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Isolation, identification and pathogenicity test of pathogens causing rhizome rot of ginger

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Abstract
The rhizome rot disease is the most destructive disease of ginger in India which reduces its economic and commercial value. To determine the causal agents of the disease fungal isolation was performed using PDA medium. The different fungal pathogens *viz. Pythium aphanidermatum*, *Fusarium oxysporum* f.sp *zingiberi* and *Sclerotium rolfsii* were isolated from infected rhizomes. These pathogens were identified based on their cultural, morphological and pathological characteristics and confirmed by microscopic observations. Pathogenicity of these pathogens were proved by applying various inoculation techniques like soil inoculation and rhizome inoculation. All the isolated pathogens were found to be pathogenic. While, *P. aphanidermatum* showed early mortality than other pathogens.

Keywords: Ginger, rhizome rot, *pythium aphanidermatum*, *Fusarium oxysporum* F. sp *zingiberi*, *Sclerotium rolfsii*. bioagents

1. Introduction
India is the largest producer of ginger in the world accounting for about one-third of the total world output, followed by Thailand and Japan (Kadam *et al*., 2015) [3]. Major ginger-growing states in India are Kerala, Sikkim, Meghalaya, West Bengal, Orissa, Tamil Nadu, Karnataka, Andhra Pradesh. Maharashtra, and Himachal Pradesh (Shukla and Gupta, 2015) [10].
This important crop is suffering from number of foliar and rhizome diseases. Amongst them rhizome rot complex is very important. As the disease causes severe mortality in the crop it leads to heavy losses in yield to the extent of 50% or more. All varieties of ginger are susceptible to rhizome rot disease. The disease is soil and rhizome borne. In India, this disease is a complex of *Pythium* and *Fusarium*, and it is exacerbated by the presence of nematode in the soil (Debnath *et al*., 2010) [1]. *Sclerotium rolfsii* causes basal rot, which emerges later in the season (Sagar *et al*., 2008) [9]. These fungal pathogens can cause heavy losses in yields of crop singly or in combination. The pathogen *Pythium aphanidermatum* causing soft rot of ginger is a serious bottleneck, resulting in massive crop losses of up to 70% in a single cropping season (Dohroo, 2005) [2].
Keeping in view the importance of ginger and losses incurred due to rhizome rot disease, present investigations on the aspects *viz*., isolation, identification and pathogenicity of rhizome rot pathogens were undertaken.

2. Material and Methodology
2.1 Isolation of rhizome rot pathogens
The rhizomes showing typical symptoms of rhizome rot were collected from farmers' field. Isolation of the fungal pathogens was carried out under aseptic conditions using Potato dextrose agar medium. After sterilization in an autoclave 20 ml of the media was distributed to each of the sterile Petri plate under aseptic conditions. Small sections of rot infected rhizomes and plant parts were selected, surface sterilized with 0.01 percent ($NaOCl_2$) solution for 30 seconds, then washed by giving three sequential changes with sterile distilled water in Petri plates to remove traces of sodium hypochlorite and again blot dried. Three bits of infected rhizome/plant parts were placed at equidistance in a circular manner in the plates. The Petri plates were incubated at 28±2 °C in the incubator.

2.2 Identification of the pathogens
The pathogens isolated were identified based on their cultural, morphological and pathological characteristics and confirmed by microscopic observations.

2.3 Pathogenicity of causal agents associated with rhizome rot.
Pathogenicity of rhizome rot pathogens were detected and proved individually by applying various inoculation techniques like soil inoculation and rhizome inoculation by using Cv. Mahim under controlled conditions in the screen house and laboratory.

A. Soil inoculation method
In sick soil inoculation method, pots were disinfected with 5% formaldehyde solution with potting mixture and inoculated with pure culture of the test fungus. One control pot was filled with sterilized potting mixture without the culture of test pathogens. These pots were incubated for 15 days at room temperature, frequently stirred, watered regularly and pathogens were allowed to colonize better in the pots. Then surface-sterilized rhizomes of Cv. Mahim were sown (@ 5 rhizomes /pot) and kept in a screen house at room temperature and watered regularly.
Observations on pre-emergence rhizome rot and post-emergence seedling mortality was recorded. The seedlings showing rhizome rot symptoms were subjected to re-isolation by passing through 0.01 percent ($NaOCl_2$) and three changes of sterile water and then placed on sterile PDA media Petri plates. Growth of the re-isolated test pathogens were compared with the original pure culture of the test fungus obtained from naturally diseased plants.

B. Rhizome inoculation method
Pathogenicity of the test pathogens was confirmed by the rhizome soaking method under *in vitro* conditions. The healthy rhizomes of ginger Cv. Mahim were surface sterilized with 0.01 percent ($NaOCl_2$) solution for 5 minutes, followed by three sequential washings with sterilized water in Petri plates to remove traces of the chemical. The rhizomes were then blot dried and soaked in spore cum-mycelial suspension of fungal pathogens. These pre-inoculated rhizomes were then shade dried and transferred onto sterilized glass Petri plates bottom disc lined with three layers of moistened blotter paper. Healthy rhizomes were surface sterilized with sodium hypochlorite, but were kept un-inoculated in another Petri plate lined with moistened blotter paper, which served as control. All the plates were incubated at room temperature (28±2 °C) for 7 days. Blotter paper in Petri plates was moistened by the addition of sterilized water as per requirement.
Observations on number of rhizome pieces showing a growth of the test pathogen and disease-free rhizome pieces were recorded on the 7th day after incubation. Cultural characters of test pathogen were observed and compared with their pure culture growth.

3. Results and Discussion
The findings of the present study as well as relevant discussion have been presented under the following heads

3.1 Isolation of pathogens
The different fungal pathogens *viz*. *Pythium aphanidermatum*, *Fusarium oxysporum* f.sp *zingiberi* and *Sclerotium rolfsii* were isolated from infected rhizomes.

3.2 Identification of pathogens causing rhizome rot of Ginger.
The identification of isolated pathogens was confirmed on the basis of their cultural, morphological, microscopic observations

1. ***Pythium aphanidermatum*:** The colony of the fungus was white, fluffy and had dense mycelium. The mycelium was coenocytic, hyaline, well branched, and colourless to white. Zoospore and zoosporangium were observed under the microscope. Similar morpho-cultural characters were earlier reported by Parveen and Sharma, (2015) [5]; Rajlakashmi *et al.* (2016) [7]; Kawathe, (2020) [4] and Patil, (2021) [6].
2. ***Fusarium oxysporum* f. sp *zingiberi*:** The mycelium of the isolated fungus showed woolly to cottony growth. Hyphae were septate, hyaline and colour varied from white to dull white with slightly yellowish. The micro conidia and macro conidia were observed under the microscope. These morphological characters are in accordance with the morphological characters earlier reported by Kawathe (2020) [4] and Patil, (2021) [6].
3. ***Sclerotium rolfsii*:** The mycelium of isolated pathogen had white, dense, radiating growth. The fungus produced silky white mycelium which gradually lost its lustre and became dull in appearance. Initiation of formation of sclerotial bodies were obtained from fifth day after inoculation. In the beginning, the sclerotial bodies were white but gradually turned to buff brown colour and then to chocolate brown. At maturity the sclerotial bodies were spherical to ellipsoidal. This type of observations on morpho cultural characters were earlier reported by Sagar (2006) [8]; Kawathe (2020) [4] and Patil, (2021) [6].

Table 1: Primary characters of isolated pathogens

Sr. No.	Pathogen	Colony characters				
		Colour	Pigmentation	Elevation	Septation	Spores
1	*Pythium aphanidermatum*	White	Yellowish	Flat	Aseptate	Zoospore
2	*Fusarium oxysporum* f. sp. *zingiberi*	White	Yellowish	Flat	Septate	Macro-conidia, Micro-conidia
3	*Sclerotium rolfsii*	White	Whitish	Flat	Septate	Sclerotia

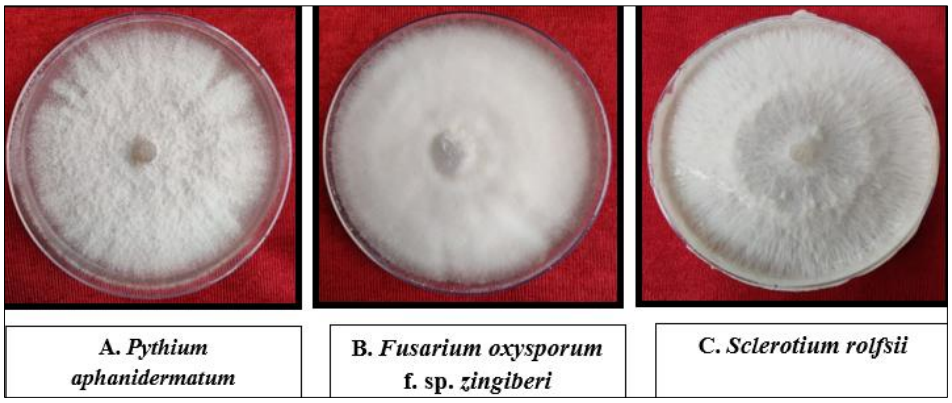


Plate 1: Ginger rhizome rot pathogens

3.3 Pathogenicity

Pathogenicity of all the isolated pathogens was proved by two methods under controlled conditions

1. Soil inoculation method
2. Rhizome inoculation method

A. Soil inoculation method

In soil inoculation method inoculum broth of *Pythium aphanidermatum*, *Fusarium oxysporum* f. sp *zingiberi and Sclerotium rolfsii* were mixed thoroughly with sterilized potting mixture and filled in pots, watered adequately and incubated in screen house for two weeks. One pot filled with sterilized potting mixture and without culture was maintained as un-inoculated control. These pots were incubated for 15 days at room temperature, frequently stirred, watered regularly and the pathogens were allowed to colonize in the pots. Surface sterilized (1% NaOCl) rhizomes of ginger (Cv. Mahim) were then sown (@ 5 rhizomes /pot) and kept in screen house at room temperature and watered regularly. The characteristic symptoms of ginger rhizome rot exhibited on naturally and artificially diseased ginger (Cv. Mahim) and cultural and morphological characteristics of the test pathogen isolated from naturally and artificially diseased rhizomes of Cv. Mahim were found exactly similar.

The results revealed that all the test pathogens were pathogenic to the ginger. Among the three test pathogens the pots inoculated with *Pythium aphanidermatum* showed lowest rhizome germination with highest infection. In case of *P. aphanidermatum* inoculated pots infection began at 45 days after planting and was highest at 90 days after planting. The pots inoculated with *Fusarium oxysporum* f. sp *zingiberi* showed initial infection 60 days after qplanting and moderate infection at 75 days after planting. The pots inoculated with *Sclerotium rolfsii* showed no infection until 60 days after planting and moderate infection was established 90 days after planting. The un-inoculated control pot showed 100 percent germination with no mortality over 165 days after planting.

Table 2: Pathogenicity by soil inoculation method

Sr. No.	Pathogens	Infection / day				
		30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
1	*P. aphanidermatum*	-	+	+	++	+++
2	*F. oxysporum* f. sp. *zingiberi*	-	-	+	++	++
3	*S. rolfsii*	-	-	+	+	++
4	Control	-	-	-	-	-

- = No infection + = less infection ++ = moderate infection +++ =higher infection

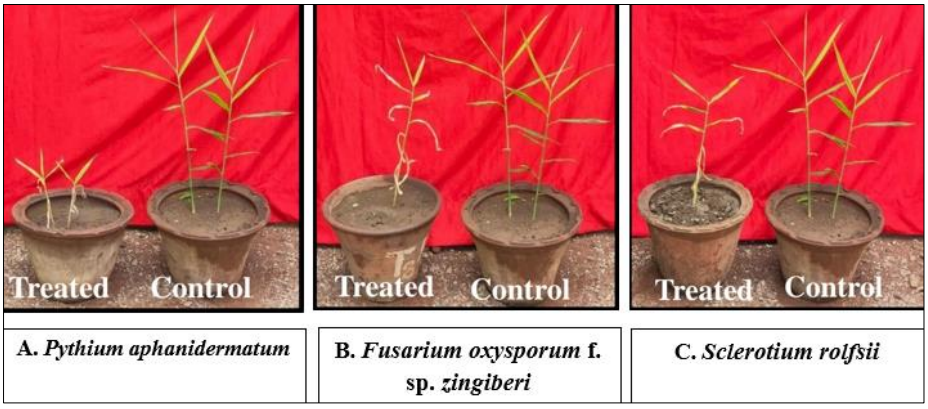


Plate 2: Pathogenicity by soil inoculation method

B. Rhizome inoculation method

Pathogenicity of the isolated pathogens was proved using rhizome dip method under *in vitro* conditions. The degree of rotting of inoculated rhizomes at different days after inoculation was recorded. The scale of observations were no symptoms (-), less rotting (+), moderate rotting (++) and higher rotting (+++).

The results revealed that rhizomes dipped in *Pythium aphanidermatum* inoculum showed earliest rotting on 3rd day after inoculation with moderate rotting at 6th day after inoculation and highest rotting at 7th day after inoculation followed by *Fusarium oxysporum* f. sp *zingiberi* which showed initial rotting at 3rd day after inoculation with moderate rotting at 7th day. *Sclerotium rolfsii* showed initial

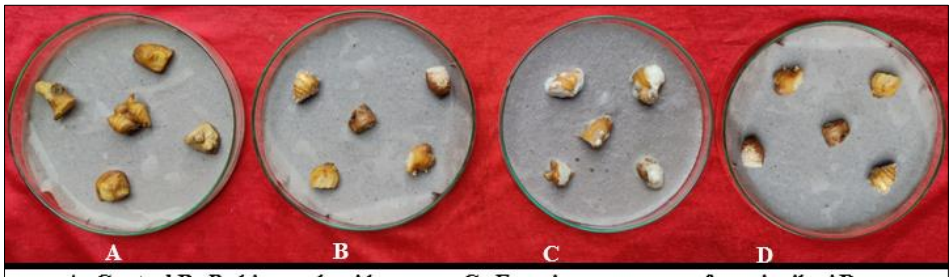
rotting symptoms after 4th day of inoculation. None of the pathogens showed infection before 3rd day after inoculation. Un-treated control rhizomes did not show any rotting. The pathogens were re-isolated from these rotted rhizomes and the morpho-cultural characters were matched to the original characters and thus pathogenicity of these pathogens was proved.

These studies revealed that *P. aphanidermatum* was more pathogenic and showed early infection as compared to other pathogens.

Table 3: Pathogenicity by rhizome dip method

Sr. No.	Pathogens	Infection / day						
		1st	2nd	3rd	4th	5th	6th	7th
1	*P. aphanidermatum*	-	-	+	+	+	++	+++
2	*F. oxysporum* f. sp. *zingiberi*	-	-	+	+	+	+	++
3	*S. rolfsii*	-	-	-	+	+	+	+
4	Control	-	-	-	-	-	-	-

- = No rotting + = less rotting ++ = moderate rotting +++ =higher rotting



A: Control B: *Pythium aphanidermatum* C: *Fusarium oxysporum* f. sp *zingiberi* D: *Sclerotium rolfii*

Plate 3: Pathogenicity by rhizome inoculation method

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