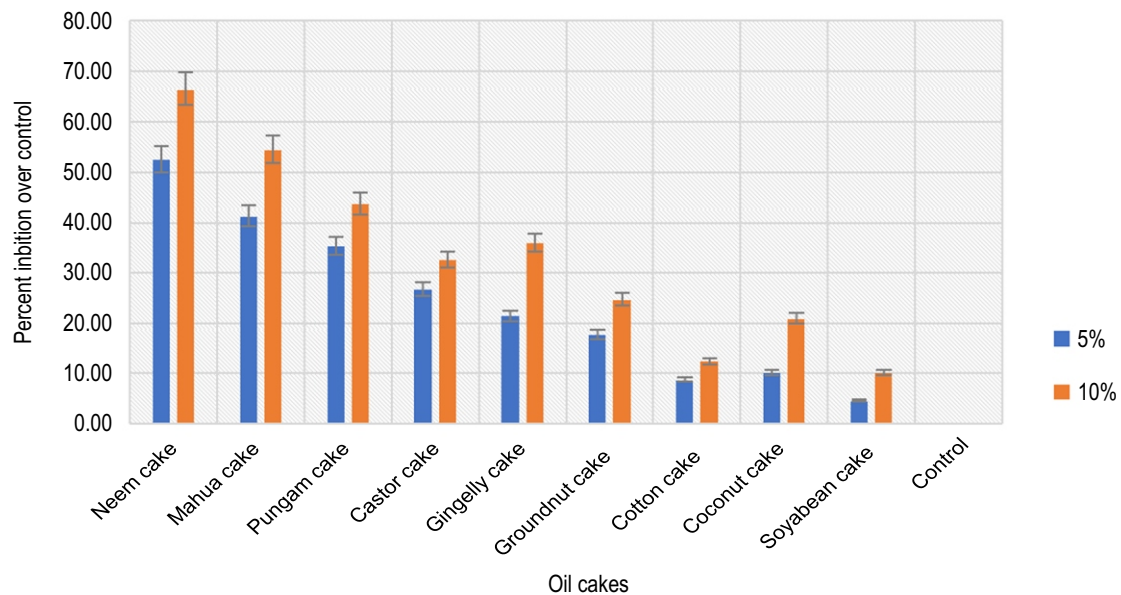


Fig. 4: Bioefficacy of oil cakes against *P. aphanidermatum*.

Table 1: Culture characteristics and pathogenicity of *Pythium aphanidermatum*

Villages	Culture codes	Culture characters	Pathogenicity (PDI)
Madampatti	PA1	Rapid, dull whitish with slightly raised growth	54.20 <sup>a</sup>
Devarayapuram	PA2	Rapid, dull white with sparse growth	42.07 <sup>c</sup>
Karadimadai	PA3	Rapid, whitish with slightly raised growth	45.21 <sup>b</sup>
Nachipalayam	PA4	Moderate growth, dull white with aerial flat mycelium	41.67 <sup>c</sup>
Arisipalayam	PA5	Moderate growth, dull white with aerial flat mycelium	33.33 <sup>e</sup>
Vadasithur	PA6	Rapid, whitish with slightly raised growth	36.11 <sup>d</sup>
Arasampalayam	PA7	Moderate growth, dull white with aerial flat mycelium	24.21 <sup>g</sup>
Sencherimalai	PA8	Rapid growth along with raised fluffy growth	36.97 <sup>d</sup>
Sokkampalayam	PA9	Moderate, dull white with sparse growth	33.13 <sup>e</sup>
Thekkampatti	PA10	Moderate growth, dull white with aerial flat mycelium	28.27 <sup>f</sup>
CD Value (p=0.01)			1.52

Values are mean of three replications. Means in the same column followed by same letter are not significantly different at  $P = 0.01$  according to Duncan's Multiple Range Test

(2001); Khare and Upadhyay (2009); Muthukumar *et al.* (2011); Kamala and Indira (2011); Jeyaseelan *et al.* (2012) who reported the antagonistic potential of *Trichoderma* spp. against the *Pythium*. *Trichoderma* spp. control phytopathogens through a variety of mechanisms, like degrading host cell wall followed by assimilation of its cellular content, primarily mycoparasitism, formation of specialised structures like haustoria, competition for food and space, antibiotic secretion, and production of fungal cell wall degrading enzymes (Harman *et al.*, 2004; Benitez *et al.*, 2004; Zeilinger and Omann, 2007). The volatile organic compounds emitted by *Trichoderma* sp. inhibits phytopathogens by triggering amino acid metabolism (Sridharan *et al.*, 2021). In this study, the antagonistic effect expressed by the *Trichoderma* spp. in dual

culture method might be due to the one or combination of all the above mechanisms and also interestingly, the antagonistic assay of *Pseudomonas* depends on their ability to produce antibiotic metabolites such as pyoluteorin, pyrrolnitrin, phenazine 1-carboxylic acid, 2, 4 diacetylphloroglucinol, hydrogen cyanide, kanosamine, pyocyanin and viscosinamide (Prabhukarthikeyan and Raguchander, 2016). Further research might be carried out using tested fungi and bacteria that have an antagonistic impact on *P. aphanidermatum* as a direct biological controlling agent or extracted and purified antimicrobial compounds. In agriculture, oil cakes provide a bigger advantage that no pesticide or synthetic fertiliser can match. They feed and protect the host plant in a gradual and steady manner (Parihar *et al.*, 2015).

**Table 2:** *In vitro* efficacy of native biocontrol agents against *P. aphanidermatum*

Villages	<i>Trichoderma viride</i>			<i>Pseudomonas fluorescens</i>			<i>Bacillus subtilis</i>		
	CC	MG	% Inhibition over control	CC	MG	% Inhibition over control	CC	MG	% Inhibition over control
Madampatti	TV1	47.33 <sup>c</sup>	46.82	PF1	60.00 <sup>b</sup>	32.58	BS1	56.70 <sup>bc</sup>	36.06
Devarayapuram	TV2	34.67 <sup>f</sup>	61.04	PF2	53.33 <sup>e</sup>	40.08	BS2	34.00 <sup>h</sup>	61.66
Karadimadai	TV3	44.67 <sup>cd</sup>	49.81	PF3	44.67 <sup>e</sup>	49.81	BS3	56.67 <sup>bc</sup>	36.09
Nachipalayam	TV4	17.67 <sup>h</sup>	80.15	PF4	33.33 <sup>g</sup>	62.55	BS4	48.67 <sup>f</sup>	45.11
Arisipalayam	TV5	37.47 <sup>f</sup>	57.90	PF5	40.67 <sup>f</sup>	54.30	BS5	45.67 <sup>g</sup>	48.49
Vadasithur	TV6	26.33 <sup>g</sup>	70.42	PF6	55.33 <sup>e</sup>	37.83	BS6	50.67 <sup>ef</sup>	42.86
Arasampalayam	TV7	43.33 <sup>de</sup>	51.31	PF7	50.00 <sup>d</sup>	43.82	BS7	55.00 <sup>cd</sup>	37.97
Sencherimalai	TV8	41.00 <sup>e</sup>	53.93	PF8	48.00 <sup>d</sup>	46.07	BS8	49.67 <sup>f</sup>	43.98
Sokkampalayam	TV9	53.33 <sup>b</sup>	40.08	PF9	53.33 <sup>e</sup>	40.08	BS9	58.33 <sup>b</sup>	34.22
Thekkampatti	TV10	47.00 <sup>c</sup>	47.19	PF10	47.67 <sup>de</sup>	46.44	BS10	53.00 <sup>de</sup>	40.23
Control	C	89.00 <sup>a</sup>		C	88.67 <sup>a</sup>		C	88.67 <sup>a</sup>	
CD Value (P=0.01)		2.90			3.04			2.63	

Values are the mean of three replications. Means in the same column followed by same letter are not significantly different at P = 0.01 according to Duncan's Multiple Range Test. MG- Mycelial growth; CC- Culture code and %- Percent

In the present study, an insight into the bioefficacy data reveals that all the oilcakes significantly inhibited mycelial growth of *P. aphanidermatum* at 5% and 10% concentrations. Irrespective of all the treatments, increased level of 10% concentration of all the nine oilcakes showed maximum inhibition percentage. Maximum inhibition percentage was observed to be in neem cake with 66.20% at 10% concentration and 52.44% at 5% concentration followed by mahua cake at both the concentrations (Fig. 4). Similar observations were recorded in the previous studies that organic amendments reduce the plant pathogens and increased some of the potential bio-control agents in the soil (Abbasi *et al.*, 2005; Haritha *et al.*, 2010). The mechanism behind the effective management of soil borne diseases by organic amendments includes the alteration of the soil suppressiveness (Bonanomi *et al.*, 2018) and activation of beneficial microbiota such as Rhizobacteria, *Trichoderma* and *Pseudomonas* species that creates a competition with the pathogen during the decomposition (Panth *et al.*, 2020). Particularly, the toxicity of neem is because of the effects of azadirachtin a tetranortriterpenoid in the limonoid group and other allied chemicals such as alkaloids, glycosides, flavonoids, and saponins, which are common antibiotics found in plants that control the plant pathogens (Abbasi *et al.*, 2005, Pandey *et al.*, 2014).

The present study indicates the bio-efficacy of biocontrol agents as well as organic amendments against the damping off pathogen *P. aphanidermatum* *in vitro*. In future, the results can be further utilized for combined study of biocontrol agents with organic amendments for the successful management of damping off disease in tomato. Moreover, an improved formulation with microbial consortium of *T. viride* and *P. fluorescens* on oilcake will enhance the biocontrol efficacy. The findings of the study may be further explored for sustainable organic farming of vegetable crops.

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## Add-on Information

**Authors' contribution:** **B. Madhumitha:** Conducted the study and wrote the manuscript; **S. Gnanaprakash:** Data interpretation and statistical analysis; **C. Jayapradha:** Conducting laboratory experiments; **S. Thankappan:** Editing manuscript; **S. Rathikannu:** Conducting laboratory experiments; **T. Priyanka:** Reference.

**Research content:** The research content is original and has not been published elsewhere.

**Ethical approval:** Not applicable.

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Data from other sources:** Not applicable.

**Consent to publish:** All authors agree to publish the paper in *Journal of Environmental Biology*.

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