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Fungal diversity in cabbage rhizosphere: A study in Shirpur, Dhule district

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Abstract

Rhizosphere holds thousands of different bacteria specific to the plant species and root zone. There is a good amount of researches on cabbage related diseases and their mitigation strategies but no research has actually analysed fungal diversity in Cabbage rhizosphere. Thus, assessing fungal diversity in the cabbage rhizosphere is crucial for sustainable agriculture, disease management, and soil health. It helps in identifying both beneficial and harmful fungi, leading to better crop management strategies. Thus, the present research studies the fungal diversity and did soil analysis to understand the rhizosphere for cabbage in Shirpur Tehsil, District Dhule, Maharashtra, India.

The soil samples of cabbage rhizosphere were collected from 18 villages across Shirpur, Dhule District, Maharashtra, India between 2022-23. The isolation of soil fungi was done via Soil dilution method. Soil analysis suggested adequate water-holding capacity and aeration along with some scope for optimisation of soil for better cabbage growth and yield. A total of ten fungal species were identified. The most dominant fungi were found to be *Aspergillus niger*. Further, the research found maximum species richness in Kharde region.

Keywords: Rhizosphere, cabbage, soil, fungal diversity, soil analysis

Introduction

Rhizosphere is the layer of soil that is influenced by root secretions and soil microorganisms. The soil pores in this layer of the soil are home to multiple bacteria and fungi which are responsible for influencing soil biochemical properties. These soil microbes help in determining soil quality, sustainability of agriculture, and ecosystem since they play major role in mediating soil organic matter decomposition and nutrient cycling (Manpoong *et al.*, 2020) ^[16]. Abundance of dominant bacteria like *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Plantomycetes* are found in this layer (Lisuma *et al.*, 2020) ^[14]. *Rhizobium*, rare fungi *Kurtzmanomyces*, *Ascomycota*, and *Basidiomycota* are certain fungi which are commonly found in the rhizosphere layer (Essel *et al.*, 2019) ^[4]. *Trichoderma* is a fungal genus that is known for rapid growth and high stress tolerance. *Trichoderma harzianum* strains from crop rhizosphere are more likely to be effective biocontrol agents since they are in close contact with the plant roots. It also acts as an efficient antagonist against soil borne pathogens. There are multiple other species of *Trichoderma harzianum* such as *T. afroharzianum*, *T. inhamatum*, *T. lentiforme*, *T. camerunense*, *T. asperellum*, *T. asperelloides*, *T. erinaceum*, *T. atroviride*, *T. ghanense*, and *T. longibrachiatum*. All these species are efficient and have good growth promotion potentials (Jambhulkar *et al.*, 2024) ^[10].

It is important to understand the microbial community of rhizosphere soil to maintain the soil's health and its fertility, thereby improving the crop yield. In this direction some researchers in past have assessed microbial community of rhizosphere soil as discussed in the succeeding section.

Research conducted by Wei *et al.*, (2023) ^[24] thoroughly investigated the microbial community and how it affects the soil growth and nutrients by using high throughput sequencing technologies. Based on the investigations and analysis, it was finally concluded that rhizosphere soil had richer contents of nutrients, but when pH value is considered, it showed the reverse trend.

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The research by Guleri *et al.*, (2021) ^[7] analysed the physicochemical properties including soil texture, soil reaction (pH), and organic carbon in the rhizosphere plantation. Different textures of soil with different pH were observed in various plantation sites even in the same areas. These physicochemical parameters affect the changing population number and relative abundance of fungal species. Soil moisture enhances the micro fungal activity by making organic carbon available. *Aspergillus* and *Penicillium* appeared abundantly in collections because of their prolific sporulating capacity. Fungi are much more resistant to the acidic environment than any other group of soil microorganisms.

Cabbage is one crop that has unique interactions with soil fungi due to its susceptibility to diseases, high nutrient demand, unique root exudates, and monoculture risks. Studying fungal diversity in its rhizosphere can lead to better disease control, soil health management, and sustainable farming practices. Cabbage crops are prone to various fungal infections like Clubroot, Black rot, Damping-off and Fusarium wilt to name a few. As per Ping *et al.*, (2024) ^[19], there is a soil borne fungus known as *Fusarium Oxysporum f. sp. Conglutinans* (Foc) that causes a terrible disease known as Cabbage Fusarium Wilt (CFW). CFW can be managed by usage of tolerant / resistant cabbage varieties. There are species of rhizobacteria that have been identified which seems to resist diseases in susceptible cabbages. Assessing fungal diversity in cabbage thus helps in detecting and managing pathogenic species before they cause serious yield losses. Many researchers have studied cabbage and related.

As per the research by Begum & Sarma, (2024) ^[3], the population in the rhizosphere soil of cabbage is higher than the population of fungi. The most dominant genera of fungus prevailing over cabbage were species of *Aspergillus*, *Mucor*, *Rhizopus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Curvularia*, and *Mortierella*. The study also observed that the cultivation system may affect the fungal distribution regarding factors that affect fungal density under organic and conventional agricultural systems. The increased fungal population in the rhizosphere layer in cabbage plantation is due to the combination of organic and inorganic fertilizers. There are helpful for the internal nutrient cycling and circulation within the soil and forms the parameter for species richness and evenness.

Khan & Khan, (2021) ^[11] in the research, they worked on the microscopic fungal population present in the rhizosphere layer of soil in cabbage plantation. It was found that the fungi play a very important role and is responsible for decomposition of organic carbon and cycling of phosphorous and nitrogen. A survey was also conducted with the purpose of soil sampling. There were a wide range of fungus such as *Trichoderma*, *Fusarium*, *Aspergillus*, *Alternaria*, *Curvularia*, *Mucor*, *Penicillium*, and *Mycelia sterilia* that were mostly obtained.

Based on similar observations, Sarkar *et al.*, (2024) ^[20] stated that bio-inoculants and fungicides are beneficial for the growth, yield, quality, and disease incidence in cabbage plantation. Cabbage responded better to the dual inoculation of *Trichoderma asperellum* and *Bacillus subtilis*. This dual inoculation also supported with the head yield of crop and enhanced its nutritional value. Better disease control has been observed with this combination thereby increasing the economic return on its cultivation. Such microbes are

essential for sustainable agricultural production. They help meet population need for food, preserve the environment, and ensure excellent yields at reasonable prices.

As per the research conducted by Wang *et al.*, (2023) ^[23], if there is continuous cropping of cabbage, then it would decrease the yield as well. There were ten different combinations of treatments set up to minimize the effect of this damage. High throughput technology was used in this study to analyze and observe the variation in soil nutrients, fungal community structure and diversity and composition of the soil. It was noted that there was an abundance of fungal species across these ten treatments. It was also observed that *Diaportheales* might have been the main reason for cropping disorder.

The research by Hu *et al.*, (2021) ^[8] stated that one of the highest destructive diseases was identified as Clubroot for cabbage. Out of the various biological control agents, *Streptomyces Alfalfae XY25* was identified as one of the most effective biological control agents to reduce the impact of Clubroot disease, regularizing the rhizosphere communities, thereby promoting growth in greenhouses. The use of these agents can help increase the quality of the soil and its matter, by adding in phosphorous, potassium, thereby enhancing its activities. There was a decrease and an increasing trend noticed during the Chinese cabbage growth. Sequential analysis provided the main bacterial and fungal phyla such as *Proteobacteria* and *Ascomycota* respectively. Adding to this, Liu *et al.*, (2019) ^[15] noted that *Plasmodiophora brassicae* is one highest devastating pathogen that causes Clubroot disease in cabbage. As per the study, fungicide fluazinam was used for the treatment of Clubroot disease. It was found out that there was a decreasing trend in microbial communities during the two growth stages, but was found to recover at the rosette stage. It was concluded that the effect of fluazinam on the fungal community in the Chinese cabbage rhizosphere was transient. High throughput pyrosequencing was suggested as an appropriate method for characterizing cabbage rhizosphere in the microbial communities. Lastly, the researcher also pointed out that various microbial communities could help manage the clubroot disease and develop strategies accordingly in the future for cabbage.

It can be inferred from the above discussion there are researches available on cabbage related diseases and their mitigation strategies but no research has actually analysed fungal diversity in Cabbage rhizosphere. As a matter of fact, rhizosphere holds thousands of different bacteria specific to the plant species and root zone (Manpoong *et al.*, 2020; Peiffer *et al.*, 2013) ^[16, 18]. Thus, assessing fungal diversity in the cabbage rhizosphere is crucial for sustainable agriculture, disease management, and soil health. It helps in identifying both beneficial and harmful fungi, leading to better crop management strategies.

Materials and Methods

Collection of Soil Sample

The soil samples of cabbage rhizosphere were collected from 18 villages (Table 1) across Shirpur Tehsil, Dhule District, Maharashtra, India. It was collected from 2022-23. For collecting, soil was dug to 15 cm to 20 cm depth. These were then stored in sterilized polythin zip lock bag and for further experiment, the samples were transported to the laboratory

Table 1: Collection Sites for Soil Samples

Sample Number	Collection Site	Season of Sample Collection
Sample 1	Boradi	Winter
Sample 2	Borpani	Winter
Sample 3	Balkuwe	Summer, Rainy
Sample 4	Fattepur	Rainy, Winter
Sample 5	Gartad	Rainy, Winter, Summer
Sample 6	Hingoni	Winter
Sample 7	Kuwe	Summer, Rainy
Sample 8	Kharde	Rainy, Winter, Summer
Sample 9	Nimzari	Winter
Sample 10	Pimpri	Winter, Rainy
Sample 11	Sangavi	Rainy, Winter, Summer
Sample 12	Savalde	Rainy, Winter
Sample 13	Shingave	Rainy, Winter
Sample 14	Shirpur	Rainy, Winter
Sample 15	Varul	Rainy, Summer
Sample 16	Wadi	Rainy, Winter, Summer
Sample 17	Waghadi	Rainy, Winter, Summer
Sample 18	Wasardi	Rainy, Winter

Isolation of fungi from the soil samples

The isolation of soil fungi was done via Soil dilution method. The isolation was done on two different mediums namely potato dextrose agar (PDA) and CzapeksDox Agar (CZA).

Soil dilution method: 1 gm of soil sample was diluted in 10 ml of sterilized distilled water to make microbial suspension 10^{-1} to 10^{-5} . Dilution of 10^{-3} to 10^{-5} was used to isolate fungi. 1 ml of dilution was taken from each serial dilution sample in triplicate form in petriplate which is already filed approximately 15 ml medium. The incubation of petriplate was done for 72 hours.

Identification of soil fungi

The identification of fungus was done by considering the work of Subramanian, (1971) ^[21], Gilman, (1945) ^[6] and Barnett & Hunter, (1972) ^[2].

Statistical analysis of isolated soil fungi

The percentage contribution was calculated via following formula-

$$\% \text{ contribution} = \frac{\text{Total no. of CFU of an individual species} \times 100}{\text{Total No. of CFU of all species}}$$

Further, the number of colonies forming was calculated as follows-

Number of colonies forming unit/gram of soil =

$$\frac{\text{Number of Colonies}}{\text{Dry Weight of Soil}} \times \text{Dilution Factor}$$

Results and Discussion

Soil Analysis

Soil analysis is crucial to assess for modern agriculture and land management. It provides insights into efficient nutrient management, sustainable farming, and environmental protection (Ansari *et al.*, 2024) ^[1]. Soil analysis facilitates the farmers in assessing the soil health thereby making data-driven decisions for improving crop yield, conserving resources, and protecting the environment. Regular soil

analysis can lead to improved soil health, crop productivity, and enhanced profitability.

The present research has conducted soil analysis of rhizosphere of Cabbage fields across Shirpur Tehsil, Dhule District, Maharashtra, India (Table 2). The identified physical properties of the analysed soil have been discussed below-

Table 2: Physical Properties of Soil

Soil Testing Property	Amount Obtained in the test	Analysis
pH	8.52	Alkaline
Alkalinity (ms/Cm)	0.43	Normal
Moisture (%)	2.56	-
Coarse Sand (%)	41.90	-
Clay (%)	23.08	-
Slit (%)	12.82	-
Fine Sand (%)	35.79	-
Water Holding Capacity (%)	53.59	-
Virtual density (Gm/cubic)	1.34	-
Standard Density (Gm/cubic)	2.41	-
Porosity Amount (%)	33.27	-
Texture	9.00	Loamy Loam Slit

Cabbage prefers slightly acidic to neutral soil. It can be inferred from Table 2 that the pH of the soil is 8.52 which presents alkaline nature of this soil. This might reduce nutrient availability in the soil. As alkalinity is normal; it is unlikely to cause major nutrient imbalances. The observed moisture content in the collected samples is low which can stress plants and reduce nutrient absorption. Further, moderate water holding capacity is beneficial, ensuring moisture retention without waterlogging.

The loamy loam texture of the sample collected with moderate clay and sand content points towards a good drainage and aeration which is suitable for cabbage growth. Also, a porosity of 33.27% is moderate, indicating good aeration for root growth and microbial activity.

Table 2: Chemical Properties of Soil

Soil Testing Property	Amount Obtained in the test	Analysis
Organic Carbon (%)	0.39	Minimum
Phosphorus (kg/Hector)	17.40	Medium
Potassium (kg/Hector)	397.02	Most Abundant
Calcium Carbonate (%)	7.40	Most Limestone
Calcium (Ca)	46.50	Abundant
Magnesium (Mg)	6.27	Abundant
Sodium (Na)	25.92	Abundant
Nitrogen (N)	132.92	Minimum

It can be inferred from Table 2 that percentage of organic carbon is low (0.39<1%). This indicates poor microbial activity and reduced nutrient-holding capacity. Further, Phosphorus is available in medium level (17.40 kg/ha) which is adequate. Potassium was found to be most abundant (397.02 kg/ha) in the collected samples. High levels of Potassium are usually beneficial, but excess potassium can affect calcium and magnesium uptake. Similarly, high levels of calcium were found which is good for cabbage growth as it increases cell wall strength and disease resistance. There was also high calcium carbonate registered indicating calcareous soil, which can lead to

micronutrient deficiencies like iron or zinc. Further, high levels of magnesium (6.27%) and sodium (25.92%) were found. High level of magnesium can compete with calcium uptake while high levels of sodium can lead to salinity issue. Finally, nitrogen was found to be minimum (132.92 kg/ha) which can negatively impact the growth of cabbage.

Evaluation of Fungal Diversity

Diversity can be defined as the differentiations of life which can be amongst plants, animals and microorganisms. Fungi are essential constituents of biodiversity as these have played a main part in global ecological processes. This research identified ten fungal species in the rhizosphere of cabbage crop in Shirpur tehsil, Dhule District. These are *Aspergillus niger*, *Aspergillus flavus*, *A.ochraceus*, *Curvularia stolonifer*, *Alternaria brassicola*, *Colleototrichum sp.*, *Fusarium oxysporum*, *Trichoderma harzianum*, *Mucor racemosus* and *Penicillium bilaiae*.

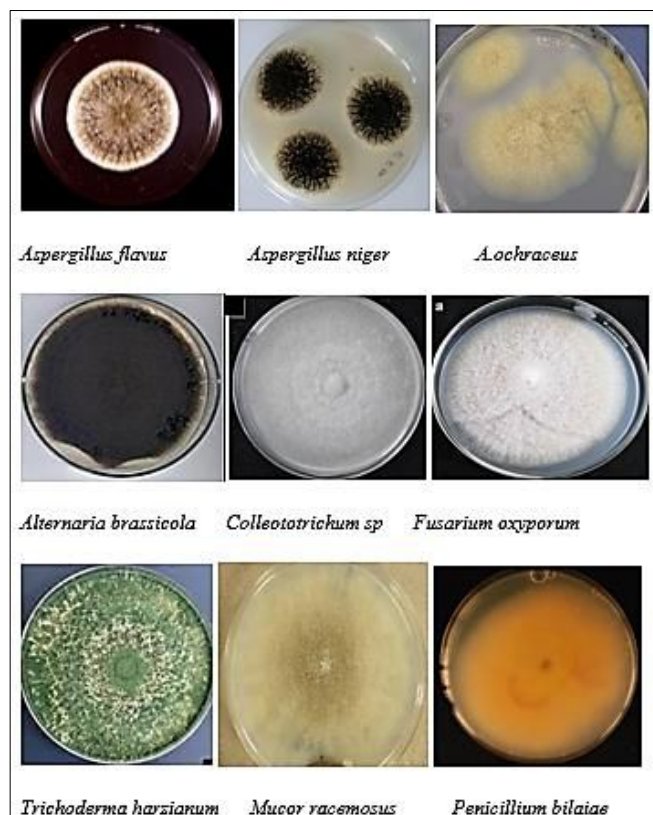


Fig 1: Different Fungus Identified in the Cabbage Rhizosphere

In the present research, 175 fungal colonies of 9 fungal species (identified in the rhizosphere of cabbage fields) were isolated from cabbage crops fields in Shirpur tehsil.

Table 3: Identified Fungus Diversity, Number of Colonies and their Contribution

Fungi	Number of Colonies	Contribution (%)
<i>Aspergillus niger</i>	26	16.1
<i>Aspergillus flavus</i> 2	25	15
<i>A.ochraceus</i>	11	6.8
<i>Curvularia stolonifer</i>	10	6.2
<i>Alternaria brassicola</i> 4	21	13.1
<i>Colleototrichum sp.</i>	14	8.7
<i>Fusarium oxysporum</i> 5	18	11.1
<i>Trichoderma harzianum</i> 6	13	8.1
<i>Mucor racemosus</i>	22	13.6
<i>Penicillium bilaiae</i>	15	9.2

The dominant fungi in the rhizosphere of cabbage can vary depending on soil conditions, climate, and microbial interactions. It can be inferred from Table 2 that the most dominant fungi were *Aspergillus niger*, *Aspergillus flavus*, *Mucor racemosus*, *Alternaria brassicola* and *Fusarium oxysporum* in the rhizosphere of cabbage. *Aspergillus niger* was reported to be the most dominant fungus in Cabbage rhizosphere in the region under research. It is a common soil-borne filamentous fungus *Aspergillus niger* is an essential component of the cabbage rhizosphere, contributing to phosphate solubilisation (Hussain *et al.*, 2024) ^[9], decomposition and nutrient cycling (Nayak *et al.*, 2020) ^[17], promotes production of plant growth-promoting metabolites (Galeano *et al.*, 2021) ^[5] and acts as a biocontrol agent against soil pathogens (V. Kumar *et al.*, 2022) ^[13]. Further, *Curvularia stolonifer* was reported to be minimum. *Curvularia stolonifer* is a fungal species belonging to the genus *Curvularia* in the family Pleosporaceae. It is known for its ability to cause foliar, stem, and root infections in cabbage.

In a research conducted by Umboh *et al.*, (2016) ^[22] also reported *Aspergillus niger* and *Aspergillus flavus* to be dominant fungi in the fields of cabbage plantation. Further, in a research conducted by Kumar *et al.*, (2015) ^[12] in agricultural fields at Tekkali Mandal in Srikakulam District, also found *Aspergillus*, *Fusarium* and *Penicillium* to be dominant. In the present research, *Penicillium* was not a dominant fungus. This might be due to specific nature of rhizosphere of cabbage crop.

Fungi Species Richness in Cabbage Rhizosphere in Different Fields

Species richness states the number of diverse species existing in an ecosystem. It plays a vital role in soil health, nutrient cycling, disease subdual, and plant growth. The present research has assessed the fungi species richness in different species from where sample of cabbage rhizosphere was collected (Table 5).

Table 4: Identified Fungi Species Richness in Different Species in Collected Samples

Field	Species Richness
Boradi	5.98
Borpani	5.83
Balkuwe	6.79
Fattepur	6.27
Gartad	5.61
Hingoni	6.35
Kuwe	2.24
Kharde	7.01
Nimzari	5.46
Pimpri	4.06
Sangavi	2.24
Savalade	4.50
Shingave	5.61
Shirpur	5.98
Varul	3.54
Wadi	4.20
Waghadi	4.87
Wasardi	4.24

It can be inferred from Table 5 above that maximum species richness was observed in Kharde (7.01), followed by Balkuwe (6.79), Hingoni (6.35), Fattepur (6.27). The minimum species richness was observed at Kuwe (2.24).

Thus, it can be stated cabbage Rhizosphere in Kharde region has enhanced nutrient cycling when compared to other regions considered in the present research. This region has superior microbial diversity and is more resilient. The graphical representation has been presented below-

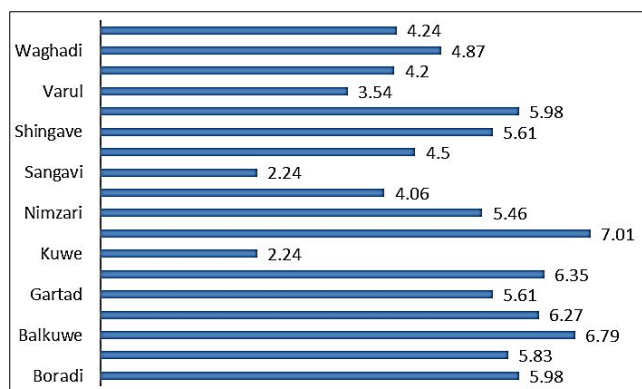


Fig 2: Graphical Representation of Species Richness

Conclusion

The present study on fungal diversity in the cabbage rhizosphere across different fields in Shirpur tehsil, Dhule District, presents significant species richness and variation in fungal communities. A total of ten fungal species were identified, with *Aspergillus niger* (16.1%), *Aspergillus flavus* (15%), *Mucor racemosus* (13.6%), and *Alternaria brassicola* (13.1%) being the most dominant species. The presence of *Fusarium oxysporum* (11.1%) and *Colletotrichum* sp. (8.7%) suggests potential phytopathogenic risks, while *Trichoderma harzianum* (8.1%) is a beneficial biocontrol agent.

The research also did soil analysis of rhizosphere and found good amounts of potassium and calcium levels for plant health; identified loamy loam texture is suitable for cabbage root development along with adequate water-holding capacity and aeration. On the down side, alkaline pH levels, low organic carbon and nitrogen levels along with high sodium levels indicated scope for optimisation of soil for better cabbage growth and yield.

Species richness across different fields varied significantly, ranging from 2.24 (Kuuwe, Pimpri) to 7.01 (Kharde). This variation suggests differences in soil conditions, crop management practices, and environmental factors influencing fungal populations.

The high fungal diversity observed in some fields indicates a well-balanced microbial ecosystem that may support plant growth through nutrient cycling and pathogen suppression. However, the presence of pathogenic fungi highlights the need for sustainable disease management strategies, such as biological control and soil amendments, to maintain soil health and improve cabbage productivity.

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