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Research Article

IN VITRO EVALUATION OF BOTANICALS AND BIO-CONTROL AGENTS AGAINST MUSTARD DAMPING OFF CAUSED BY *Pythium aphanidermatum*

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Abstract: Mustard (*Brassica* sp.) is one of the important plant species in the family Brassicaceae. *Pythium aphanidermatum* is a soil-borne plant pathogen that causes damping-off in mustard. *Pythium aphanidermatum* was isolated from the infected mustard plants, showing typical symptoms of damping off. They were collected from the Kolli hills, Namakkal. Six different solid media were tested for the growth of pathogens, with maximum radial growth of mycelium (topography) on potato dextrose agar (9 cm). Among the two bio-control agents, *Trichoderma viride* and *Bacillus subtilis, Trichoderma viride* shows the maximum percent inhibition of mycelial growth (76.33%) and strong antagonistic activity against *Pythium aphanidermatum* under *in vitro* conditions. Among the various botanicals (Neem leaf extract, Pungam leaf extract, and Ginger extract) used against *Pythium aphanidermatum* at different concentrations (5%, 10%, and 15%), Ginger extract with a 15% concentration shows 51.87% inhibition of mycelial growth of *Pythium aphanidermatum*.

Keywords: *Mustard, Damping-off, Pythium aphanidermatum, Bio-agents, Botanicals*

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Introduction

Mustard (*Brassica* sp.) is one of the important plant species in the family Brassicaceae. India is the world's fourth largest producer of oilseeds, with rapeseed and mustard accounting for approximately 28.6% of total oilseed production [1]. Damping-off produces rotting symptoms on stems and root tissues at and below the soil line. In most cases, infected plants will germinate and grow just fine, but within a few days they become water-soaked, mushy, collapse at the base and die [2]. *Pythium aphanidermatum* is a soil-borne plant pathogen that causes damping-off in mustard. Oomycetes are not true fungi as their cell walls are made of cellulose instead of chitin [3].

Pythium aphanidermatum avoiding by several effective cultural management methods. The pathogen increases in a damp environment, so it is important to avoid an excessive amount of moisture from building up in the plant media. Frequent irrigation and cultivation in soil that has poor drainage are common mistakes that result in disease incidence. In addition to that, poor ventilation and insufficient exposure to sunlight can cause the plants themselves to accumulate moisture, potentially spreading the disease.

Fungicides are also effective control methods. Systemic and contact fungicides can be used, but in order to prevent the pathogen from becoming resistant to the chemical treatment, it is good to utilize alternate methods like biological control measures to manage the damping-off in mustard.

Material and Methods

Experiments related to work have been carried out in the Plant Pathology laboratory, Department of Crop Protection, PGP College of Agricultural Sciences, Namakkal, which is situated at 11.229545° latitude and 78.200957° longitude and at an elevation of 218m above MSL. The details of materials used and the methods adopted in the present research are described in brief hereunder.

Experiment I

Survey for the intensity of the disease

A roving survey was conducted during 2021-2022 at mustard fields in and around the Kolli Hills region of Namakkal district. Observations were recorded with respect to the intensity of damping-off disease in terms of percent disease incidence (PDI) [4]. The percent disease incidence was calculated by the following formula [5].

Disease Incidence as a Percentage = (Number of Infected Plants/Total Plants) × 100

Experiment II

Isolation and characterization of *Pythium aphanidermatum*

Isolation of the pathogen

Mustard crops showing typical symptoms of damping-off were collected from crop fields in and around the Kolli Hills region of Namakkal district in May 2022. A standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues were cut and surface sterilised for 30 seconds with a 0.1 percent mercuric chloride solution before being transferred three times to Petri dishes containing sterile water, drained the water, and the hits were placed on sterile tissue paper before being transferred into Petri dishes containing 15 ml of potato dextrose agar medium and incubated at 28°C for seven days. A pure culture of the fungus was obtained by the hyphal tip method [4].

Hyphal tip technique

Infected tissues are placed in the PDA. After the 3rd day, a 9mm disc of well sprouted or mycelium growth culture at the hyphal tip end was taken with the help of a cork borer and then transferred into the medium. Such cultures stored at 4°C in a refrigerator were used for further studies.

Pathogen identification and characterization

The cultures were identified based on cultural and morphological characters seen in the Petri plates. Cultural characters are mycelial growth, topography, colour of mycelium, and pigmentation, and the morphological characters like shape, size, and colour of sporangia, sporangiophore, and oospores are also noticed [3].

Maintenance and Preservation of Culture

The slant culture of *Pythium aphanidermatum* was maintained at 4°C in the refrigerator and subcultured periodically at an interval of 30 days during investigation [6].

Experiment III

Cultural Studies of Different Growth Media

Consistent media expansion

The growth characteristics of *Pythium aphanidermatum* were studied on six solid media, *viz*., Potato dextrose agar, Sabouraud's agar, Carrot juice agar, Water agar, Richard's agar, and Czapek's agar. All the media were sterilised at 1.1 kg/cm^2 pressure for 15 minutes in the autoclave. To carry out the study, 15ml of each of the mediums was poured into each Petri plate separately. Such Petri plates were aseptically inoculated with 9 mm disc cut-outs from the periphery of an actively growing culture and incubated at 28°C for a period of seven days. Each treatment was replicated thrice. Observations were made with respect to colony size at 5 days after inoculation. The mycelial colour, substrate colour, margin of the colony, topography, and centre of the colony were recorded at 5 days after inoculation. The records on radial growth were analyzed statistically. The composition and preparation of different media were followed as given in (Potato dextrose agar and Czapek's agar medium) [7], (Water Agar) [8], (Richards Agar) [9], (Sabouraud's Agar Medium and Carrot Juice Agar Medium) [10].

Experiment IV

Isolation of bio-control agents: The bio-control agents were purchased from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, and stored at 4°C until used. *Trichoderma viride* and *Bacillus subtilis* cultures were sub cultured using potato dextrose agar (PDA) and nutrient agar (NA) medium by the fungal disc method and later pure cultures were obtained. The purified isolates were preserved at 4°C and used during the course of the study.

Experiment V

Pythium aphanidermatum in vitro botanical screening (Poisoned food technique)

The relative efficacy of three extracts, *viz*., *Azadirachta indica, Pongamia pinnata,* and *Zingiber officinale*, was tested against the pathogen *Pythium aphanidermatum* in the laboratory. The selected plants were collected from the surrounding areas and washed thoroughly with tap water and air dried. Using a pestle and mortar, one hundred grammes of plant parts were ground with an equal amount (100 ml) of sterilised distilled water (1: 1, w/v). The pulverised mass was strained through cheese cloth, and the extracts were centrifuged at 10000 rpm for 5-10 minutes before being used as a stock solution. To study the antifungal property of plant extracts, the poisoned food technique was adopted [11]. The stock solutions of different botanicals (5, 10, and 15%) were mixed with 95, 90, and 85 ml of PDA media, respectively, to get 5, 10, and 15 percent concentrations, and sterilized. Twenty ml of such medium was poured under aseptic conditions into sterile Petri plates and allowed to solidify. Mycelial discs (5 mm) were cut out using a sterile cork borer from the periphery of an actively growing culture of *P. aphanidermatum* and one such disc was placed on the centre of each Petri plate. The treatments were replicated thrice. Control was maintained by growing the pathogen on PDA plates without plant extracts. Plates were incubated at room temperature (28 + 2°C) for 7 days, and radial growth was taken at the time when maximum growth occurred in the control plates.

Dual plate technique for *Pythium aphanidermatum* biocontrol agent screening *in vitro*

The antagonists, such as *Trichoderma viride* and *Bacillus subtilis*, were tested against *Pythium aphanidermatum*. The bio-control agent and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. On the potato dextrose agar medium, 9 mm of the fungal disc of the antagonist along with the test fungus were kept in the opposite direction. The plates were incubated for a week at 28°C. The growth of antagomatic fungus and the growth of pathogens were also recorded separately. The inhibition zone was measured. The observation of the interaction zone or inhibition zone was recorded. After the period of incubation, the growth of the Pythium mycelial growth was recorded and the percent inhibition of the mycelial growth over control was calculated [12].

Results and Discussion

Damping-off disease of mustard is caused by *Pythium aphanidermatum* and occurs in mustard at pre-emergence and post-emergence stages that reduce overall plant growth and destroy much of the main root system. The present investigations into this disease include surveys on damping-off disease, its symptomology, cultural studies on the growth of *Pythium aphanidermatum*, and *in vitro* evaluation of botanicals and bio-control agents' efficacy against *Pythium aphanidermatum*.

Damping-off Disease Survey

A survey was conducted to assess the incidence of *Pythium aphanidermatum* in mustard in Namakkal district during the year 2022. Dry root rot infected samples were collected from the Kolli Hills region of Namakkal district, *viz*., Semmedu, Karavalli, Solakkadu, Ariyurnadu, and Devanurnadu. The survey conducted revealed that the overall disease incidence of 37.62 percent was recorded in the Namakkal district. Similar observations were made by Sati and Tiwari (1992) [13], who studied and surveyed five different species of Pythium in mustard and cabbage.

Symptomology

Damping-off in mustard results in seed rot that leads to failure in seedling emergence. The first symptoms appear as elongated, water-soaked areas on the roots 1-3 weeks after planting. The pathogen will extensively prune the roots, reducing overall plant growth and destroying much of the main root system. The water-soaked region may extend above the soil surface with some visible evidence of the fungal mycelium. The water-soaked region eventually dries out, becomes sunken and tan to brown in color. Above ground, symptoms include stunting and yellowing of leaves that eventually cause wilting and lead to plant death. These observations agree with the findings of Schwartz and Gent (2004) [14] and Horst (2013) [15].

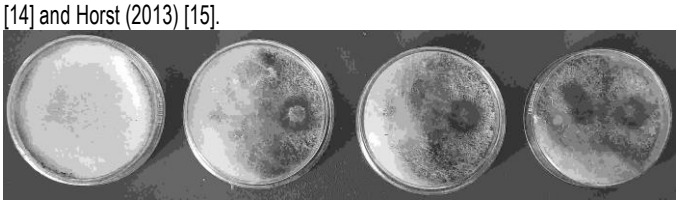

Fig-1 Antagonistic effect of *Trichoderma viride* on mycelial growth of *Pythium aphanidermatum*

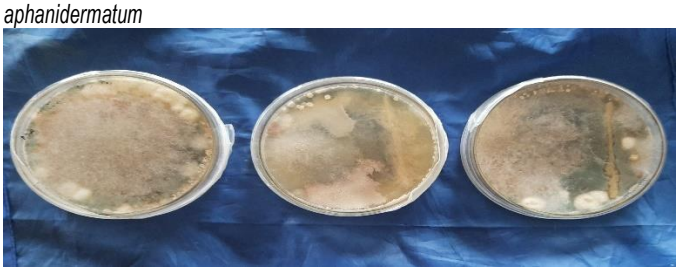

Fig-2 Antagonistic effect of *Bacillus subtilis* on mycelial growth of *Pythium aphanidermatum*

***Pythium aphanidermatum* isolation, identification, and cultural characteristics**

Standard tissue isolation technique was followed to obtain *Pythium aphanidermatum* from mustard, showing typical symptoms of the disease [Fig-1]. The description of the fungus isolated from the mustard seedling is as follows: Colonies first appeared as white aerial mycelial growth on PDA medium when

Fig-3 Effect of Neem leaf extract on mycelial growth of *Pythium aphanidermatum*

incubated at 28°C for 5 days. After 5 days, the pure culture appeared as a fully grown whitish spongy culture [Fig-2]. The mycelial topography was raised by aerial mycelium. The hyphae, sporangia, and sporangiophore are observed in the microscope clearly, but the oospores produced by *Pythium aphanidermatum* are not observable in the microscope. The findings are consistent with Subharathinam *et al.* (2020) [4 research on the isolation, identification, and morphological characterization of Pythium species.

Fig-4 *Pythium aphanidermatum*

Growth Behavior of *Pythium aphanidermatum* on Various Solid Media

The cultural characteristics of *Pythium aphanidermatum* were studied on different solid media as described in materials and methods. Observations on radial growth, mycelial colour, topography, and substrate colour on different media were recorded for 7 days after inoculation. The radial growth of *Pythium aphanidermatum* was maximum (9 cm) on Potato dextrose agar and Carrot juice agar, followed by Sabouraud's agar medium (8.93 cm). The lowest mycelial growth was observed on Richard's agar and water agar media.

In vitro* screening of botanicals against *Pythium aphanidermatum

The effects of different botanicals against *Pythium aphanidermatum* were studied by the poison plate technique under *in vitro* conditions. The results on the percent inhibition of pathogens at various concentrations revealed that Ginger extract at 15% concentration shows maximum mycelial inhibition (51.87%), followed by Pungam leaf extract at 15 % concentration (43.33%) [Table-1]; [Fig-3 & 4].

Table-1 *Effect of Various Plant Extracts on Mycelial Growth of Pythium aphanidermatum*

Treatment	Mycelial Growth* (mm)			Per Cent Inhibition of Mycelial Growth*		
	5%	10%	15%	5%	10%	15%
Pungam	7.06	5.66	5.10	21.48	37.03	43.33
Ginger	8.66	7.13	4.53	3.70	20.74	51.87
Neem	8.63	8.16	6.73	4.07	9.25	25.18
Control	9.00	9.00	9.00	0.00	0.00	0.00
CD (P=0.05)	0.7733			8.594		

Fig-5 Effect of Ginger extract on mycelial growth of *Pythium aphanidermatum*

Fig-6 Symptoms of Mustard damping off caused by *Pythium aphanidermatum*

In vitro* screening bio-control agent against *Pythium aphanidermatum

The antagonist effect of *Trichoderma viride* and *Bacillus subtilis* against the pathogen *Pythium aphanidermatum* was studied by the dual plate technique under *in vitro* conditions. The results revealed that *Trichoderma viride* screened against the pathogen recorded 76.33 %mycelial inhibition, whereas *Bacillus subtilis* recorded 25.92 percent mycelial inhibition [Table-2]; [Fig-5 & 6]. The result shows that *Trichoderma viride* has a maximum percent inhibition of *Pythium aphanidermatum*. The present results corroborate with the findings of [16] and [12], who reported that *Trichoderma viride* was effective against *Pythium aphanidermatum*.

Table-2 *Effect of Various Bio-control Agents on Mycelial Growth of Pythium aphanidermatum*

Treatment	Mycelial Growth* cm	Percent Inhibition of Mycelial Growth*
Trichoderma viride	2.10	76.33
Bacillus subtilis	6.67	25.92
Control	9.00	0.00
CD (P=0.05)	0.7416	8.2407

Conclusion

The significant results of this study show that *Trichoderma viride* is the effective bio-agent against *Pythium aphanidermatum*, followed by ginger extract at a 15% concentration. So, in the upcoming days, the use of chemicals can be reduced by using bio-agents and botanicals to save our earth from environmental hazards.

Application of research: Organic method of control measures is study to control the plant diseases to reduce chemical use which causes environmental hazards.

Research Category: Botanicals and Bio-Control Agents

Abbreviations: g-Gram, cm-Centimetre

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Study area / Sample Collection: Crop Protection Department, Namakkal

Cultivar / Variety / Breed name: Mustard, *Azadirachta indica, Pongamia pinnata, and Zingiber officinale*

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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