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Guarding the ridge gourd: Characterization and *in vitro* management of *Phytophthora nicotianae* causing fruit rot of *Luffa acutangula* in North Kerala

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Abstract
Fruit rot disease, a significant threat to ridge gourd cultivation, was prevalent in the North Zone of Kerala in 2019 from May to August. The prevalence of the disease was attributed to warm and humid climatic conditions, resulting in an incidence of 47 percent. This led to a devastating economic loss, accounting for 43.75 percent of the produce. Extensive studies encompassing symptomatology, cultural, morphological, and molecular characterisation were conducted to identify the causative pathogen, which was identified as *Phytophthora nicotianae* (Gen Bank Accession No. MK789292.1). In a bid to manage the disease, the efficacy of various fungicides, including mancozeb 75WP, carbendazim (12%) + mancozeb (63%) (Saaf), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), and tebuconazole 250EC (Folicur), was assessed through *in vitro* experimentation. Remarkably, cent percent pathogen inhibition was achieved across different concentrations, demonstrating their promising potential. Furthermore, botanicals such as garlic extract, azadirachtin 0.1 percent (Neemazal), and ready-to-use neem oil garlic soap (Raksha) were evaluated. These botanicals exhibited varying levels of inhibition, with garlic extract displaying the highest efficacy at 77.77 percent, followed by azadirachtin at 22.22 percent and ready-to-use neem oil garlic soap at 16.66 percent across different concentration ranges. *Trichoderma viride* and PGPM have emerged as strong contenders in investigating potential biological control agents, demonstrating complete inhibition against *Phytophthora nicotianae*. Additionally, *Pseudomonas fluorescens* and *Bacillus subtilis* exhibited 61.1 percent and 50 percent inhibition rates, while PGPR Mix-II exhibited an inhibition rate of 16.66 percent.

Keywords: Ridge gourd, *Phytophthora nicotianae*, MBIT, *T. viride, Bacillus subtilis, P. fluorescens*

Introduction

Ridge gourd is one of the most popular and widely cultivated cucurbitaceous vegetables in South India. It is highly nutritious and rich in fibre, vitamins, and minerals such as Vitamin B2, Vitamin C, carotene, niacin, calcium, phosphorus, and iron. In addition, it also contains trace amounts of iodine, which is known to be beneficial for human health. The soft pulp and ridge gourd skin are used in various recipes, especially in South Indian cuisine (Manikandaselvi *et al*., 2016) [10]. The warm, humid tropical climate favours various diseases in ridge gourd. Fruit rot caused by *Phytophthora nicotianae* is one of them, which affects the quality and quantity of the produce. *Phytophthora nicotianae* is a highly destructive plant pathogen that has a widespread impact on agriculture globally. This pathogen is known to cause rotting of leaves, fruits, and roots in various economically significant crops. (Erwin and Ribiero, 1996) [3]. Fruit rot disease of ridge gourd, caused by *Phytophthora nicotianae*, caused a massive loss of produce with a percent disease incidence of 47% in 2019 due to the warm, humid climatic conditions during May-August. *P. nicotianae* had a broad host range, including over 255 plant genera in 90 families (Mammella *et al*., 2013) [9]. Choudappa *et al*., 2016 [1] reported that since 2011, fruit rots caused by *Phytophthora nicotianae* had become increasingly prevalent during the south-west monsoon period (June to September) in the Southern zone of India, posing a significant threat to ridge gourd production. Fungi-induced fruit rot diseases have been causing substantial economic losses in Kerala, owing to the highly conducive climatic conditions for disease proliferation.

Hence, this study was designed to identify, characterise, and manage the fungal pathogen associated with ridge gourd fruit rot disease.

Materials and Methods

Survey and collection of diseased samples

A purposive sampling survey was carried out in North Kerala at various locations in the different regions of Kasargod district, *viz*., Periya, Karakode, and Kurunthur, during the South West monsoon period (June-August) in the context of the prevalence of the fruit rot disease in ridge gourd. The survey recorded the Percent Disease Incidence using the formula given by Wheeler (1969) [28] and the Percent Disease Severity using the formula given by Al-Sadi *et al*. (2011) [1].

Percent disease incidence (PDI) = (Number of plants infected/Total number of plants observed) x 100

Percent disease severity (PDS) = (Number of fruits affected per plant/Total number of fruits per plant) x 100

Then, the fruit rot-affected samples were collected, and the associated fungal pathogen was isolated under aseptic conditions.

Isolation and pathogenicity studies

The standard isolation method was followed to isolate the fungal fruit rot pathogen from the collected samples. The collected samples were washed with running tap water, and small bits of healthy and infected portions were cut using a sterile blade. The bits were then surface sterilised using mercuric chloride (0.1%) and rinsed thrice with sterile water under aseptic conditions. The filter paper-dried bits were subsequently inoculated in a Petri plate containing sterilised, solidified Potato Dextrose Agar (PDA) media impregnated with streptomycin and kept for incubation at room temperature (26±2 °C). Finally, the pathogenicity of the fungal isolate was immediately demonstrated to establish Koch’s postulate by utilising the Mycelial Bit Inoculation technique (MBIT) on the detached fruit. In MBIT, ten-day-old isolates were used for inoculation. Fresh, healthy, non-infected fruits were collected, brought to the laboratory, and washed under running water, followed by surface sterilisation using 70% ethanol. Artificial inoculation of the fungal isolate was done using a sterile needle after making an injury (pinpricks) on healthy fruits. Mycelial bits were placed at the site of pinpricks. Moist cotton was used to cover the site of inoculation. The fruits were then placed in an incubation chamber with high humidity at room temperature until the symptoms emerged in the inoculated areas. The control comprised healthy plant parts injured but not inoculated with the fungal mycelium. Symptoms appeared on the third day of inoculation as dark water-soaked lesions with white mycelial growth on the surface of the fruits. Later, these lesions enlarged, resulting in soft, watery rot. The artificially inoculated fruits were used to re-isolate the fungal pathogen. A comparison was made between the cultural and morphological characteristics of the re-isolated pathogen and the original isolate to establish Koch's postulate.

Symptomatology studies

The symptomatology of fungal fruit rot disease in ridge gourd was assessed in natural and artificial conditions. Observations under natural conditions were made during purposive sampling surveys conducted in various locations. Under artificial conditions, the Mycelial Bit Inoculation Technique (MBIT) was used to study the symptom development. To track the symptom development, mature detached fruits artificially inoculated with a mycelial bit were incubated in a polythene cover for one week.

Characterisation and identification of the pathogen

We identified the fungal fruit rot pathogen by conducting cultural, morphological, and molecular characterisation. The pathogen was grown on PDA for cultural analysis, and its mycelial growth pattern and rate were recorded. Microscopic observation was utilised to study the pathogen's morphological characteristics, including the mycelium type, branching pattern, spore size, shape, and colour, and the presence of sexual structures. The production of sporangia was achieved through the biting method. This method used autoclaved sterile new flush growth of *Cynadon dactylon* grass as bait. The leaf bite was then incubated with mycelial bits in sterile distilled water for three days to produce sporangia and sporangiospores.

The ZEN imaging software was utilised to obtain microscopic images and measurements of diverse fungal structures. The cultural and morphological features compared with the CMI Descriptions of Pathogenic Fungi and Bacteria descriptions. The identified isolate was provisionally categorised up to the genus level. Molecular characterisation was performed at Rajiv Gandhi Center for Biotechnology (RGCB), Thiruvananthapuram, Kerala, by sequencing the ITS region. The NCBI-BLASTn program was employed to scrutinise the sequence and nucleotide homology of the fungal pathogen. The ITS sequence analysis confirmed the identity of the pathogen up to the species level. The ITS sequence was assigned with the Accession number MK789292.1. in NCBI-Gen bank.

Efficacy of fungicides, botanicals, and biocontrol agents against fruit rot pathogen of ridge gourd under *in vitro* conditions

The efficiency of different fungicides, botanicals, and biocontrol agents against the fruit rot pathogen of ridge gourd under *in vitro* conditions was tested. Contact, combination, and systemic fungicides *viz*., mancozeb 75WP, carbendazim (12%) + mancozeb (63%) (Saaf), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), tebuconazole 250EC (Folicur), botanicals such as Azadiractin 0.1%, garlic extract, and ready to use neem oil garlic soap, biocontrol agents namely, *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis*, PGPR Mix-II and PGPM (both are microbial consortiums released from Kerala agricultural University) were selected for the study. The efficiency of chemical fungicides, botanicals, and biocontrol agents was tested using the poisoned food technique (Zentmeyer, 1955) [24] and dual culture technique (Skidmore and Dickinson, 1976). To calculate the percentage inhibition of mycelial growth in both methods, Vincent's formula (1927) was employed.

$$I = C\text{-}T/C x100$$

I: Percent inhibition
C: Mycelial growth in control (mm)
T: Mycelial growth in treatment (mm)

The data was subjected to statistical analysis using ANOVA and analysed using OPISTAT software. Data sets were determined for significance levels, means, and standard error. Multiple comparisons between the treatment means were conducted using DMRT in cases where the F test was significant. Furthermore, the data was transformed appropriately, as Gomez and Gomez (1984) [5] recommended.

Results and Discussion

Survey and collection of diseased samples

A purposive sampling survey was carried out to investigate fruit rot incidence in ridge gourd during the southwest monsoon season (May-August) in North Kerala. The study was conducted across several locations in different regions of Kasargod district, namely Periya, Karakode, and Kurunthur. The survey revealed the prevalence of the fruit rot disease with an average percent disease incidence of 47 and an average percent disease severity of 43.75. Chowdappa *et al*. (2016) [1] reported that *Phytophthora* fruit rot was many crops' most widespread and destructive disease. In addition, he recorded the incidence of the pathogen in the ridge gourd in South India. Our finding of *Phytophthora nicotianae* as a fruit rot pathogen in the Kasargod district agrees with the findings of Chowdappa *et al*. (2016) [1].

Symptomatology of fruit rot disease

The onset of the disease symptom was characterised by the appearance of dark water-soaked lesions on the fruits, accompanied by the growth of white mycelia. Eventually, this resulted in watery soft rot of the whole fruit (Fig. 1). Occurrence of this pathogen in ridge gourd was common during the warm, humid climate with frequent rains. These findings agreed with reports by Chowdappa *et al.* (2016) [1] on ridge gourd and Sharma and Sohi (1975) [20], Singh (1999) [25], Gupta and Paul (2001) [6], Housebeck (2004) [7], Granke (2012) [4], Shankar *et al*. (2014) [21] and Panabieres *et al*. (2016) [14], in other vegetable crops. Our findings validate the positive correlation between warm, humid climatic conditions and the fruit rot disease.

Isolation and pathogenicity studies

The pathogen was subjected to standard isolation and subsequently cultured in PDA media. Artificial inoculation of a mycelial bit of pathogen on detached mature fruits produced typical fruit rot symptoms after three days of incubation (Fig. 2). Koch's postulates were validated through the re-isolation of the pathogen from artificially inoculated fruits and its comparison with cultural and morphological characteristics of original isolate. A study conducted by Kousik *et al*. (2014) [11] found that mycelial bit inoculation of *Phytophthora capcisi* isolates induced typical fruit rot symptoms in bitter gourd fruits, which agrees with our result.

Characterisation and identification of the pathogen

Cultural characterisation revealed white-coloured colonies with irregular borders. Colonies had no specific pattern and produced dense or low-spreading aerial mycelium on PDA. The same white colour pattern was also observed on the bottom side of the petri dish (Fig. 3a). The total growth of the 90 mm petri dish took nine to eleven days. The hypha was branched, hyaline, and coenocytic. The isolate produced noncaducous and ovoid or lemon-shaped papillate sporangia. The dimensions of sporangia varied between 24-50 × 22-35 µm (Fig. 3b). On PDA, isolate produced spherical chlamydospores (Fig. 3c) that range from 20 to 50 µm diameter; it may be either terminal or intercalary. The pathogen-causing disease in ridge gourd was identified based on its cultural and morphological characteristics as *Phytophthora* sp. Further molecular characterisation confirmed its identity as *Phytophthora nicotianae*, with the It's sequence of the isolate deposited in Gen Bank under accession number MK789292.1. The isolate showed 100% homology with strains of *Phytophthora nicotianae* in BLASTn analysis. This finding is consistent with previous research by Chowdappa *et al*. (2016) [1], who identified papillate sporangia, dense low-spreading mycelium, and chlamydospores as characteristic features of this pathogen in ridge gourd.

In vitro efficiency evaluation of *P. nicotianae* with fungicides, botanicals and biocontrol agents against fruit rot pathogen

In vitro evaluation of the pathogen with contact, combination, and systemic fungicides *viz*., mancozeb 75WP, carbendazim (12%) + mancozeb (63%) (Saaf), cymoxanil (8%) + mancozeb (64%) (Curzate M-8), tebuconazole 250EC (Folicur) was done. Cent percent inhibition was noted with all fungicides at all concentrations (lower, recommended, and higher) (Table 1 and Fig. 4). These results agree with the findings of Praveen *et al*. (2017) [16] where cymoxanil (8%) + mancozeb (64%) (Curzate M-8), carbendazim (12%) + mancozeb (63%) (Saaf), tebuconazole 250EC (Folicur) showed cent percent inhibition against *Phytophthora* sp. Gupta and Bharath (2008) [9] worked on Buckeye rot disease of tomato caused by the pathogen *Phytophthora nicotianae* var. *parasitica and* found that cymoxanil+mancozeb is the most effective fungicide. The data on the botanicals revealed that Garlic extract shows maximum percent inhibition of radial mycelia growth (66.66%, 77.77%, 100%) at all three concentrations (0.5%, 1%, 2%) (Table 2 and Fig. 5). Among botanicals best treatments were Azadirachtin 0.1% and Ready to use neem oil garlic soap, these were significantly not different. These results accorded with the results of Pente *et al*. (2015) [15]. Using a poisoned food technique, he evaluated neem and garlic at 5% concentration *in vitro* against *Phytophthora parasitica*. He observed 100 percent inhibition of mycelial growth of *Phytophthora parasitica* with garlic extract. According to Nagadze's research conducted in 2014, the aqueous extracts of *Allium sativum* (garlic) and *Azadirachta indica* (neem) exhibited significant effectiveness against *Phytophthora infestans*. This plant pathogen causes devastating damage to crops such as tomatoes and potatoes.

As biocontrol agents, *Trichoderma viridae* and Plant Growth Promoting Microbes (PGPM) were the best treatments and showed cent percent inhibition. *Pseudomonas fluorescens*, *Bacillus subtilis*, and PGPR Mix-II followed it. Here the least effective biocontrol agent against *Phytophthora nicotianae* was Plant Growth Promoting Rhizobacteria Mix-II (Table 3 and Fig. 6). The above findings matched with Mushrif *et al*. (2005) [11], who tested five bacterial antagonists (two *Bacillus spp*. and three *Pseudomonas* spp.) from the rhizosphere and phyllosphere of *Hevea* antagonistic to *Phytophthora*. *Bacillus* spp. The result showed a higher efficacy of bacterial antagonists in disease control under laboratory and field conditions. Sharma *et al*. (2018) [18] found that *T. viride* is very effective (67.43 percent inhibition) against *Phytophthora nicotianae* obtained from tomato, while *Pseudomonas fluorescens* was least effective with 53.67

percent mycelial growth inhibition. The study by Vithya *et al*. (2018) [23] revealed that *Trichoderma harzianum* was most effective in managing *Phytophthora* in black pepper nurseries.

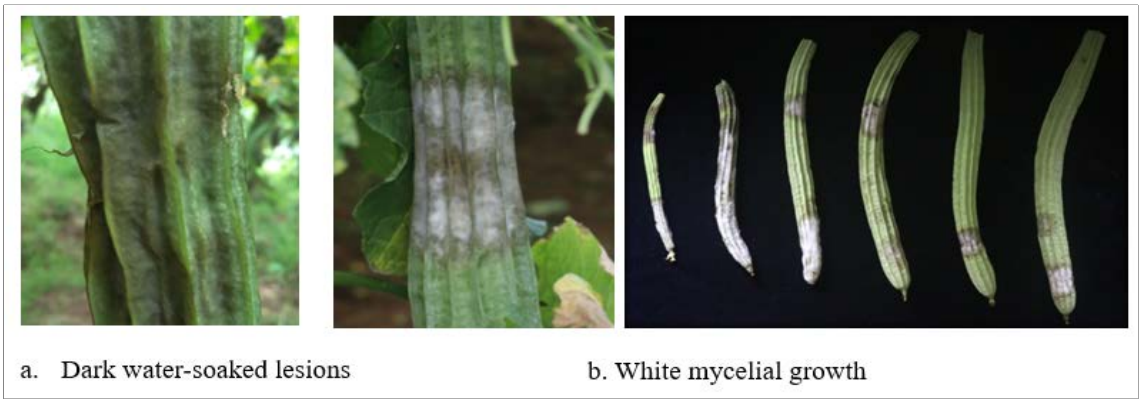

Fig 1: Symptomatology

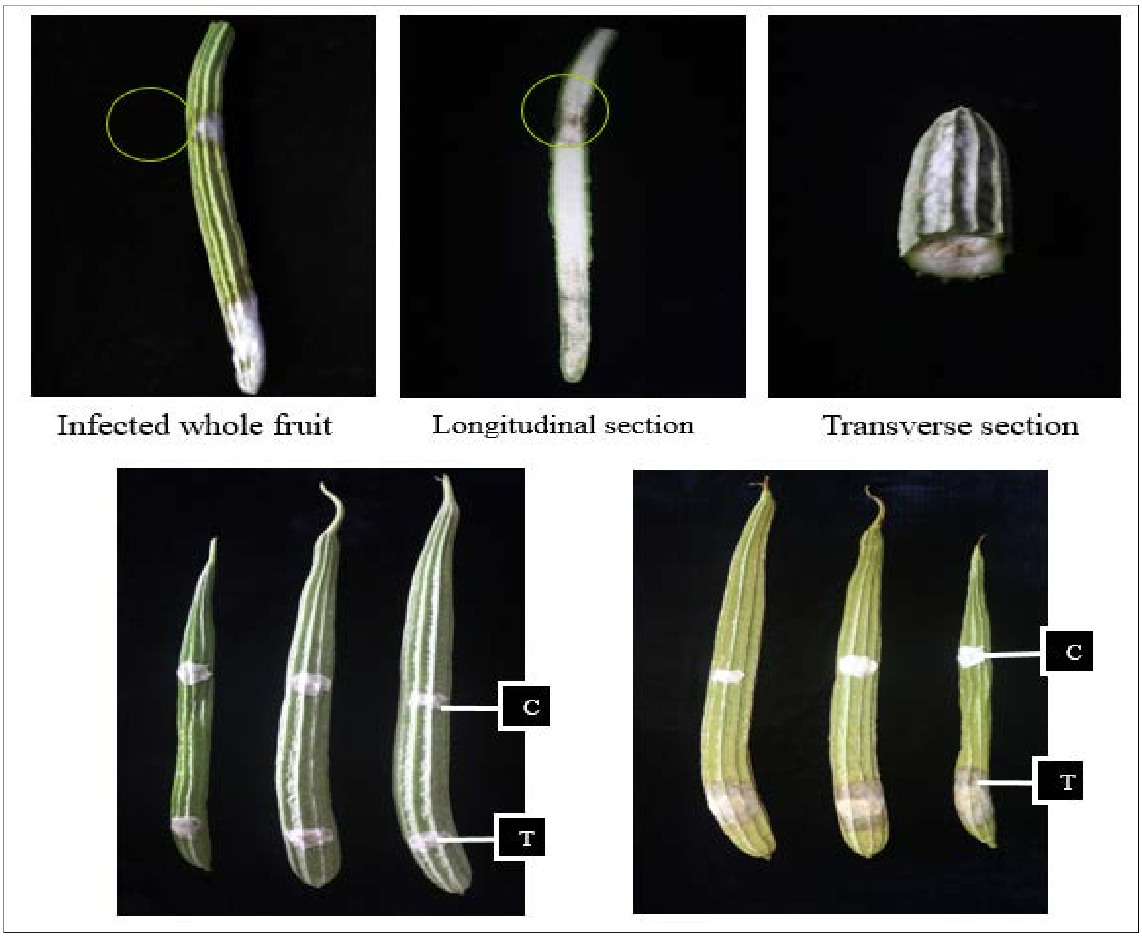

Fig 2: Pathogenicity test

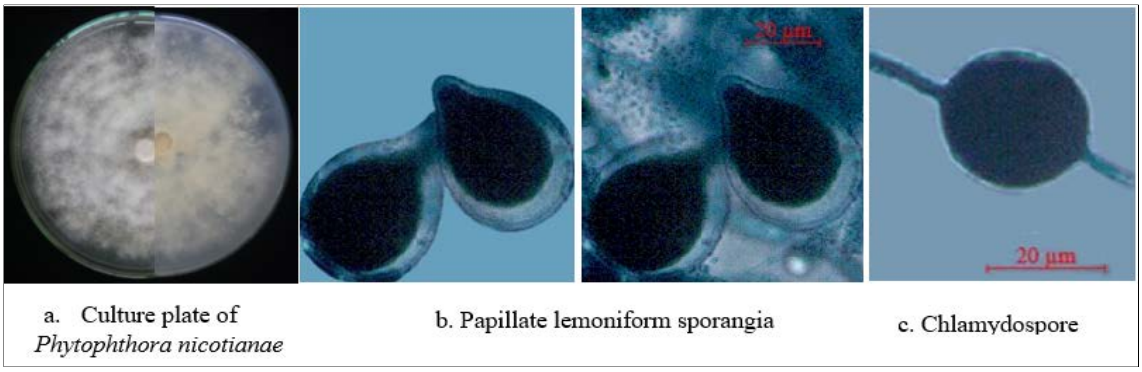

Fig 3: Cultural and morphological characters

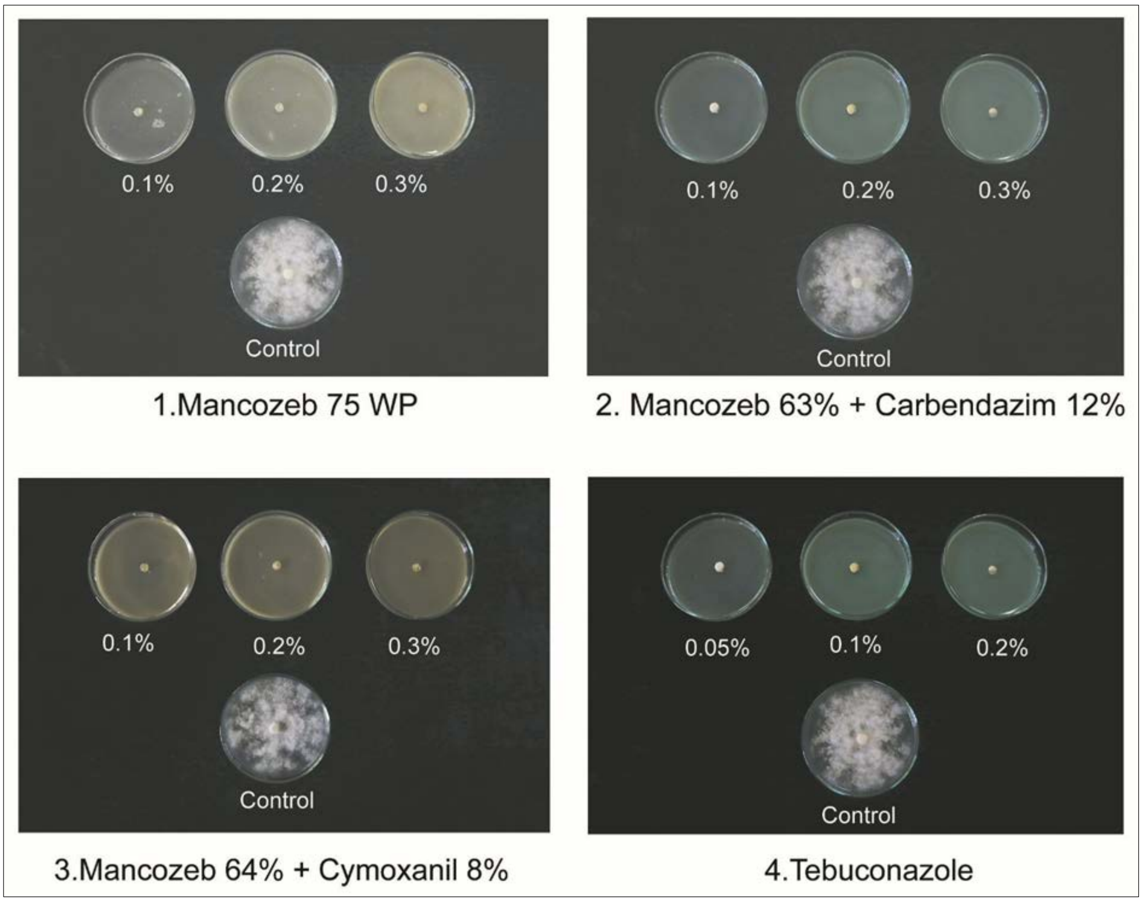

Fig 4: Effect of chemical fungicides on the radial growth of *Phytophthora nicotianae*

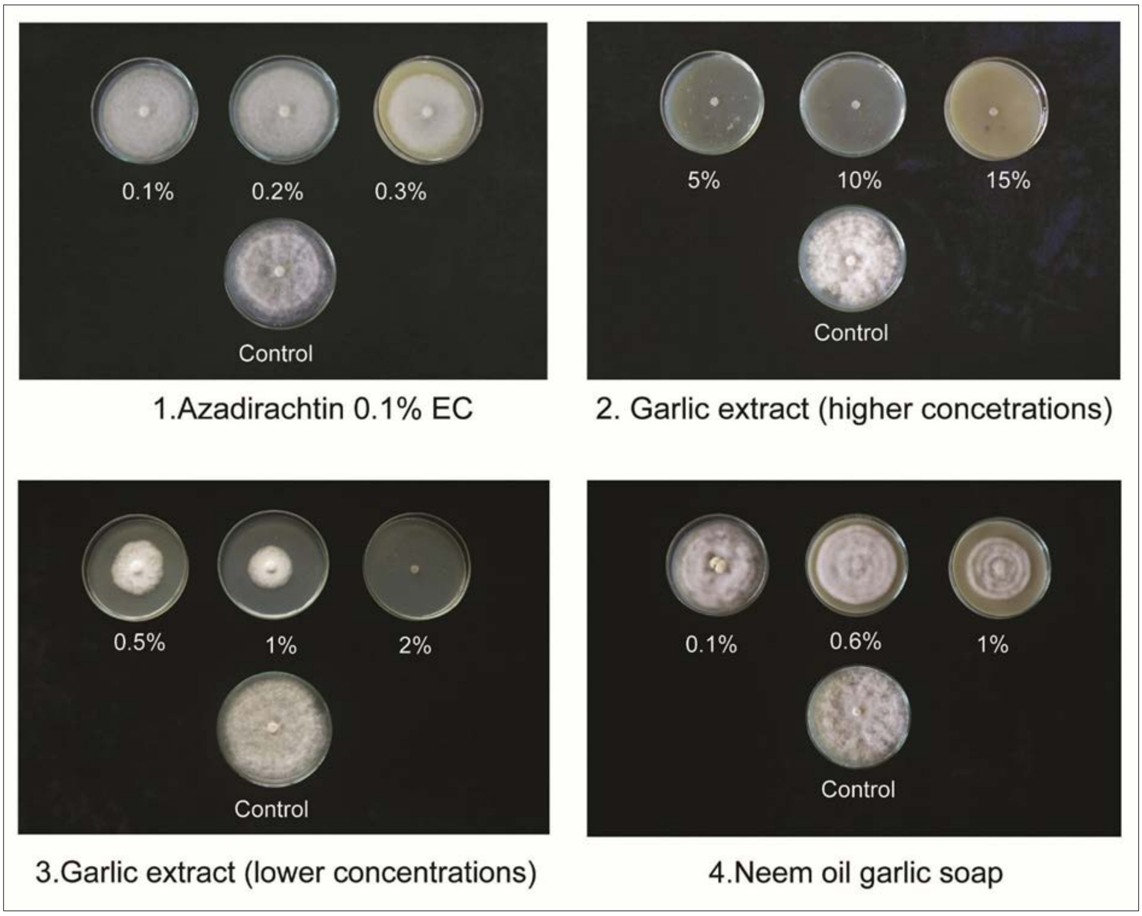

Fig 5: Effect of botanicals on the radial growth of *Phytophthora nicotianae*

Fig 6: Effect of biocontrol agents on the radial growth of *Phytophthora nicotianae*

Table 1: *In vitro* evaluation of chemical fungicides on the inhibition of mycelial growth of *Phytophthora nicotianae*

Treatments	**Chemical fungicides**	**Trade name**	**Inhibition of mycelial growth of *Phytophthora nicotianae* (%)**		
			C-1	**C-2**	**C-3**
T_1	Mancozeb 75% WP(0.1%, 0.2%, 0.3)	Macoban-M 45	100* (90.00)**	100 (90.00)	100 (90.00)
T_2	Mancozeb63%+Carbendazim 12% (0.1%, 0.2%, 0.3%)	Saaf	100 (90.00)	100 (90.00)	100 (90.00)
T_3	Mancozeb 64%+Cymoxanil 8% (0.1%, 0.2%, 0.3%)	Curzate M8	100 (90.00)	100 (90.00)	100 (90.00)
T_4	Tebuconazole 5% EC (0.05%. 0.1%, 0.2%)	Folicur	100 (90.00)	100 (90.00)	100 (90.00)
CD					
SE					

* Mean of three values
** Values in parenthesis are angular transformed values
C- Concentration

Table 2: *In vitro* evaluation of botanicals on the inhibition of mycelial growth of *Phytophthora nicotianae*

Treatments	Botanicals	Trade name	Inhibition of mycelial growth of *Phytophthora nicotianae* (%)		
			C-1	C-2	C-3
T_1	Azadiractin 0.1% (0.1%, 0.2%, 0.3%)	Neemazal	5.55* (11.02)**	22.22 (28.11)	33.32 (35.19)
T_2	Garlic extract (0.5%, 1%, 2%)		66.66 (54.71)	77.77 (61.96)	100 (90.00)
T_3	Ready to use neem oil garlic soap (0.1%, 0.6%, 1%)	Raksha	0 (0.00)	16.66 (23.88)	27.77 (31.71)
CD			11.74	6.81	5.78
SE			3.32	1.93	1.64

* Mean of three values
** Values in parenthesis are angular transformed values
C- Concentration

Table 3: *In vitro* evaluation of biocontrol agents on the inhibition of mycelial growth of *Phytophthora nicotianae*

Treatments	Biocontrol agents (2%)	Inhibition of mycelial growth of *Phytophthora nicotianae* (%)
T_1	*Trichoderma viride*	100* (90.00)**
T_2	*Pseudomonas fluorescens*	61.10 (51.42)
T_3	*Bacillus subtilis*	50 (44.98)
T_4	PGPR Mix-II	16.66 (23.88)
T_5	PGPM	100 (90.00)
CD		4.71
SE		1.40

* Mean of three values, ** Values in parenthesis are angular transformed values
C- Concentration

Conclusion

This study has unveiled new information about the fruit rot that affects ridge gourds in North Kerala during the southwest monsoon season, which causes significant yield losses. Using morpho-molecular analysis, the study identified the pathogen responsible for the disease as *Phytophthora nicotianae*. *In vitro* experiments have shown that chemical fungicides and botanicals, particularly garlic extract, can effectively manage the pathogen. Additionally, biological control agents like *Trichoderma viride* and PGPM have also showed significant inhibition, offering promising eco-friendly disease management strategies.

To ensure sustainable cultivation of this economically important crop, future research should focus on developing resistant ridge gourd varieties and exploring novel eco-friendly disease management practices. This study can serve as a foundation for further research into the complex interactions between ridge gourd and *Phytophthora nicotianae*, with the aim of securing the future of ridge gourd cultivation.

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