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(RESEARCH ARTICLE)

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Isolation, identification, and enzyme screening of fungi infested various vegetables in the local markets of Visakhapatnam, Andhra Pradesh

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Abstract

This study aimed to examine the fungi present in fruits and vegetables obtained from the local vegetable markets, Visakhapatnam and assess their enzymatic activity. seven fungal pathogens responsible for vegetable spoilage were isolated using sterilization and spread plate techniques on potato dextrose agar (PDA) medium. The isolated fungi were identified based on macroscopic and microscopic characteristics. The identified fungal strains included *Aspergillus, Curvularia, Alternaria, Cladosporium, Fusarium, sclerotinia, Mucor, Penicillium*, and *Rhizopus*. Only three selected strains were tested for extracellular amylase, pectinase and cellulase activity, which were showing 15mm,20mm,30mm clear zone capability. These findings provide essential information about the degradative properties of filamentous fungi and their secretion of extracellular enzymes. The study also highlights the presence of toxigenic fungi in local vegetables, emphasizing the importance of removing spoiled fruits to prevent the accumulation of toxins associated with fungal growth.

Keyword: Enzyme activity; Spoilage; PDA; Degradative; Extracellular; Filamentous.

1. Introduction

In the Indian states of Andhra Pradesh and Telangana, there is a type of farmers' market called Rythu bazaar. It is managed by the Andhra Pradesh and Telangana governments for small-scale farmers with modest farmlands. In the month of January 1999, Chief Minister N. Chandrababu Naidu opened the inaugural market [1]

This encouraged small and medium-sized farmers to sell their products directly to end-users at a profit and helped them maintain a modest level of financial stability. Customers in towns and cities can purchase these goods directly from farmers for a lower price. This allowed farmers to sell their products directly to consumers in towns and cities without the use of intermediaries, which helped them economically to some extent. Additionally, consumers can get high-quality agricultural products at reasonable prices.

Vegetables play a vital role in human nutrition, providing essential vitamins, minerals, and dietary Fiber. However, the presence of plant pathogenic fungi in vegetables can pose significant risks to both crop productivity and human health. These fungi are known to cause devastating diseases in plants (table-1), resulting in economic losses for farmers and affecting food security. Moreover, emerging research suggests that plant pathogenic fungi can also impact human health directly through infections and indirectly through the production of mycotoxins. Understanding the prevalence and implications of plant pathogenic fungi in vegetables is crucial for developing strategies to ensure both crop safety and human well-being.

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Table 1 Different types Fungal diseases in vegetable crops

Vegetable Names	**Fungal Disease**	***Organism***	**Symptoms and effecting organ**
Green Amaranth	Leaf Spot	*Cercospora spp.* *Cladosporium spp.*	Necrotic lesions on leaves
Tomato	Leaf spot White Mold	*Alternaria alternata* *Sclerotinia sclerotiorum* *Sclerotinia minor*	white, cottony mycelium
Banana flower Plantain (raw banana)	Panama Disease (Fusarium Wilt)	*Fusarium oxysporum*	yellowing and wilting of the leaves
Beetroot	Rhizopus Root Rot	*Rhizopus arrhizus* *Rhizopus stolonifer*	fungal mycelium may be white and fluffy,
Bell Pepper (Capsicum) Green chili	Cercospora Leaf Spot Anthracnose	*Cercospora capsici* *Colletotrichum spp*	Circular lesions on fruit
Bitter gourd Snake gourd Ridge gourd Bottle gourd Pumpkin Cucumber	Alternaria Leaf Blight Anthracnose Downy Mildew Gummy Stem Blight Powdery Mildew	*Alternaria cucumerina* *Colletotrichum orbiculare* *Pseudoperonospora cubensis* *Didymella bryoniae* *Erysiphe cichoracearum*	light brown spots, sunken lesions wilting, withering, and dying of tissues resembling mosaic Gray-green to black circular leaf spots White or brown mealy growth
Broad beans (Fava beans, lima beans), Butter beans, Cluster beans, French Beans (Green beans)	Alternaria Leaf and Pod Spot	*Alternaria alternata*	small irregular brown lesions with concentric zones
Cabbage Cauliflower	Black Spot Disease	*Alternaria brassicicola*	black spots on their leaves.
Carrot	Cottony Soft Rot	*Sclerotinia sclerotiorum*	white, fluffy fungal growth on the surface of the infected tissues
Coconut (fresh)	Nut Fall	*Fusarium verticillioides*	Sunken black or dark brown lesions on rachis
Curry leaves	Leaf Spot	*Cercospora spp.*	a few round spots or lesions.
Eggplant (Brinjal or Aubergine)	Colletotrichum Fruit Rot Phomopsis Fruit Rot	*Colletotrichum melongenae* *Phomopsis vexans*	Sunken lesions on the fruit filled with pinkish fungal ooze. soft, watery & decay.

Fenugreek leaves	Powdery Mildew Fusarium Wilt	*Erysiphe polygoni DC* *Fusarium oxysporum*	Powdery white growth can be seen on both the surfaces of the leaves
Ginger	Fusarium Rot Disease Phyllosticta Leaf Spot	*Fusarium oxysporum* *Phyllosticta zingiberi*	yellowing of the margins of the older leaves
Lemon (Lime)	Alternaria Brown Spot of Citrus	*Alternaria alternata*	brown spot attacks leaves, twigs, and young fruit.
Mint leaves	Mint Rust	*Puccinia menthae*	Orange, yellow and black spots on leaves
Okra (Ladies finger)	Powdery Mildew Fusarium Wilt	*Erysiphe cichoracearum* *Fusarium oxysporum*	subtle, small, round, whitish spots on leaves and sometimes stems.
Onion Garlic Green onion (Spring onion)	Onion Rust Garlic Rust Onion Smut	*Puccinia allii* *Urocystis cepulae*)	Light yellow to orange or reddish, powdery pustules develops
Radish (Daikon)	White Rust Alternaria Blight	*Albugo candida* *Alternaria raphani*	small, yellowish, slightly raised lesions
Spinach	Anthracnose	*Colletotrichum spp.*	Small water-soaked spots on leaves

Vegetables, including leafy greens, root crops, and vegetables, are susceptible to infections by various plant pathogenic fungi. These fungi can infect different parts of the vegetables, such as the leaves, stems, roots, and flowers, leading to visible symptoms such as wilting, rotting, and discoloration. The diseases caused by these pathogens can result in significant yield reduction, economic losses, and compromised nutritional value of the vegetables.

Apart from the direct impact on vegetable crops, the presence of plant pathogenic fungi can also pose risks to human health. These fungi can produce mycotoxins, toxic compounds that can contaminate vegetables during cultivation, storage, and processing. Consumption of mycotoxin-contaminated vegetables can have severe health consequences, including carcinogenic, mutagenic, immunosuppressive, and hepatotoxic effects [2][3]. It is essential to understand the occurrence and levels of mycotoxins in vegetables to mitigate health risks associated with their consumption.

Fungi is a diversified eukaryotic organism. They can be found in natural habitats such as water, soil, and decaying organic matter. Some fungi establish symbiotic relationships with animals, plants, and others act as parasites, causing diseases or even death. Fungi are characterized by their rigid cell walls containing chitin, mannan, and other polysaccharides. They reproduce both sexually and asexually [4].

Fungi have the ability to produce both intracellular and extracellular enzymes. While small molecules like monosaccharides, disaccharides, fatty acids, and amino acids can be directly utilized by fungi, larger complex compounds such as cellulose, starch, and pectin, require the secretion of extracellular enzymes for breakdown. [5] The production of extracellular enzymes by fungi plays a crucial role in facilitating the degradation process. This ability allows fungi to efficiently obtain nutrients from complex substrates and participate in nutrient cycling within ecosystems. Understanding the enzymatic capabilities of fungi can have significant implications in various fields, including biotechnology, agriculture, and environmental sciences. In this study, we explore the diverse enzymatic activities exhibited by fungi and their relevance to different applications and processes [6].

2. Materials and methods

2.1. Collection of samples

Vegetables available at the bazaar were identified, and a comprehensive list of the different vegetable varieties was compiled, and Fungal-infected vegetables were targeted for observation and analysis. The identified vegetables were carefully examined for any visible signs of fungal infection, including discoloration, spots, and Molds growth. Samples of both the infected and non-infected vegetables were collected to facilitate a comparative analysis, and Multiple samples were collected to ensure representative results. Each sample was appropriately labelled with essential information, such as the date, location, and type of vegetable, to maintain accurate records. The collected samples were stored in sterile containers or bags. The Storage conditions involved maintaining a cool and dry environment to prevent contamination and minimize further fungal growth or decay. The collected samples were subjected to laboratory analysis. In the laboratory, the samples were examined to identify the specific type(s) of fungi present and determine the extent of the infection.

2.2. Isolation of fungal pathogen

The diseased leaves were washed thoroughly under running tap water to remove soil particles and the infected portions of the leaves were cut into 1.0 to 1.5 cm. fragments. The pieces were surface sterilized by 70% ethyl alcohol for 1-2 minutes and then rinsed in sterile distilled water for three to four times. Finally, the leaf bits were washed with 0.01% mercuric chloride for 1 or 2 minutes followed by washing with sterile autoclaved double distilled water 2 or 3 times. These fragments were transferred onto Potato dextrose agar (PDA) plates supplemented with 1.0% streptomycin sulphate (antibiotic solution) under completely sterile conditions in an inoculation chamber.

2.3. Identification of fungal pathogens

Identification of the fungal isolates was made with help of the relevant literature [7][8][9][10][11]. The diagnostic characteristics of each isolate were compared with the previous work of the various authors. Fungal morphology was studied macroscopically by observing colony features (colony diameter, color, texture, and pigmentation) and microscopically by staining with Lacto phenol cotton blue (mounting fluid). A small pinpoint mycelium of each isolate was examined under a compound microscope for the observation of the conidia, conidiophores, and arrangement of spores.

2.4. Enzyme screening

The screening was performed by plate assay method. Isolated fungal strains were primarily tested for enzyme activity by culturing on CZA medium supplemented with 1% Carboxymethyl cellulose (CMC), 1% pectin, and 1% starch as a carbon source. Tetracycline was added to the media to control the bacterial contamination (pH 7.0). The plates were incubated at 30°C for 3 days. After incubation, the plates are flooded with following reagents (table no. 2). The presence of clear zones around the fungal colonies indicates the production of respected enzymes.

Table 2 Different types enzyme screening reagents and subtracts

S. No.	Enzyme	Substrate added in the medium	Reagents used for detection	Observation for positive isolates
1	Amylase	Starch 1%	Lugol' s iodine	Clear zone
2	Cellulase	Cellulose 1%	Gram's iodine	Clear zone
3	Pectinase	Pectin 1 %	Potassium iodide iodine solution	Clear zone

3. Results

Rhythu Bazaar is a farmer's vegetable market located at Visakhapatnam, Andhra Pradesh. This markets hub for local farmers to sell their fresh produce directly to consumers, cutting out the middlemen and allowing farmers to earn a fair price for their crops. The term "Rythu Bazaar" translates to "Farmer's Market" in the local language.

Figure 1 Field survey on Rhythu Bazaar

In the survey conducted at Rythu Bazaar, A total variety of vegetables was recorded and categorized in the following table (Table 3), including both taxonomical and vernacular information. Additionally, during the survey, fungal diseased vegetables were identified and are represented in Figure 1, as outlined in Table 4.

To determine the plant pathogenic species responsible for the fungal infections, the isolated fungal species were identified. The results, presented in Table 3, show the following fungal species*: Alternaria, Curvularia, Rhizopus, Penicillium, Aspergillus flavus, Aspergillus niger,* and *Mucor.* These fungal species were associated with specific host plants.

Table 3 Different types of vegetables present in the Rythu bazaar at Visakhapatnam

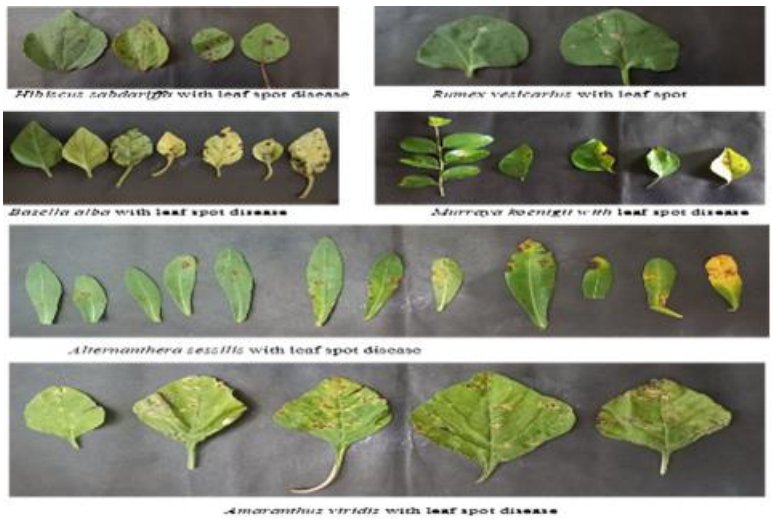
Vegetable Name	**Scientific name**	**Family**	**Telugu Name**
Green Amaranth leaves	*Amaranthus viridis*	Amaranthaceae	Thotakura
Amla	*Phyllanthus emblica*	Phyllanthaceae	Vusiri
Ash gourd	*Benincasa hispida*	Cucurbitaceae	Bhudidha Gummadi
Tomato	*Solanum lycopersicum*	Solanaceae	Rammulakkaya
Banana flower	*Musa acuminata*	Musaceae	Arati Puvu
Beetroot	*Beta vulgaris (subsp. vulgaris Conditiva)*	Amaranthaceae	Beetroot
Bell Pepper (Capsicum)	*Capsicum annuum*	Solanaceae	Capsicum
Bitter gourd	*Momordica charantia*	Cucurbitaceae	Kakara Kaya
Bottle gourd	*Lagenaria siceraria*	Cucurbitaceae	Sorakaya
Butter beans	*Phaseolus lunatus*	Fabaceae	Pedda Chikkudukai
Broad beans (Fava beans, lima beans)	*Vicia faba*	Fabaceae	Chikkudukai

Table 4 List of Identified fungal pathogens from infected vegetables

Vegetable Names	**Fungal Disease**	**Organism**	**Symptoms and effecting organs**
Green Amaranth	Leaf Spot	*Cercospora spp.*	Necrotic lesions on leaves

Cabbage	*Brassica oleracea var. capitata*	Brassicaceae	Cabbage
Carrot	*Daucus carota*	Apiaceae	Carrot
Cauliflower	*Brassica oleracea var. botrytis*	Brassicaceae	Cauliflower
Cluster beans	*Cyamopsis tetragonoloba*	Fabaceae	Goruchikkudulu
Coconut (fresh)	*Cocos nucifera (L.)*	Arecaceae	Kobbari Kaya
Colocasia leaves (Taro leaves)	*Colocasia esculenta*	Araceae	Chama Akulu
Corn	*Zea mays*	Poaceae	Mokka Jonna
Cucumber	*Cucumis sativus*	Cucurbitaceae	Dosa Kaya
Curry leaves	*Murraya koenigii*	Rutaceae	Karivepaku
Drumsticks	*Moringa oleifera*	Moringaceae	Mulakaya
Eggplant (Brinjal or Aubergine)	*Solanum melongena L.*	Solanaceae	Vankaya
Elephant Yam	*Amorphophallus paeoniifolius*	araceae	Kanda Dumpa
Fenugreek leaves	*Trigonella foenum-graecum L.*	Fabaceae	Menthi Kura
French Beans (Gre.beans)	*Phaseolus vulgaris*	Fabaceae	Sanna Chikkudu
Garlic	*Allium sativum*	Amaryllidaceae	Vellulli
Ginger	*Zingiber officinale*	Zingibarace	Allam
Green chili	*Capsicum annuum*	Solanaceae	Pachi Mirchi
Green onion (Scallian or Spring onion)	*Allium fistulosum*	Amaryllidaceae	Ullikadalu
Green peas	*Pisum sativum*	Fabaceae	Batani
Lemon (Lime)	*Citrus limon*	Rutaceae	Nimma Kaya
Mango	*Mangifera indica*	Anacardiacea	Mamidi Kaya
Mint leaves	*Mentha piperita*	Lamiaceae	Pudhina
Mushroom (button)	*Agaricus bisporus*	Agaricaceae	Putta Godugulu
Mustard leaves	*Brassica juncea*	Brassicaceae	Avala Aaku
Okra (Ladies finger)	*Abelmoschus esculentus*	Malvaceae	Bendakaya
Plantain (raw banana)	*Musa paradisiacal*	Musaceae	Kura Aarati Kaya
Onion	*Allium cepa*	Amaryllidaceae	Uliipaaya
Radish (Daikon)	*Raphanus sativus*	Brassicaceae	Mullangi
Ridge gourd	*Luffa acutangula*	Cucurbitaceae	Birakaya
Snake gourd	*Trichosanthes cucumerina*	Cucurbitaceae	Potla Kaya
Potato	*Solanum tuberosum*	Solanaceae	Bangala Dumpalu
Pumpkin	*Cucurbita pepo*	Cucurbitaceae	Gummadikaya
Spinach	*Spinacia oleracea*	Amaranthaceae	Palakura
Sorrel leaves	*Rumex acetosa L.*	Polygonaceae	*Naga Gorinta*
Shallot (pearl onion)	*Allium cepa, variety aggregatum*	Amaryllidaceae	Ullipaaya

		Cladosporium spp.	
Tomato	White mould	*Sclerotinia minor* *Mucor mucedo*	white, cottony mycelium
Beetroot	Root Rot	*Rhizopus arrhizus* *Rhizopus stolonifer*	fungal mycelium may be white and fluffy,
Bell Pepper	Anthracnose	*Colletotrichum spp*	Circular lesions on fruit
Bottle gourd	Leaf Blight	*Alternaria cucumerina*	light brown spots, sunken lesions
Broad beans (Fava beans, lima beans)	Leaf and Pod Spot	*Alternaria alternata*	small irregular brown lesions with concentric zones
Cabbage	Black Spot Disease	*Alternaria brassicicola*	black spots on their leaves.
Carrot	Cottony Soft Rot	*Sclerotinia sclerotiorum*	white, fluffy fungal growth on the surface of the infected tissues
Curry leaves	Leaf Spot	*Cercospora spp.*	a few round spots or lesions.
Eggplant (Brinjal or Aubergine)	Fruit Rot	*Colletotrichum melongenae*	Sunken lesions on the fruit filled with pinkish fungal ooze.
Lemon (Lime)	Brown Spot of Citrus	*Alternaria alternata*	brown spot attacks leaves, twigs, and young fruit.
Onion Garlic	Onion Rust Garlic Rust	*Puccinia allii* *Urocystis cepulae*	Black powdery spots on layers
Radish	Alternaria Blight	*Alternaria spp.*	Black spots on leaves



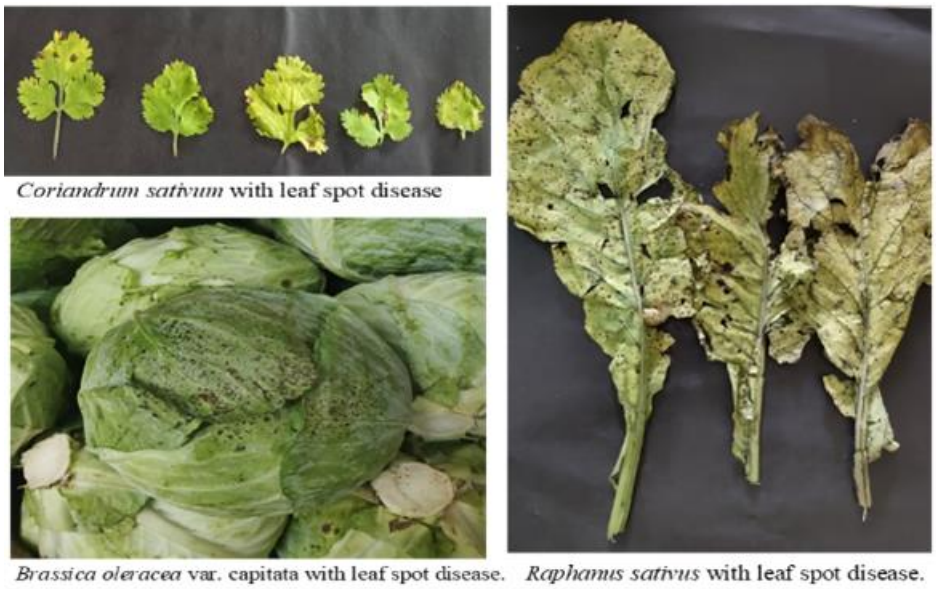
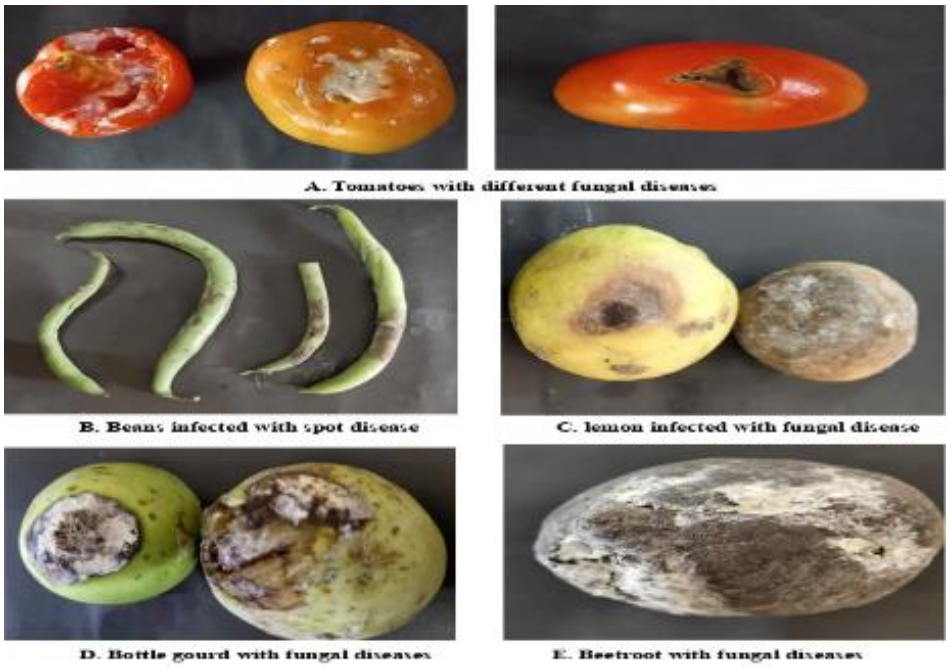


Figure 2 Different types of fungal infected leafy vegetables



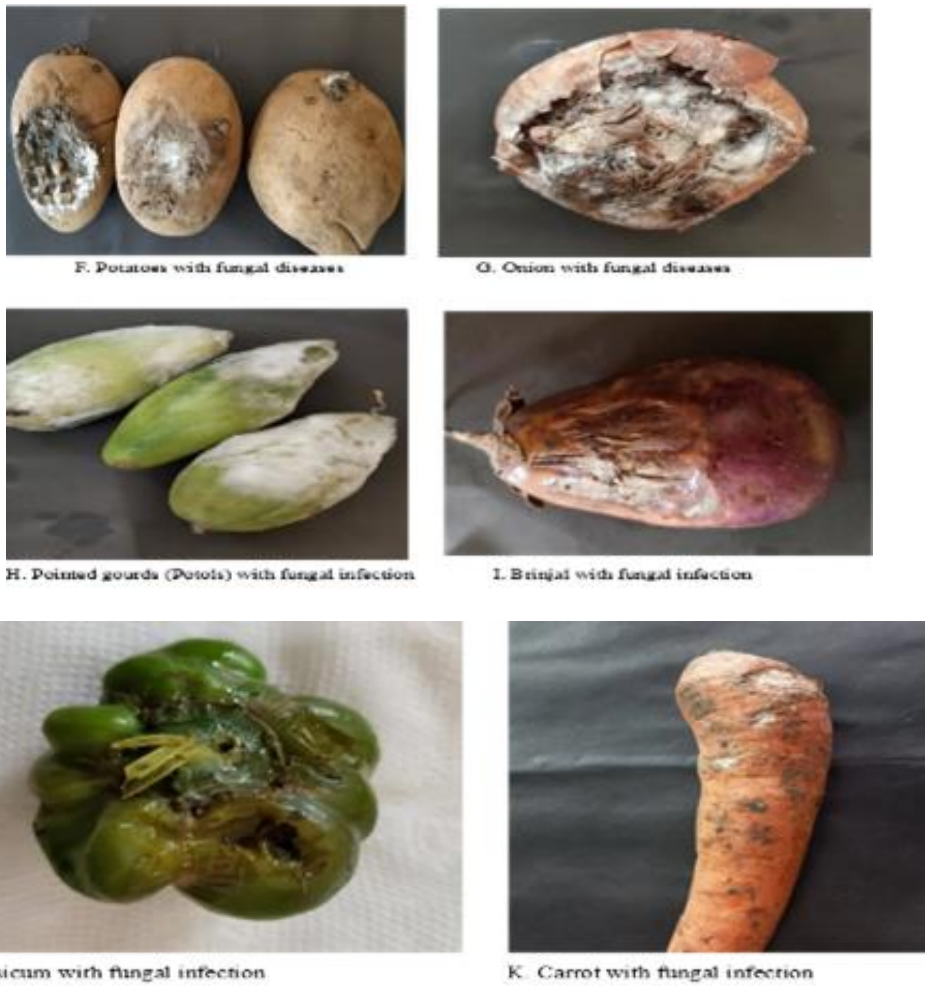


Figure 3 Different types of fungal infected fruits and normal vegetables

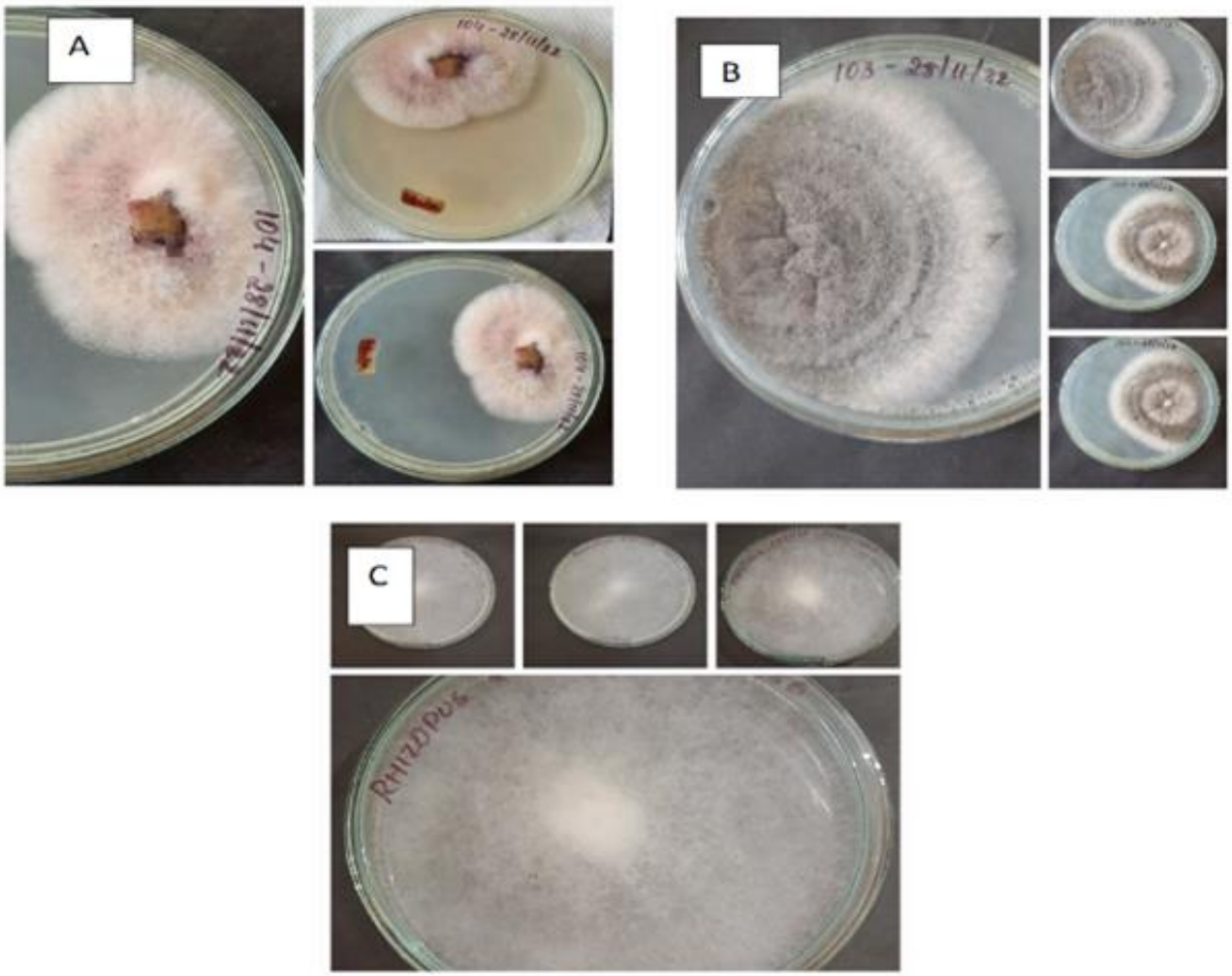


Figure 4 Isolated fungal pathogens grown on PDA medium plates, *A. Colletotrichum spp. B. Sclerotinia spp. And C. Rhizopus spp*

Table 4 Enzyme screening assay with isolated plant pathogens

S.no	Enzyme	Subtract	Positive or Negative	Clear Zone size in mm		
				Sclerotinia spp.	***Rhizopus spp.***	***Colletotrichum spp.***
1	Amylase	Starch	(+ + +)	30	13	31
				32	13	28
				31	12	30
2	Cellulase	Cellulose (CMC)	(+ + +)	39	20	20
				37	19	15
				38	20	20
3	Pectinase	Pectin	(- + +)	-	30	10
					28	13
					32	15

The table above summarizes the results of the enzyme screening for amylase, cellulase, and pectinase activities in three different (figure 7) fungal species: *Rhizopus, Colletotrichum, and Sclerotinia*. The screening was conducted by testing for the presence or absence of enzyme activity using standard assays.

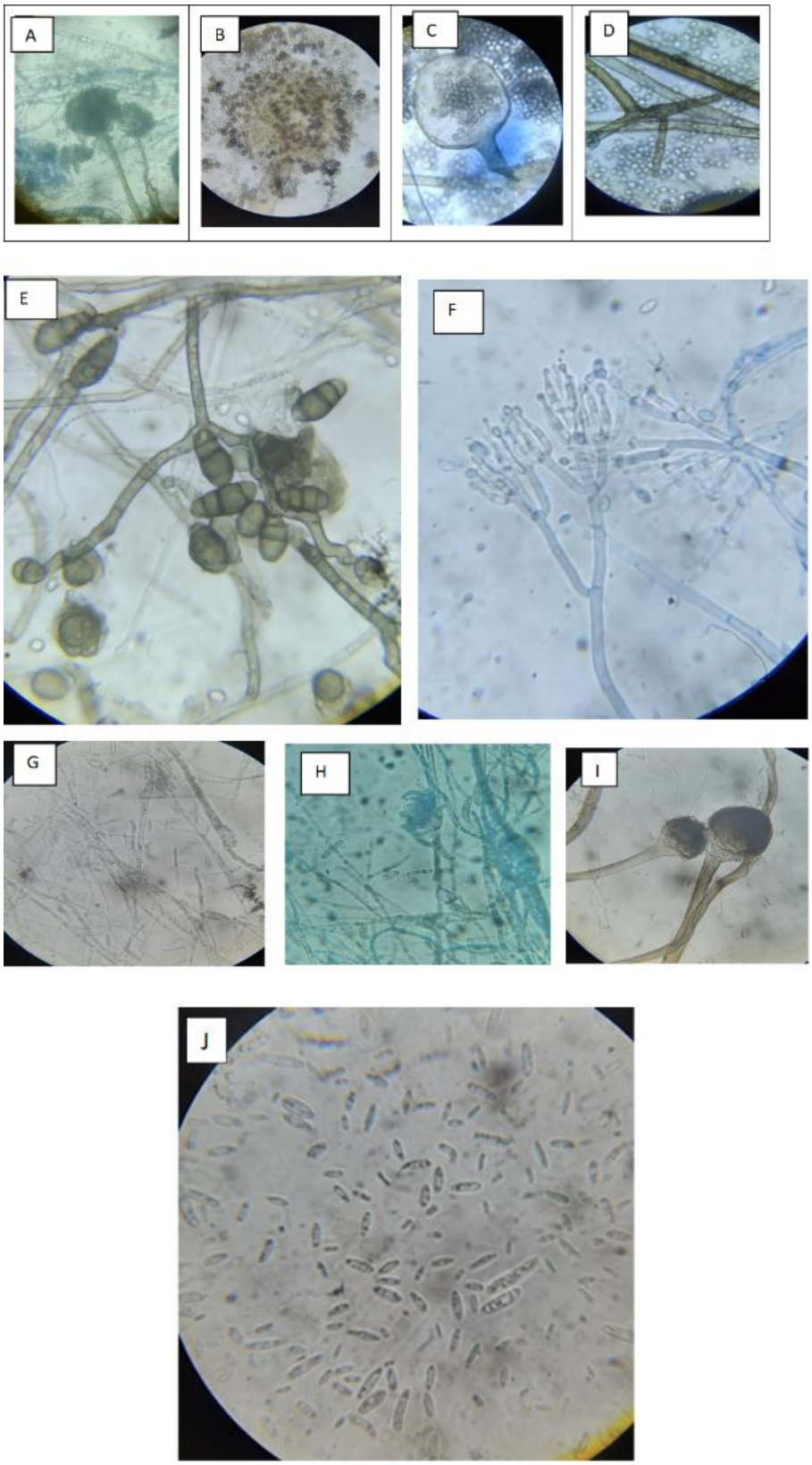


Figure 4 Isolated fungal pathogens grown on PDA medium plates, A. *Colletotrichum spp*. B. *Sclerotinia spp*. And C. *Rhizopus spp*.

The results show *that Rhizopus and Colletotrichum* species have all three types of enzyme activities, these findings suggest that *Rhizopus and Colletotrichum* species may be useful sources of enzymes for industrial processes that require the breakdown of complex carbohydrates, such as starch, cellulose, and pectin. However, further studies are needed to determine the specific properties and potential applications of these enzymes.

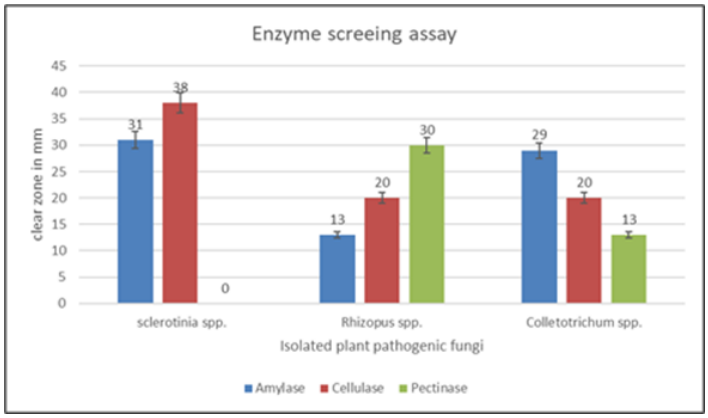


Figure 5 Graphical representation of enzyme screening with isolated plant pathogenic fungi

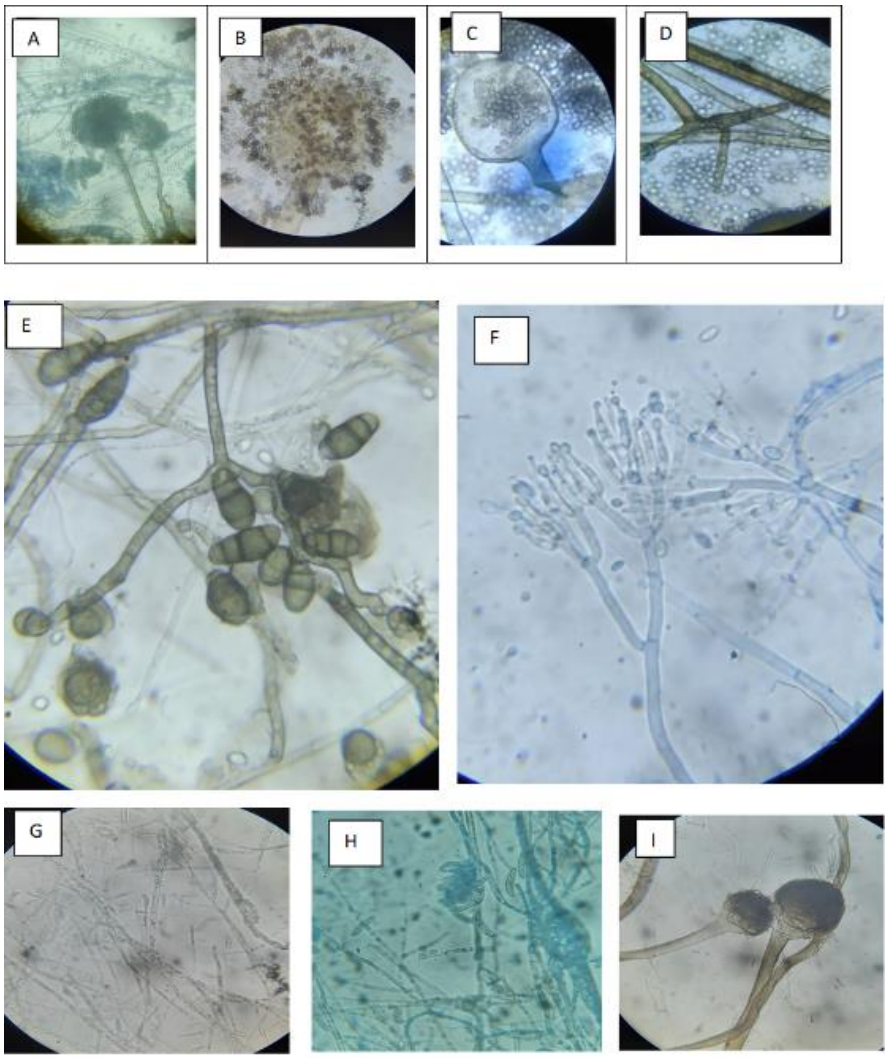


Figure 6 Isolated fungal spp. (A-B) *Aspergillus spp*. (C-D) *Rhizopus spp*. (E) *Curvularia spp*. (F) *Penicillium spp*. (G) *Fusarium spp*. (H) *Colletotrichum spp*.(I)*mucor spp*. (J) *Sclerotinia spp*.

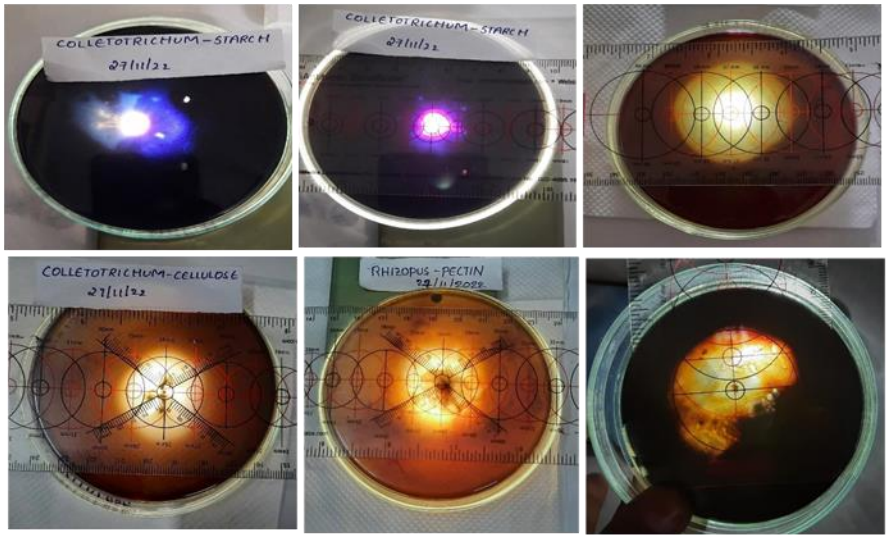


Figure 7 Enzyme activity of isolated fungal spp. With amylase, cellulase and Pectinase clear zones.

4. Discussions

In total, 13 fungal endophytes were isolated from 13 different vegetables verities and five different tissue types like leaf, stem, root, flower and fruits. Our findings align with the results reported by [12] in their study on Soil Mycoflora in different soil samples from crop fields in Chintalapudi Mandal, West Godavari District, Andhra Pradesh, India. They also isolated genera such as *Aspergillus, Mucor, Curvularia, Fusarium, Penicillium, and Rhizopus*, which are similar to the genera we isolated during our investigation.

In another study conducted by [13] in agricultural fields of Tekkali Mandal, Srikakulam District, Andhra Pradesh, they identified 18 species belonging to 6 genera in Soil Mycoflora. They found that *Aspergillus and Penicillium* were dominant in their study. In our current study, we isolated a total of 27 fungal species belonging to 19 genera, and we also observed that *Aspergillus and Fusarium* were the dominant genera in all the agricultural fields we examined.

[14] examined the fungal diversity in rhizospheric soils from various fields in Nanjangud taluk of Mysore district, Karnataka. They identified 10 fungal species representing 7 genera, including *Aspergillus, Alternaria, Mucor, Curvularia*, *Fusarium, Penicillium, and Rhizopus*. Similarly, in our study, we isolated 27 species belonging to 19 genera, and the dominant genera we observed were *Aspergillus, Fusarium, and Alternaria*.

To summarize, our findings are consistent with previous studies, highlighting the presence of common fungal genera such as *Aspergillus, Fusarium, Alternaria*, and *Penicillium* in agricultural.

Among the isolated fugal isolates, *Colletotrichum and Rhizopus* species had the greatest production cellulase, amylase and pectinase enzymes these results corroborated [15], All isolates evaluated in this study showed cellulolytic activity as a yellow halo around the colony in plates including stained with Congo red and secured with NaCl. C. *chrysophilum* showed a significantly larger mean degradation halo of 8 mm in cellulolytic activity assay when compared to *C. noveboracense* and *C. fioriniae* (mean halo zone 6 and 6.5 mm, respectively).

Our findings also align with the results reported by Pectinases are produced by different fungi, including *Aspergillus spp., Fusarium spp., Penicillium spp., Rhizopus spp., Trichoderma spp., Rhizomucor spp., Aureobasidium spp., Thermotoga spp., Saccharomyces spp., Candida spp., Pichia spp., and Kluyveromyces spp.,* have been documented as producers of alkaline pectinases [16][17][18][19].

5. Conclusion

Firstly, the presence of fungal infestations in vegetables sold in local markets indicates a potential health hazard to consumers. The fungi isolated from the vegetables belonged to different genera, including *Aspergillus, Alternaria, Colletotrichum, Penicillium, Rhizopus, and Mucor*, which are known to produce mycotoxins that can cause food poisoning and other health problems.

Secondly, the study showed that the incidence of fungal infestation was higher in leafy vegetables, spinach and coriander, than in root vegetables, carrot and potato. This suggests that leafy vegetables may be more susceptible to fungal infections, possibly due to their higher moisture content and surface area.

Thirdly, the enzyme screening of the isolated fungi revealed that they produced a wide range of extracellular enzymes, including amylase, protease and cellulase. This indicates the potential of these fungi for industrial applications, such as bioremediation and bioprocessing.

These findings suggest that *Rhizopus and Colletotrichum* species may be useful sources of enzymes for industrial processes that require the breakdown of complex carbohydrates, such as starch, cellulose, and pectin. However, further studies are needed to determine the specific properties and potential applications of these enzymes.

Overall, the study highlights the need for improved hygiene and quality control measures in local markets to prevent the spread of fungal infections in vegetables. It also underscores the importance of further research on the enzymatic properties of fungi and their potential applications in biotechnology.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflicts of interest

All authors declare that they have no conflicts of interest.

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