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Hydrolytic Enzyme Activity Of Pathogenic Fungi Associated With Turmeric (Curcuma Longa): Implications For Tissue Degradation And Disease Progression

[1]Ranveersingh Rajendra Ambhure, [2]Prashant Pangarikar

[1]Research Scholar, [2]Head of Department

[1]Department of Botany, R. B. Attal Arts, Science and Commerce College, Georai, District Beed, Maharashtra, India

Abstract: Turmeric (Curcuma longa), a widely cultivated medicinal crop, is highly susceptible to fungal infections that impact its yield and quality. This study aimed to isolate and characterize pathogenic fungi from infected turmeric rhizomes and leaves collected across nine talukas in the Parbhani district. Morphological and microscopic identification revealed ten fungal species: Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum curcumae, Colletotrichum gloeosporiodes, Curvularia lunata, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani and Rhizoctonia solani. The hydrolytic enzyme activity of the fungal isolates was assessed to evaluate their pathogenic potential. Cellulase and protease activity were quantified using spectrophotometric assays, revealing significant enzymatic variability among species. Colletotrichum gloeosporioides, Colletotrichum capsici, and Pythium aphanidermatum exhibited the highest cellulase and protease activities, suggesting a strong capacity for tissue degradation and pathogenicity. Fusarium solani, Fusarium proliferatum, and Rhizoctonia solani also demonstrated considerable enzymatic activity, further supporting their role in host tissue colonization. In contrast, Aspergillus flavus and Aspergillus niger exhibited lower enzyme activity, indicating the possibility of alternative pathogenic mechanisms. The results highlight the role of hydrolytic enzymes in fungal virulence, contributing to turmeric crop deterioration. Understanding these enzymatic capabilities can aid in developing targeted disease management strategies. This study provides essential knowledge into the pathogenic behaviour of fungal isolates, offering a foundation for future research on biocontrol approaches and resistant cultivar development to mitigate turmeric diseases.

Index Terms **- Curcuma longa, pathogenic fungi, hydrolytic enzyme, cellulase, protease.**

I. INTRODUCTION

Turmeric (Curcuma longa L.), a rhizomatous herbaceous plant belonging to the Zingiberaceae family, is widely cultivated for its medicinal, culinary, and industrial applications (Kaliyadasa & Samarasinghe, 2019). It is valued for its bioactive compounds, particularly curcuminoids, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties (Rubió et al., 2013). However, turmeric production is often compromised by fungal infections, which significantly affect yield and quality (Kumar, 2023). Various soil-borne and foliar fungal pathogens infect turmeric, leading to rhizome rot, leaf spot, and wilting diseases, ultimately causing economic losses to farmers (Sagar, 2006).

Fungal pathogens exert their virulence through the secretion of hydrolytic enzymes, which play a crucial role in host tissue colonization and disease progression (Schaller et al., 2005). Among these, cellulase and protease are key enzymes responsible for degrading plant cell wall components and host proteins, facilitating fungal invasion (Lorrai & Ferrari, 2021). Cellulase hydrolyzes cellulose into glucose monomers,

weakening the structural integrity of plant cell walls, while protease degrades host proteins, disrupting cellular function and enhancing fungal pathogenicity (Lorrai & Ferrari, 2021). The enzymatic activity of different fungal species varies, influencing the extent of damage caused to the host plant (Usha Rani & Jyothsna, 2010).

Several fungal species have been reported to be associated with turmeric diseases, including Fusarium spp., Colletotrichum spp., Alternaria alternata, Rhizoctonia solani, and Aspergillus spp. (Fulano, 2016). These pathogens are known to produce hydrolytic enzymes that contribute to pathogenicity, making their identification and characterization critical for disease management strategies. Understanding the enzymatic activity of these fungi can provide knowledge into their virulence mechanisms and aid in developing targeted control measures (Shahid et al., 2012).

The present study aims to isolate and morphologically identify fungal pathogens from infected turmeric rhizomes collected from nine talukas in the Parbhani district. Furthermore, the study investigates the hydrolytic enzyme activities, specifically cellulase and protease, of the isolated fungi to assess their role in host tissue degradation. The results will contribute to a better understanding of the pathogenic potential of these fungi, facilitating the development of effective disease management strategies for turmeric cultivation;

II. Materials and Methods

2.1. Isolation of Fungi from the Explant

Fungi were isolated from Curcuma longa (turmeric) explants collected from nine talukas in Parbhani district. The infected leaf and rhizome samples were first surface-sterilized to eliminate external contaminants. After sterilization, small sections (5 mm) containing both healthy and infected tissues were excised and placed on sterile water agar medium supplemented with streptomycin (100 µg/ml) to prevent bacterial contamination. The infected portion was embedded within the agar, while the healthy section remained on the surface to promote fungal growth. The plates were then sealed with paraffin tape to maintain sterility and incubated at 28°C for five days or until fungal growth was observed. This method ensured the successful isolation of pathogenic fungi while minimizing bacterial interference, facilitating accurate identification and further analysis (Richardson & Fedoroff, 2001). Once isolated, the fungal cultures were assessed for hydrolytic enzyme activity, specifically cellulase and protease, to determine their potential for tissue degradation and pathogenicity. These enzymatic assays provided knowledge into the role of fungi in disease progression by evaluating their ability to break down plant cell walls and proteins.

2.2. Hydrolytic Enzyme Activity

Hydrolytic enzymes produced by fungi are crucial for their adaptability in environments where nutrients may not be readily available (Magan, 2007). These enzymes enable fungi to degrade carbon sources present in host materials, converting them into usable energy. The presence of genes coding for these enzymes and their expression can vary depending on environmental factors. Studying the production of these hydrolytic enzymes is essential for characterizing the pathogenicity of fungal isolates.

2.2.1. Quantitative estimation of Cellulase

The cellulase activity in healthy and pathogen-infected turmeric rhizomes was assessed using a spectrophotometric method (Shuangqi et al., 2011). Turmeric rhizome tissues, both healthy and infected, were collected and homogenized in ice-cold 50 mM sodium acetate buffer (pH 5.0) at a ratio of 1 g tissue per 5 mL buffer. The homogenate was centrifuged at 12,000 rpm for 15 minutes at 4°C, and the supernatant was collected as the crude cellulase extract. A 1% (w/v) solution of carboxymethyl cellulose (CMC) in 50 mM sodium acetate buffer (pH 5.0) was prepared as the substrate. For the assay, 0.5 mL of enzyme extract was mixed with 0.5 mL of the CMC solution and incubated at 50°C for 30 minutes. The reaction was stopped by adding 1.0 mL of dinitrosalicylic acid (DNS) reagent, which was used to estimate the reducing sugars released due to cellulase activity. The reaction mixture was then heated in a boiling water bath for 10 minutes to develop the color and subsequently cooled to room temperature. Absorbance was measured at 540 nm using a UV-Vis spectrophotometer. A blank containing only substrate and DNS reagent (without enzyme extract) was used for baseline correction. Negative controls, in which the enzyme extract was inactivated by boiling for 5 minutes prior to the assay, were also included to account for non-enzymatic reactions. A glucose standard curve, prepared using known concentrations of glucose ranging from 0.1 to 1.0 mg/mL, was used to quantify the reducing sugars released. Cellulase activity was calculated as the amount of reducing sugar released, expressed in µmol per minute per gram of fresh weight (U/g FW) or per milligram of protein (U/mg protein). This analysis provided a comparison of cellulase activity between healthy and infected rhizomes, revealing the potential physiological impacts of pathogen infection on cellulase production.

2.2.2. Quantitative estimation of Protease

The quantitative determination of protease activity, along with the assessment of pectinase activity in healthy and pathogen-infected turmeric rhizomes, was carried out using spectrophotometric methods. Protease activity was measured as per the method of McDonald and Chen (1965). The reaction mixture consisted of 0.5

mL of crude extract containing a known amount of protein (50 µg) in Tris–HCl buffer (50 mM, pH 7.2) and 0.5 mL of casein (2% w/v) dissolved in citrate-phosphate buffer (50 mM, pH 6.8). The mixture was incubated at 37°C for 1 hour. The reaction was terminated by adding 1 mL of 10% (w/v) ice-cold trichloroacetic acid (TCA). Unhydrolyzed casein was removed by centrifugation at 5,000 rpm for 5 minutes. An aliquot (0.5 mL) of the supernatant was mixed with 2.5 mL of a reagent containing 2.9% sodium carbonate and 0.3 N sodium hydroxide, followed by the addition of 0.75 mL of Folin–Ciocalteu's phenol reagent (diluted 1:3 with distilled water). The samples were incubated at 37°C for 20 minutes, and absorbance was recorded at 650 nm using a UV-Vis spectrophotometer (Ultra Spec 1100 Pro, Amersham Pharmacia Biotech, US). A standard curve constructed using tyrosine was used to quantify the protease activity. One unit of protease activity was defined as the amount of enzyme that liberates 1 µmol of tyrosine equivalent per minute under the specified assay conditions.

III. RESULTS AND DISCUSSIONS

3.1. Morphological identification of fungi

The fungal hyphae and spore structures were distinct in identifying the fungal species. Through detailed microscopic examination, the morphological characteristics of the hyphae, such as branching patterns, septation, and pigmentation, along with the spore morphology including shape, size, and arrangement, were analyzed and found to be Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum curcumae, Colletotrichum gloeosporiodes, Curvularia lunata, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani and Rhizoctonia solani.

3.2. Study of hydrolytic enzymes

3.2.1. Cellulase

The cellulase activity of fungal isolates from turmeric rhizomes exhibited significant variability, reflecting the cellulolytic potential of each species and their contribution to pathogenicity. The highest cellulase activity was recorded in Colletotrichum gloeosporioides (36.8 ± 0.05 U/mg protein), followed by Colletotrichum curcumae (33.73 ± 0.07 U/mg protein), which showed their strong enzymatic capability to degrade cellulose as shown in table 1 and figure 1. Curvularia lunata showed an activity of 30.77 ± 0.07 U/mg protein, closely followed by Fusarium solani (30.27 ± 0.1 U/mg protein) and Fusarium proliferatum (29.6 ± 0.09 U/mg protein). Rhizoctonia solani displayed a cellulase activity of 29.63 ± 0.03 U/mg protein, while Alternaria alternata and Fusarium oxysporum exhibited similar activities of 28.47 ± 0.17 U/mg protein and 28.43 ± 0.07 U/mg protein, respectively. Aspergillus flavus and Aspergillus niger showed comparatively lower activities of 25.7 ± 0.09 U/mg protein and 24.57 ± 0.07 U/mg protein, respectively, but still notably higher than the baseline. In contrast, the healthy rhizome demonstrated significantly lower cellulase activity at 10.3 ± 0.05 U/mg protein, representing the basal enzymatic levels in non-infected tissue. The negative control and blank samples exhibited minimal or no activity at 1.33 ± 0.12 U/mg protein and 0 ± 0 U/mg protein, respectively, confirming the accuracy of the experimental conditions. The highest cellulase activity was observed in Colletotrichum gloeosporioides and Colletotrichum curcumae, the results were similar when Lakshmesha et al. (2005) did the study of hydrolytic enzymes on capsicum. Therefore, it reflected their strong enzymatic ability to degrade cellulose. This heightened activity suggested an important role in tissue maceration and pathogenesis, as cellulase is a key enzyme for breaking down plant cell walls and facilitating fungal invasion (Reignault et al., 2008). Curvularia lunata, Fusarium solani, and Fusarium proliferatum exhibited substantial cellulase activity, further supporting their capacity to degrade host cell walls and contribute to disease progression. Studies carried out by Vinayarani and Prakash (2018), on the effect fungal endophytes of Curcuma longa against the pathogens Curvularia lunata and Rhizoctonia solani aligned with the present study. The high cellulolytic potential of these species aligns with their known pathogenic behavior in various crops, including turmeric. Rhizoctonia solani, Alternaria alternata, and Fusarium oxysporum displayed moderate cellulase activities, which, although lower than those of Colletotrichum species, still indicated their potential to compromise plant tissue integrity. Research done by Dwivedi (2015), suggested that cellulase activity increased when the diseased brinjal and tomato affected by Fusarium oxysporum and Fusarium solani, which contrasted with the present study. These results suggest a varying degree of dependency on cellulase-mediated degradation for pathogenicity among different fungal isolates (Sornakili et al., 2020). In contrast, Aspergillus flavus and Aspergillus niger exhibited relatively lower cellulase activity, indicating that their pathogenic mechanisms might rely on other enzymatic or non-enzymatic factors. In contrast, studies by Bamidele and Oladiti (2013), investigated that highest cellulase acvtivity was seen by Aspergillus niger cultured on potato peels. The comparatively low activity of these species does not negate their pathogenicity but rather highlights the diverse arsenal of tools employed by fungi to infect host plants (Doohan & Zhou, 2017).

Table 1 Cellulase activity (U/mg) of isolated fungi

Sample	Cellulase activity (U/mg protein)
Healthy Rhizome	10.30 ± 0.05
Alternaria alternata	28.47 ± 0.17
Aspergillus flavus	25.70 ± 0.09
Aspergillus niger	24.57 ± 0.07
Colletotrichum curcumae	33.73 ± 0.07
Colletotrichum gloeosporioides	36.80 ± 0.05
Curvularia lunata	30.77 ± 0.07
Fusarium oxysporum	28.43 ± 0.07
Fusarium proliferatum	29.60 ± 0.09
Fusarium solani	30.27 ± 0.10
Rhizoctonia solani	29.63 ± 0.03
Negative Control	1.33 ± 0.12
Blank	0.00 ± 0.00

Mean values followed by ± is the standard error of the mean

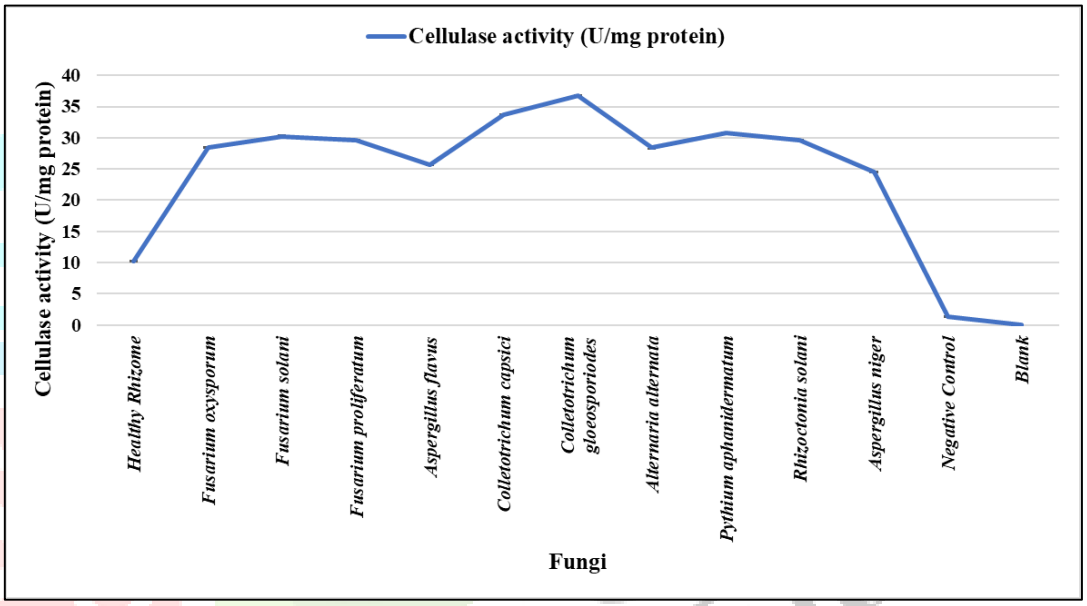

Figure 1 Cellulase activity (U/mg) of isolated fungi

3.2.1 Protease

The protease activity of fungal isolates from turmeric rhizomes revealed substantial differences among the species, underscoring their proteolytic potential and contribution to pathogenicity as shown in table 2 and figure 2. The highest protease activity was observed in Colletotrichum gloeosporioides (60.57 ± 0.05 U/mL protein), followed by Colletotrichum capsici (55.2 ± 0.05 U/mL protein) and Curvularia lunata (53.7 ± 0.09 U/mL protein), indicating their strong ability to hydrolyze proteins. Fusarium solani displayed an activity of 52.77 ± 0.19 U/mL protein, while Fusarium proliferatum and Rhizoctonia solani exhibited similar activities of 50.3 ± 0.05 U/mL protein and 49.7 ± 0.05 U/mL protein, respectively. Fusarium oxysporum and Alternaria alternata demonstrated moderate protease activities of 48.63 ± 0.07 U/mL protein and 46.43 ± 0.15 U/mL protein, respectively. Aspergillus flavus and Aspergillus niger showed comparatively lower activities of 42.63 ± 0.1 U/mL protein and 40.7 ± 0.05 U/mL protein, respectively, although significantly higher than the basal level. The healthy rhizome displayed a protease activity of 18.77 ± 0.19 U/mL protein, representing the normal enzymatic activity in uninfected tissue. The negative control exhibited minimal protease activity at 2.3 ± 0.16 U/mL protein, while the blank recorded no activity (0 ± 0 U/mL protein), confirming the validity of the assay. The highest protease activity was recorded in Colletotrichum gloeosporioides, followed by Colletotrichum capsici and Curvularia lunata, indicating their strong ability to hydrolyze proteins. The studies done by Muthukumar et al. (2016), aligned with the present study which recorded increased protease activity in solanaceous vegetables on pythium attack. This high enzymatic activity emphasized their aggressive pathogenic behavior, as proteases play a crucial role in breaking down host proteins, facilitating tissue colonization, and suppressing host defenses (Ramírez-Larrota & Eckhard, 2022). Fusarium solani, Fusarium proliferatum, and Rhizoctonia solani displayed substantial protease activities, further supporting their ability to degrade host proteins and contribute to infection. The comparable activity levels of these fungi suggested

a shared reliance on protease-mediated degradation as a pathogenic strategy. The results aligned with Li et al. (2017) who studies the protease activity of Fusarium proliferatum on banana fruit. Moderate protease activities were observed in Fusarium oxysporum and Alternaria alternata. These findings indicate that these species may rely on a combination of enzymatic and other factors to establish infection. The results aligned with the research carried out by Ramírez-Cariño et al. (2020), who investigated the biocontrol of Alternaria alternata and Fusarium proliferatum on tomato plant. In contrast, Aspergillus flavus and Aspergillus niger exhibited relatively lower protease activities, although their activity levels were still significantly higher than those of healthy rhizome tissues. In contrast to the present study, research done by Asis et al. (2009), stated that protease activity was higher in Aspergillus flavus and A. parasiticus on peanut seed infection.

Table 1 Protease activity (U/mL) of isolated fungi

Sample	Cellulase activity (U/mg protein)
Healthy Rhizome	18.77 ± 0.19
Alternaria alternata	46.43 ± 0.15
Aspergillus flavus	42.63 ± 0.10
Aspergillus niger	40.70 ± 0.05
Colletotrichum curcumae	55.20 ± 0.05
Colletotrichum gloeosporioides	60.57 ± 0.05
Curvularia lunata	53.70 ± 0.09
Fusarium oxysporum	48.63 ± 0.07
Fusarium proliferatum	50.30 ± 0.05
Fusarium solani	52.77 ± 0.19
Rhizoctonia solani	49.70 ± 0.05
Negative Control	2.30 ± 0.16
Blank	0.00 ± 0.00

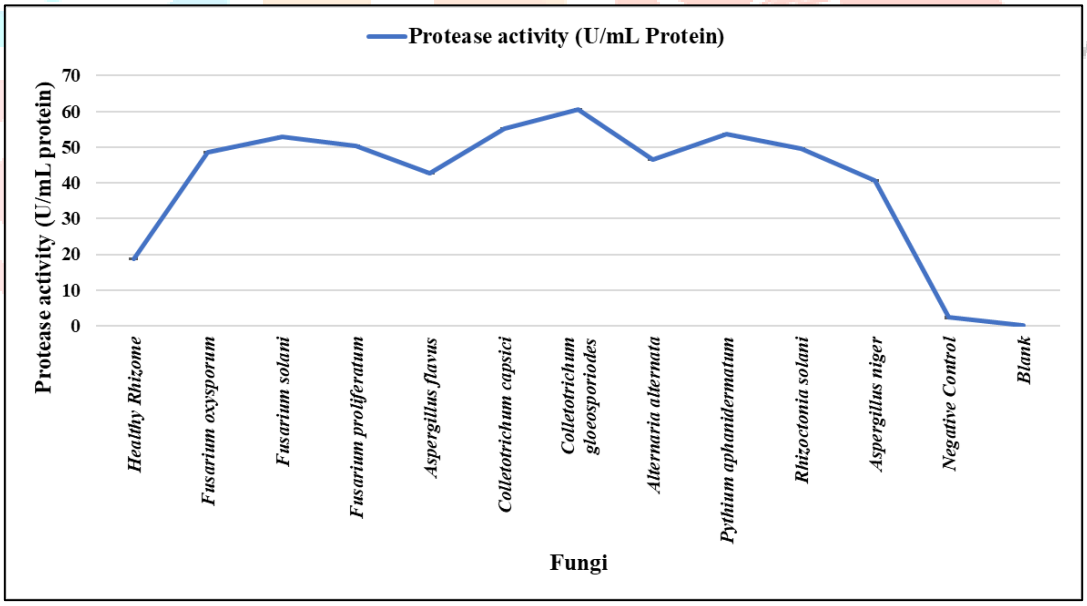

Figure 2 Protease activity (U/mL) of isolated fungi

IV. CONCLUSION

This study successfully isolated and identified pathogenic fungi associated with turmeric (*Curcuma longa*) infections from nine talukas in the Parbhani district. The morphological and microscopic characterization revealed ten fungal species, including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum curcumae*, *Colletotrichum gloeosporiodes*, *Curvularia lunata*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium solani* and *Rhizoctonia solani*. The quantitative assessment of hydrolytic enzyme activity showed that cellulase and protease production varied significantly among the isolates, directly influencing their pathogenic potential. *Colletotrichum gloeosporioides*, *Colletotrichum curcumae*, and *Curvularia lunata* exhibited the highest cellulase and protease activities, indicating their strong ability to degrade plant tissues and facilitate infection. Moderate enzyme activity observed in *Fusarium* and *Rhizoctonia* species suggested their involvement in disease progression through enzymatic degradation. In contrast,

Aspergillus flavus and *Aspergillus niger* showed lower enzyme activity, implying alternative pathogenic mechanisms.

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