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Isolation and identification of fungal community in the rhizosphere of *Cucumis sativus* L.

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This study examines the influence of soil physicochemical properties on fungal populations in the rhizosphere of *Cucumis sativus* L. Soil samples were collected from the agricultural fields at Aliba Village, Mokokchung district, Nagaland, India during the spring season of April 2024.The soil at the experimental site was classified as silty loamwith moderate acidity, with pH ranging from 5–6.3, and high electrical conductivity (EC) ranging from 241.6 to 263 ds/m^{-1}. The soil organic carbon was 1.22%, and the total organic matter was 2.103.Fungal species were isolatedusing plating and dilution techniques and identified based on their morphological features observed under a microscope with lactophenol cotton blue.A totalof 147 fungal colonies from 17 species across 8 genera were isolated from the study area, with *Penicillium* species being the most dominant. Among the identified genera,*Penicillium frequentans*was the most frequently encountered, while *Aspergillus* species especially,*Aspergillus niger*, also showed considerable abundance.

Keywords: Agriculture, *Cucumis sativus*, fungi, soil

INTRODUCTION

Cucumis sativus L., commonly known as cucumber, is a widely cultivated vegetable from the Cucurbitaceae family widely cultivated in India for its nutritional value, such as vitamins B and C, water, proteins, fats, carbohydrates, and minerals. With a 96% water content, cucumbers are highly beneficial for human health.India produces over 1.6 million metric tons of cucumbers annually on over104,000 hectares. In Northeast India, particularly Nagaland, farming cucumber (*Cucumis sativus* L.) is of significant agricultural and economic importance. The region's diverseagro-climatic conditions foster the cultivation of unique cucumber cultivars, highly priced both locally and globally(Jami *et al.* 2021).The state's total production and cucumber area account for 21,617 MT and 714 ha, respectively, with Mokokchung district leading of total output of 18,070 MT and an area of 280 ha from 2016-2017 (Walengpong and Mazhar, 2023). To guarantee its accessibility and enhance commercial revenue, farmers in Nagaland utilize off-season cultivation techniques beginning in January (Jami *et al.* 2021). Farmers employ their enhanced traditional technical expertise and engage in organic farming using farmyard waste or poultry manure.

Controlled climatic conditions can be obtained by greenhouse farming or polyhouse cultivation (Jha and Pongener, 2015).Soil in the region is rich in microorganisms, including fungi, which contributesignificantly to soil health and fertility (Habeeb *et al.* 2023).Soil formation is driven by the weathering of rocks and the addition of organic matter, which enriches the soil with vital nutrients and minerals essential for plant growth (Sylvia *et al.,* 2005). Soil properties like texture, moisture, and nutrient content are crucial for plant growth, and the use of chemical fertilizers can disrupt microbial communities, affecting soil health (Sylvia *et al.* 2005; RohillaandSalar, 2012; Pahalvi*et al.* 2021). In the rhizosphere, fungi play an essential role in nutrient cycling and enhancing

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plant growth by forming symbiotic relationships with plant roots (Mendes *et al.* 2017; Kumar *et al*. 2015; Barbhuiya *et al.* 2004), organic matter decomposition, and enhancing soil structure. Fungi like mycorrhizae improve nutrient uptake, while some soilborne fungi, such as *Fusarium* and *Pythium*, can act as pathogens (Gaddeyya*et al.* 2012). Organic farming practices that promote the growth of beneficial soil fungi, can enhance soil structure and nutrient availability, making the soil morefertile for *Cucumis sativus* L. cultivation. In the context of cucumber farming in Mokokchung, Nagaland, understanding the composition of soil fungal communities can help identify both beneficial and harmful microorganisms, offering valuable insights into sustainable agricultural practices (Swer*et al.* 2011).

MATERIALS AND METHODS

Study site and soil sample collection

The current study was conducted in the field ofAliba Village, Mokokchung District, Nagaland (Fig.1).The experiment was laid out in a complete randomized design in a selected cucumber field having a geographical location between 26Ú 35 ’86.38 “ N and 94^{0}C 40' 78.55” E and with an elevation of 1060 msl. The soil samples for microbial analysis were collected in early April 2024. The climate condition of the village is humid, subtropical to dry winter, with an average precipitation between 2000 and 2500 mm.The soil samples were obtained from a depth of roughly 10-15 cm from eight corners of two independent sites from the same field area and were blended to make a composite sample. Then, the samples were tagged correctly in sterilized polybags and were brought to the laboratory of the Central Department of Botany, Nagaland University, for analysis.

Soil physicochemical analysis

The composite sample collected from the study area was air-dried for 3-4 days, crushed, and sieved through a 2 mm mesh size. The fine-sieved soil was used for physicochemical analysis except for moisture. In the laboratory, different physical and chemical properties, such as moisture, organic carbon, pH, EC, and texture analysis, were analyzed. Soil moisture content was determined using the procedure suggested by Buckman and Brady (Sikora *et al.*1997). The pH of the soil was quantified using Fisher’s digital pH meter, with a soil-water mixture ratio of 1:2. The soil organic carbon content was calculated using the Walkey and Black Technique (Jha *et al*. 2014). The soil texture was determined through mechanical analysis using the pipette method.

Isolation and Identification of Soil Fungi

The Waksman (1922) method of serial dilution plate was employed to isolate fungi from the rhizosphere soil. A soil sample weighing 1 gram was mixed with 10 millilitres of sterilized distilled water to create microbial suspensions ranging from 10-1 to 10-4. One millilitre of each concentration of a microbial suspension was transferred to petri dishes containing sterilized Rose Bengal Agar (RBA) medium. The plate was then rotated gently to disperse the suspension evenly over the medium (Toma and Abdulla, 2012). The isolation was carried out in a sterilized”Laminar Air Flow Chamber” throughout the investigation process. The petri dishes that had been inoculated were subsequently labelled and placed upside down in a BOD incubator, where they were incubated at a temperature of 25±1°C for 5-7 days. The fungal isolates were purified using a single spore isolation technique by sub-culturing them with a sterilized needle (Noman *et al.* 2018; Choi *et al.* 1999) using Malt Extract Agar medium (MEA). The purified cultures of soil fungi were identified based on morphological and cultural characteristics (Simmons, 2007; Kanwal *et al.* 2017).Fungal cultures were determined by examining their morphological characteristics and colony structure under a microscope. The macroscopic features of the colonies, such as color and texture, were observed, and the microscopic examination involved staining with lactophenol cotton blue and observing the conidia, conidiophores, and spore arrangement. The fungi were identified using established literature and books (Webster & Weber 2007).

RESULTS AND DISCUSSION

Soil chemical characteristics

The investigation found that the soil at the experimental location had a moderate level of acidity and belonged to the silty loam soil type. The pH of the soil varied from 5 to 6.3and a pH of about 5 is known to be ideal for fungal growth. However, as the pH increases, bacteria often outcompete fungi (Kanwal *et al*., 2017; Sinha *et al.,* 2022).The soil samples' electrical conductivity values collected from the study area varied between 241.6 and 263 ds/m^{-1}(2.416 µS/cm–2.63 µS/cm).The soil sample demonstrated a significant electrical conductivity (EC) value, suggesting a greater concentration of salts in the soil. The soil sample under study included 1.22% soil organic carbon, and the total organic matter was assessed as 2.103% (Table 1). It indicates that the soil is enriched with an adequate concentration of organic matter, which improves its structure, moisture retention capacity, nutrient cycling, microbial activity, and overall plant growth. This, in turn, enhances agricultural productivity and contributes to long-term environmental sustainability. Soil organic matter (SOM) also impacts soil characteristics such as bulk density, aggregate stability, cation exchange capacity, and biological activity.

Soil mycoflora

A total of 147 colonies of 17 fungal species belonging to 8 genera were isolated from the rhizospheric soil of *Cucumis sativus* L. out of which 3 species *Penicillium citrinum*, *Penicillium frequentans*, and *Penicillium decumbens* belong to *Penicillium* genera, 2 species *Eupenicilliumja vanicu*m and *Eupenicillium* sp. belong to *Eupenicillium*, 2 fungal species were sterile and other species found were *Aspergillus niger*, *Paecilomyces*, *Rhizopus stolonifer, Mucor hiemalis F. silvaticus,* and *Tricoder-maviride*. Morphological and microscopic characters of the isolated fungi have been presented in Figs. 2 & 3.Among the isolates, the genus *Penicillium* was the most prevalent, and the highest number of colonies was observed in the *Aspergillus* species. The fungi displayed diverse morphology and microscopic characteristics as presented in (Table 2). The high rate of *Penicillium* sp. among the isolates may be attributed to their flexibility and versatility, as they are known to flourish in various environmental circumstances and

Table. 1: Physicochemical properties of soil samples collected from the *Cucumis sativus* L. field.

Soil temp. (ºC)	pH	EC (dS m^{-1})	Moisture (%)	Soil type	Organic matter (%)
28±0.57	5.70±0.64	241±20.55	38.084±0.414	SL	2.103%

Values are Mean ±S.D; SL-Silt Loam, Organic content: 0.3-0.5=low, 0.5-0.75= medium, 0.75-2= high

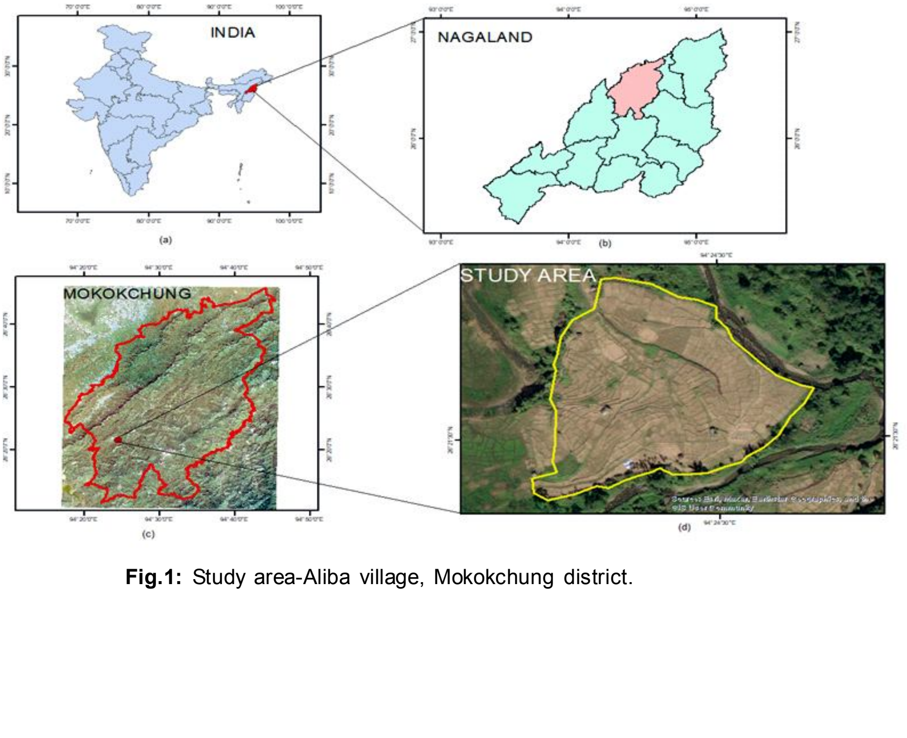

Fig.1: Study area-Aliba village, Mokokchung district.

Table. 2: Colony and microscopic characteristics of the fungal species isolated from the rhizosphere of *Cucumis sativus* L.

Colony and microscopic features	Fungal species	No.of colonies
The colonies are dark olive in colour, conidia of more uniform size and usually smooth-walled. Condiophores tripes are long (100-300μm) and is biverticillate. Mycelia are septate.	*Penicillium critnium*	36
Colonies are white light brown in color. Hyphae are septate and dark. Conidiophores are short, brown in color; bear large ovoid or ellipsoid conidia. Mycelia are both transverse and longitudinal septations.	*Alternaria alternata*	6
Colonies are brown to blackish, velvety, powder like. Conidia are globus to subglobus. Conidiophores are long, smooth walled, hyaline and become dark at the apex. The conidial head appear radial.	*Aspergillus niger*	40
Colonies are white to light greenish in color, powdery to suede-like. Conidia are subsphericial , ellipsoidal to fusiform, smooth walled. The mycelia are septate. Conidiophores usually erect bearing several whorles of flask-shaped phialides.	*Paecillomyces* sp.	7
Colonies are fast growing, light green in color and granular texture. Chains of single celled conidia and conidiogenous cell called a phialide. Condiophores and condia are oval or spherical in shape. Septate hyphae.	*Penicillium frequentans*	6
Colonies are dark greyish-brown in color. Sporangia often black in color, usually spherical and oval in shape, hyphae septate, sporangia and sporangiospores within the sporangia	*Rhizopus stolonifer*	4
Colonies are olive green in color. Condia smooth walled and cylindrical or ellipsoidal in shape, distinctive brush like appe arance due to arrangement of phialides on the conidiophores. Hyphae septate.	*Penicillium decumbens*	2
Colonies are velvety or powdery in texture and yellow-light green color. Conidia are single celled,usually spherical or ellipsoidal in shape. Conidiophores typivally brush -like or tufted appearance due to arrangement of conidia - bearing structures called phialides. Hyphae are septate.	*Eupenicillium javanicum*	1
Colonies are white -green, fluffy or cottony with a downy texture. Sporangia are typically spherical or ellipsoidal in shape. Sporangiophores are erect and unbranched. Coenocytic hyphae.	*Mucor hiemalis* f*silvaticus*	7
Colonies are initially white, woolly or cottony in texture. Conidia are single celled and produce chain along the conidiophores. Often ellipsoidal or cylindrical in shape. Conidiophores are branched and are either solitary or in clusters. Hyphae are septate.	*Tricoderma viride*	4
Colonies are white - yellowish, cottony texture. Spor angia produce spores either internally or externally. Spores often multinucleate. Shape and size vary depending on the species. The hyphae are septate.	*Mortierella* sp.	2

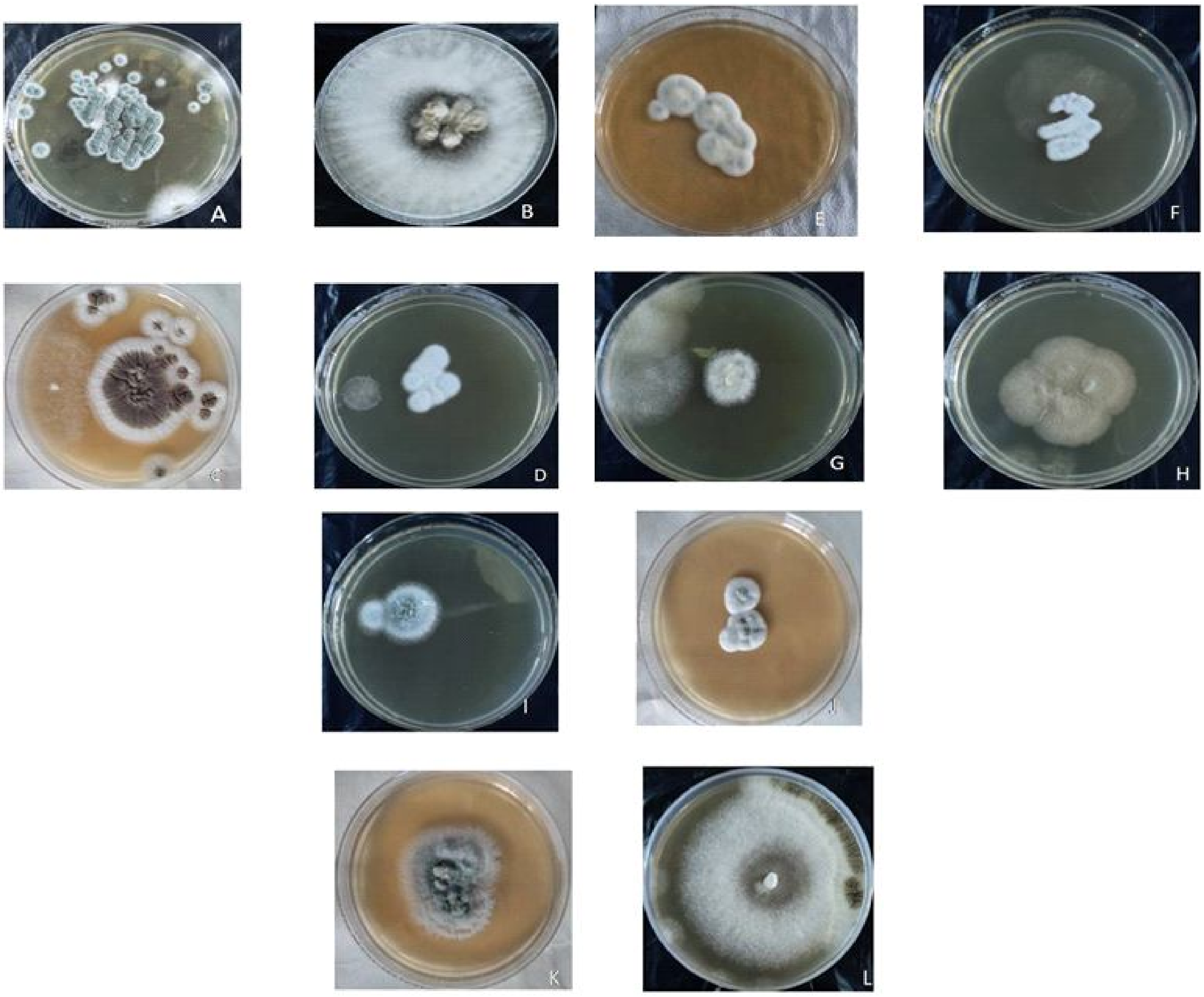

Fig. 2: Cultural view of mycoflora on PDA. A. *Penicillium citrinum*. B. *Alternaira alternate* C. *Aspergillus niger*. D. *Paecilomyces sp.* E. *Penicillium frequentas*. F. Sterile fungi G. *Mortierella* sp. H. *Rhizopus stoloniferI: Penicillium decumbens*. J: *Eupenicillium javanicum* K: *Mucor hiemalis* f. *silvaticus* L. *Tricoderma viride*

potentially outcompete other species for resources. *Aspergillus* species are frequently found in soil and can dwell and grow on decomposing organic material. The large number of colonies observed in *Aspergillus* species aligns with the findings of Qudsia and her colleagues (2017), who identified *Aspergillus* species as the most prevalent in cucumber soil. This abundance can be attributed to their ability to thrive in acidic environments, possibly influenced by other physicochemical factors(Tafinta and Hasatu, 2020). The higher concentration of organic matter in the soil creates a favorable environment for the growth of *Aspergillus*, resulting in higher numbers of *Aspergillus* colonies than other fungi.*Aspergillus* species, including *A. niger*,*Rhizopus*, and*Penicillium* have been identified as postharvest pathogens of cucumber (Amin *et al.,* 2009; Jimeta*et al*., 2022).Like *Aspergillus, Eupenicillium* sp. maythrive in slightly acidic to neutral pH conditions and moderate moisture content. *Trichoderma* sp.isolated from the soil may be controlling the wilt disease in cucumber plant fields acting as a biocontrol agent (Malavika *et al.,* 2022).The abundance, diversity, and composition of soil microbial communities play a crucial role in nutrient cycling and the decomposition of litter in arid regions around the world. These factors can be influenced by variables such as the amount of organic matter, moisture levels, and moderate temperatures, which together affect the rate at which decomposition occurs (Muslem *et al.* 2023). Several variables, including soil pH, moisture content, temperature, electrical conductivity (EC),

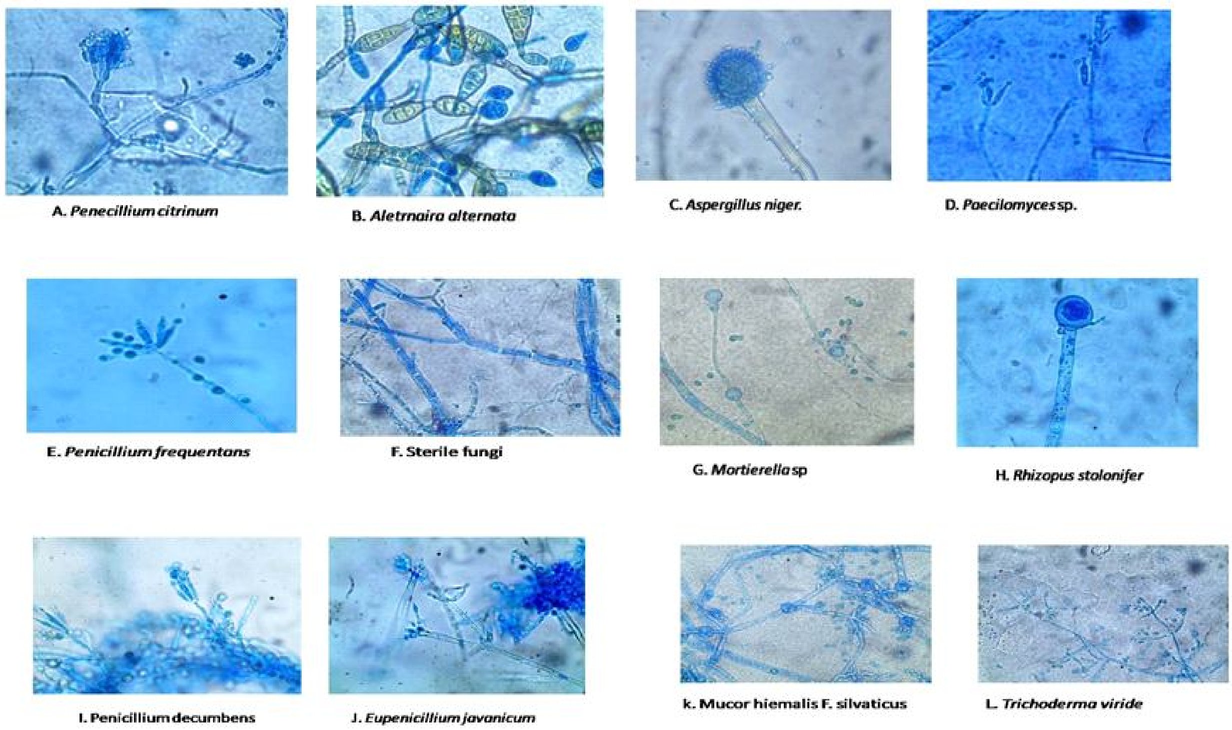

Fig. 3: . Microscopic view of various fungal species in cucumber crop field of Aliba village, Mokokchung

texture, nutrition level, and organic matter concentration, might affect fungi populations.

CONCLUSION

This study highlights the significant influence of soil's physical and chemical properties on the distribution and abundance of fungal communities in the rhizosphere of *Cucumis sativus* L. The findings demonstrate that soil characteristics such as pH, moisture, temperature, and organic matter content significantly shape fungal diversity and population dynamics. Fungi, particularly those from the *Penicillium* and *Aspergillus* genera play key roles in nutrient cycling and organic matter breakdown, influencing both soil health and plant productivity.Fungi aid plants in drought resistance by facilitating moisture absorption within plant cells.Ultimately, this study highlights the structural relationship between soil characteristics and fungal dynamics, providing useful insights for improving agricultural practices and soil management to support crop growth and soil health.While certain fungi are harmful to soil and can cause wilting in cucumber plants, beneficial fungi like *Trichoderma* serve as effective biocontrol agents. Researchers can assist in educating farmers on these differences, helping to prevent disease outbreaks in the field.Through comprehension and control of these soil attributes, farmers can exert influence over the makeup and variety of fungal populations in the soil. This, in turn, can impact the well-being and productivity of plants. By leveraging this knowledge, farmers can make well-informed decisions to maintain crop health and productivity, ultimately contributing to more resilient and productive agricultural systems.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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