

Dynamics of Microbial Populations in the Rhizosphere Soil of Chilli (*Capsicum annuum* L.) from Sowing to Harvest in the Vidarbha Region of Sakoli Tehsil, Maharashtra, India



Vaishnavi Zanzad¹  and Archana Masram^{*2} 

¹P.G.T.D. of Zoology, RTM Nagpur University, Nagpur – 440033, India

²L.A.D. & Smt. R.P. College for Women, Nagpur, India

ABSTRACT

In the current study, we have investigated changes in the microbial population of chilli (*Capsicum annuum* L.) rhizosphere soil between sowing and harvesting stages in four chilli-growing regions of the Vidarbha region in Sakoli Tehsil, Maharashtra, India. Soil samples were collected before cultivation and after crop harvesting during November 2024 to June 2025. Serial dilution and plate count techniques were employed using nutrient agar, potato dextrose agar (PDA), Azotobacter agar, Rhizobium agar, and Pikovskaya agar to enumerate total bacterial, fungal, Azotobacter, Rhizobium, and phosphate-solubilizing microbial populations, respectively. Microbial counts were expressed as CFU mL⁻¹, and paired t-tests were used to assess differences between growth stages. The total bacterial population on nutrient agar showed a numerical decline from sowing (mean 5.053×10^6 CFU mL⁻¹) to harvesting (mean 1.413×10^6 CFU mL⁻¹), but the difference was not statistically significant ($P = 0.3811$). In contrast, the fungal population on PDA increased significantly from 1.3×10^6 to 5.3×10^6 CFU mL⁻¹ ($P = 0.0029$). Azotobacter populations decreased markedly from 3.999×10^7 to 7.605×10^6 CFU mL⁻¹, showing a significant reduction ($P = 0.0092$). Phosphate-solubilizing microorganisms exhibited a non-significant increase from 2.975×10^7 to 4.365×10^7 CFU mL⁻¹ ($P = 0.2150$), while Rhizobium populations increased from 5.325×10^7 to 1.371×10^8 CFU mL⁻¹ without statistical significance ($P = 0.3083$). These findings demonstrated that chilli cultivation is associated with differential responses among rhizosphere microbial groups. Fungal populations increased significantly during crop growth, whereas Azotobacter populations declined significantly, while total bacteria, phosphate-solubilizing microorganisms, and Rhizobium populations remained relatively stable despite numerical changes. The results provide baseline information for evaluating the impact of agricultural practices, fertilizers, and biofertilizers on rhizosphere microbial ecology in chilli cultivation systems.

Keywords: Chilli rhizosphere; Microbial diversity; Azotobacter; Rhizobium, Phosphate-solubilizing microorganisms, Soil microbiology, Rhizosphere dynamics.

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Corresponding Author: Archana Masram

E-mail Address: amg123321@gmail.com

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Introduction

The rhizosphere represents a specialized soil interface where plant root exudates selectively promote distinct microbial assemblages, thereby modulating essential nutrient cycling and plant health [1]. However, prolonged monoculture practices can disrupt these ecological interactions, often precipitating shifts in soil pH and organic matter content that subsequently alter the composition of dominant bacterial and fungal communities [2,3]. Specifically, the continuous influx of root-derived carbon compounds such as organic acids and sugars selectively stimulates specialized microbial groups, which in turn influence the availability of phosphorus and nitrogen in the soil matrix [4,5]. Furthermore, the metabolic activities of these rhizosphere-resident communities often fluctuate in direct response to the developmental stages of the host, as root-associated microorganisms exhibit distinct physiological patterns from the pre-sowing phase through harvest [6,7].

Such shifts in microbial abundance and diversity underscore the complex interplay between plant development and the soil environment, where the enrichment of specific taxa can either facilitate nutrient acquisition or reflect a depletion of soil fertility [8]. Moreover, continuous monoculture and intensive agricultural systems frequently induce microbial degeneration by depleting essential organic matter and nutrients, which can destabilize beneficial bacterial communities while favoring fungal proliferation [9,10]. This niche differentiation is particularly evident in the rhizosphere, where increased fungal plasticity and heterogenic associations contrast with the relative stability of bulk soil microbial profiles [11]. Consequently, the specific selection exerted by chilli genotypes on these microbial assemblages suggests that host-mediated recruitment is a primary driver of rhizosphere community assembly [12,13].

Beyond host-mediated selection, extrinsic factors such as phosphorus fertilizer management and localized root-dipping techniques further modulate these dynamics, particularly within alkaline soil environments [14]. Furthermore, the accumulation of low molecular weight compounds, particularly organic acids, serves as a selective filter that shapes the richness of the rhizosphere microbiome by favoring dominant taxa over others. In the present study, we examine how the temporal progression from the vegetative phase to crop maturity influences the relative abundance and functional diversity of these microbial populations within the Sakoli Tehsil at Vidarbha soil ecosystem.

Materials and Methods

Collection of soil from Chilli cultivated area

In an order to study the changing bacterial diversity around the chilli cultivated soil, the soil study as before and after cultivation in the Vidarbha region of Sakoli Tehsil, Maharashtra for the four different agricultural regions in the month of November 2024 and June 2025. This sampling ensures us to understand the changing microbiological diversity so as to confirm whether the used bio fertilizers, chemical fertilizers, and additives really making any impact on microbial diversity or not. In an order to collect the soil, 100 grams of it collected from the regions in a sterile plastic bag and transported immediately into the laboratory for processing.

Serial dilution of soil

Collected soil sample (1 g) serially diluted with sterile distilled water up to 10^{-4} by serial dilution method. The dilutions of 10^{-3} and 10^{-4} with the total volume of 50 μL have been used for inoculation on various types of media, which showcase the specificity of the bacterial and fungal population growth and used to record the diversity up to different plant growth promoting microbial level.

Growth on specific media and colony count

In the present study, five microbial media have been utilized, those were nutrient agar which has been used to record the total colony-forming units for the commonly growing bacteria. Another media used as potato dextrose agar which has been used to calculate the growth potential of yeast, mould and fungi. The third media used as Azotobacter agar which is promising to isolate mannitol positive azotobacter species from the soil. The fourth media used as rhizobium media which is used for isolation of mannitol positive rhizobium species. The fifth medium used as pikovskaya agar which is promising for the detection of phosphate soluble soil microorganisms able to produce clear zone surrounding the colony and recognized as positive culture. Upon inoculation, all plates were incubated at 25-28°C for 3 to 4 days. Appeared colonies then calculated as per standard formula to record the CFU/mL.

Statistical analysis

In the present study, paired T test has been used to record the changes in the microbial count as of sowing stage and of harvesting stage for the chilli plant. Here, the P value set as <0.05 and the data recorded for minimum, median, maximum, mean, standard deviation, standard error, and mean of differences along with the P value to record the significant change if any. The significant change has been recorded as 0.05 (*), 0.01 (**), and 0.0001 (***) and non-significant as (NS).

Result and Discussion

Comparison of Microbial Population in Chilli Rhizosphere Soil at Sowing and Harvesting Stages using nutrient agar as medium. In the chilli field, microbial counts decreased from a mean of $5.053 \times 10^6 \text{ CFU mL}^{-1}$ at sowing to $1.413 \times 10^6 \text{ CFU mL}^{-1}$ at harvesting. However, paired t-test analysis revealed that the difference was not statistically significant ($P = 0.3811$), indicating that microbial populations remained relatively stable throughout the crop growth period despite a numerical decline. High variability among observations, particularly during the sowing stage, was reflected by the large standard deviation values and may have contributed to the lack of statistical significance (Table 1) (Fig. 1). This reduction, while numerically evident, likely mirrors broader trends where intensive agricultural practices and host-mediated nutrient uptake influence rhizosphere dynamics over the growing season [15]. Furthermore, the observed decline in microbial counts on nutrient agar may be attributed to the depletion of readily available root exudates as the plant reaches senescence, leading to a shift in community composition toward specialized oligotrophic taxa [16]. Consistent with these observations, the reduction in nutrient availability may necessitate reliance on secondary metabolic pathways for survival, a phenomenon frequently observed in phosphorus-limited or alkaline environments where specialized phosphate-solubilizing bacteria play a critical role [17,18].

Comparison of fungal Population in Chilli Rhizosphere Soil at Sowing and Harvesting Stages using potato dextrose agar as medium

The fungal population enumerated on PDA medium increased substantially from the sowing stage to the harvesting stage of chilli cultivation. The mean fungal count increased from $1.3 \times 10^6 \text{ CFU mL}^{-1}$ at sowing to $5.3 \times 10^6 \text{ CFU mL}^{-1}$ at harvesting, representing a mean increase of $4.7 \times 10^6 \text{ CFU mL}^{-1}$.

Paired t-test analysis revealed that this increase was highly significant ($P = 0.0029$, $P < 0.01$), indicating a marked enhancement in fungal abundance during crop growth. The higher median value at harvesting ($6.0 \times 10^6 \text{ CFU mL}^{-1}$) compared to sowing ($6.0 \times 10^5 \text{ CFU mL}^{-1}$) further supports the substantial increase in fungal populations (Table 2) (Fig. 2). This elevation in fungal propagule density likely corresponds to the increased secretion of complex root exudates, such as organic acids and flavonoids, which progressively stimulate mycofloral activity throughout the plant developmental cycle [19]. Such shifts in the rhizosphere fungal community composition are frequently driven by changing nutrient dynamics and the host plant's developmental stage, which create distinct niche environments [20]. These results align with previous reports indicating that fungal abundance is significantly stimulated by the presence and physiological activity of root systems throughout the vegetative cycle [21]. Furthermore, these successional shifts in fungal and bacterial diversity underscore the plant's capacity to define specific functional rhizosphere communities as it matures [22]. Specifically, the accumulation of recalcitrant organic matter and altered soil pH levels toward the end of the cultivation cycle may favor saprotrophic fungal proliferation over the bacterial populations initially dominant during early plant establishment [23].

Comparison of Azotobacter Population in Chilli Rhizosphere Soil at Sowing and Harvesting Stages using Azotobacter agar as medium

The population of *Azotobacter* in the chilli rhizosphere exhibited a marked decline from sowing to harvesting. The mean count decreased from 3.999×10^7 CFU mL⁻¹ at sowing to 7.605×10^6 CFU mL⁻¹ at harvesting, resulting in a mean difference of 3.238×10^7 CFU mL⁻¹.

Paired t-test analysis demonstrated that this reduction was statistically significant ($P = 0.0092$, $P < 0.01$), indicating a substantial decrease in *Azotobacter* population during crop development. The median value also declined sharply from 6.0×10^7 CFU mL⁻¹ at sowing to 1.3×10^6 CFU mL⁻¹ at harvesting, supporting the observed trend (Table 3 and Fig. 3). This reduction in free-living nitrogen-fixing bacteria likely reflects a progressive shift in the rhizosphere niche, where the plant's developing root system favors different microbial cohorts [24]. Specifically, as root exudate profiles shift from high-sugar content toward increased amino acid and phenolic concentrations during later developmental phases, nitrogen-fixing specialists like *Azotobacter* may face competitive exclusion by communities better adapted to utilizing these recalcitrant organic compounds [25]. This transition aligns with the understanding that root exudate profiles evolve significantly during plant development, thereby exerting a strong selective pressure on the composition and functional capabilities of the rhizosphere microbiome [26,27]. Furthermore, the accumulation of root-derived organic compounds throughout the growing season serves as a critical substrate for diverse microbial groups, whose degradation capacities directly influence both nutrient availability and plant performance [28].

Comparison of Phosphate-Solubilizing Microorganisms Population in Chilli Rhizosphere Soil at Sowing and Harvesting Stages using PKV agar as medium

The population of phosphate-solubilizing microorganisms (PKV medium) showed an increase from the sowing stage to the harvesting stage of chilli cultivation. The mean microbial count increased from 2.975×10^7 CFU mL⁻¹ at sowing to 4.365×10^7 CFU mL⁻¹ at harvesting, corresponding to a mean increase of 1.39×10^7 CFU mL⁻¹. Despite the numerical increase, paired t-test analysis indicated that the difference was not statistically significant ($P = 0.2150$; $P > 0.05$). Therefore, the observed increase in phosphate-solubilizing microbial population cannot be conclusively attributed to crop growth stage and may reflect natural variation among samples. The median value increased considerably from 2.45×10^7 CFU mL⁻¹ at sowing to 6.0×10^7 CFU mL⁻¹ at harvesting, suggesting a tendency toward higher microbial abundance at harvest. However, the large standard deviations observed at both stages indicate substantial variability among samples, which likely contributed to the lack of statistical significance (Table 4 and Fig 4). This pattern suggests that while plants may employ organic acid secretion to solubilize inorganic phosphorus as a coping strategy for nutrient limitation, these responses are highly dynamic and influenced by fluctuating soil characteristics and individual plant physiological status [29,30]. Moreover, the sustained release of organic acids by these microorganisms facilitates the liberation of phosphorus from complexed forms such as Fe, Al, and Ca, thereby enhancing nutrient availability as the root system matures.

This process is further augmented by the secretion of specific phenolic compounds that, depending on their concentration and composition, can either catalyze or inhibit the proliferation of distinct phosphate-solubilizing bacterial strains within the rhizosphere [31]

Comparison of Microorganisms Population in Chilli Rhizosphere Soil at Sowing and Harvesting Stages using Rhizobium agar as medium

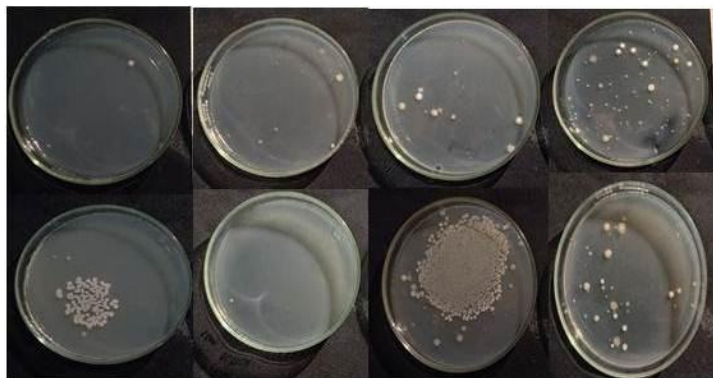
The *Rhizobium* population in the chilli rhizosphere increased from a mean of 5.325×10^7 CFU mL⁻¹ at sowing to 1.371×10^8 CFU mL⁻¹ at harvesting. Despite this numerical increase, paired t-test analysis revealed no significant difference between the two growth stages ($P = 0.3083$). The high variability observed at harvesting, as indicated by the large standard deviation, suggests that the increase was not consistent across all samples (Table 5 and Fig. 5). Such variation likely stems from the metabolic versatility of *Rhizobium* spp., which can effectively solubilize mineral phosphorus through the production of organic acids like gluconic or citric acid, adapting their activity to the specific nutritional requirements and chemical shifts of the maturing rhizosphere [32]. Moreover, these strains frequently augment iron acquisition through the secretion of siderophores, which function as high-affinity iron-chelating molecules that are vital for maintaining physiological homeostasis within the nutrient-stressed rhizosphere [33]. Furthermore, the ability of these microorganisms to hydrolyze organic phosphorus substrates via phosphatase production complements plant-driven nutrient mobilization, creating a synergistic microenvironment that sustains plant productivity despite the depletion of readily available mineral fractions. This metabolic adaptability is further reflected in their capacity to modulate soil pH through the excretion of various organic acids, including malic and oxalic acids, which facilitate the chelation of cations and subsequent solubilization of immobile nutrients [34,35].

Table 1: Comparison of microbial population (CFU mL⁻¹) in chilli rhizosphere soil at sowing and harvesting stages

	Chilly (Sowing)NA	Chilly(Harvesting)NA
Minimum	160000	20000
Median	740000	860000
Maximum	3000000	6000000
Mean	5053000	1413000
Std. Deviation	10300000	1992000
Std. Error	3640000	704400
P value	0.3811	
P value summary	ns	
Mean of differences	3640000	



A) Count on Nutrient agar with Chilli Soil at sowing stage



B) Count on Nutrient agar with Chilli Soil at harvesting stage

Fig. 1: Growth on Nutrient agar medium for microbes of chilli field at sowing and harvesting stage

Table 2: Comparison of fungal population (CFU mL⁻¹) in chilli rhizosphere soil under at sowing and harvesting stages

	Chilly (Sowing)PDA	Chilly (Harvesting)PDA
Minimum	120000	2000000
Median	600000	6000000
Maximum	6000000	6000000
Mean	1300000	5300000
Std. Deviation	2000000	1600000
Std. Error	690000	670000
P value	0.0029	
P value summary	**	
Mean of differences	-4700000	



A) Count on PDA agar with Chilli Soil at sowing stage



B) Count on PDA agar with Chilli Soil at harvesting stage

Fig. 2: Growth on PDA agar medium for fungi of chilli field at sowing and harvesting stage

Table 3: Comparison of Azotobacter population (CFU mL⁻¹) in chilli rhizosphere soil under at sowing and harvesting stages

	Chilly (Sowing)AZ	Chilly(Harvesting)AZ
Minimum	600000	60000
Median	60000000	1300000
Maximum	60000000	34000000
Mean	39990000	7605000
Std. Deviation	27860000	12300000
Std. Error	9849000	4349000
P value	0.0092	
P value summary	**	
Mean of differences	32380000	



A) Count on Azotobacter agar with Chilli Soil at sowing stage



B) Count on Azotobacter agar with Chilli Soil at harvesting stage

Fig. 3: Growth on Azotobacter agar medium for Azotobacter of chilli field at sowing and harvesting stage

Table 4: Comparison of phosphate-solubilizing microorganisms (CFU mL⁻¹) in chilli rhizosphere soil under at sowing and harvesting stages

	Chilly (Sowing)PKV	Chilly (Harvesting)PKV
Minimum	600000	2200000
Median	24500000	60000000
Maximum	60000000	60000000
Mean	29750000	43650000
Std. Deviation	26770000	24430000
Std. Error	9465000	8639000
P value	0.2150	
P value summary	ns	
Mean of differences	-13900000	



A) Count on PKV agar with phosphate-solubilizing microorganisms of Chilli Soil at sowing stage



B) Count on PKV agar with phosphate-solubilizing microorganisms of Chilli Soil at harvesting stage

Fig. 4: Growth on PKV agar medium for phosphate-solubilizing microorganisms of chilli field at sowing and harvesting stage

Table 5: Comparison of Rhizobium population (CFU mL⁻¹) in chilli rhizosphere soil under at sowing and harvesting stages

	Chilly (Sowing)Rhizo	Chilly(Harvesting)Rhizo
Minimum	6000000	60000000
25% Percentile	60000000	60000000
Median	60000000	60000000
75% Percentile	60000000	60000000
Maximum	60000000	600000000
Mean	53250000	137100000
Std. Deviation	19090000	204100000
Std. Error	6750000	77140000
P value	0.3083	
P value summary	ns	
Mean of differences	-84860000	



A) Count on Rhizobium agar with phosphate-solubilizing microorganisms of Chilli Soil at sowing stage



B) Count on Rhizobium agar with phosphate-solubilizing microorganisms of Chilli Soil at sowing stage

Fig. 5: Growth on Rhizobium agar medium for microorganisms of chilli field at sowing and harvesting stage

Conclusion

This study evaluated the dynamics of microbial populations in chilli rhizosphere soil between sowing and harvesting stages across four chilli-growing regions of Vidarbha in Sakoli Tehsil, Maharashtra. Distinct microbial groups responded differently during crop growth. Fungal populations increased significantly at harvesting, indicating enhanced fungal proliferation in the rhizosphere during the crop cycle. In contrast, Azotobacter populations declined significantly, suggesting that conditions prevailing during later stages of crop growth may not favor their persistence or activity. Although total bacterial counts, phosphate-solubilizing microorganisms, and Rhizobium populations showed numerical changes between sowing and harvesting, these differences were not statistically significant. The considerable variability among field samples indicates that environmental and management factors may strongly influence microbial abundance. Overall, the results suggest that chilli cultivation alters the rhizosphere microbial community in a group-specific manner rather than causing a uniform increase or decrease in all microorganisms. Monitoring beneficial microbial populations such as Azotobacter, Rhizobium, and phosphate-solubilizing microorganisms may therefore be useful for assessing soil health and the effectiveness of fertilizer and biofertilizer practices in chilli production systems. Future studies incorporating molecular characterization of microbial communities, larger sample sizes, and detailed analysis of soil physicochemical properties would provide deeper insight into the mechanisms driving these microbial shifts and their implications for sustainable chilli cultivation.

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