

Evaluation of Microbial Diversity at Various Growth Stages of Pepper (*Capsicum annum* L.) and Garden Egg (*Solanum melongena* L.).

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Abstract

The rhizosphere, provides a niche for the interaction between plant roots and microorganisms, whose diversity are affected by various biotic and abiotic factors. This study investigated microbial diversity in the rhizosphere of garden eggs and pepper at various growth stages. The study was carried out at the University of Port Harcourt's Faculty of Agriculture Teaching and Research Farm in Rivers State, Nigeria. Seeds of pepper and garden egg were purchased and planted in a 15 m × 15 m experimental layout using a Randomized Complete Block Design (RCBD) with four (4) replications, soil sample collection and analysis, bacterial, fungal, and nematode count and isolation were carried out before planting and at three stages of plant development. Findings in the study showed the presence of *Staphylococcus* spp, *Bacillus* spp, *Streptococcus* spp, *Aspergillus* spp, *Rhizopus*, *Yeast*, *Pratylenchus* spp before planting and the presence of *Staphylococcus* spp, *Streptococcus* spp, *Bacillus* spp, *Pseudomonas*, *Lactobacillus*, *E. coli*, *Aspergillus*, *Rhizopus*, *Yeast*, *Fusarium*, *Mucor*, *Meloidogyne* spp, *Helicotylenchus* spp, *Pratylenchus* spp, and *Tylenchus* spp at different stages of plant development with variations in their percentage occurrence. The study concluded that microbial diversity in the rhizosphere of garden eggs and pepper are affected by various growth stages. These variations in microbial diversity can explored for intercrop selection in sustainable agriculture.

Keywords: Pepper, Garden Egg, Seedling Stage, Flowering Stage, Maturity Stage, Rhizosphere, Microbial Diversity

INTRODUCTION

The genus *Capsicum* has roughly 35 species, of which the principal five cultivated species are *Capsicum annum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens* (Li *et al.*, 2024). Among these principal five cultivated species, *Capsicum annum* which is a perennial or annual herb is the most frequently farmed species in the world (Carrizo García *et al.*, 2016; Li *et al.*, 2024), and the biggest spice and most important vegetable crop in Nigeria (Zhang *et al.*, 2024). The plant is native to the tropical and temperate Americas (Carrizo García *et al.*, 2016), and its distinctive flavor and rich nutritional qualities have made it a staple in global agriculture (Choi *et al.*, 2023). Pepper has a unique pungency that is created by the accumulation of a type of alkaloids called capsaicinoids, which are synthesized in the placenta and consist of capsaicin and dihydrocapsaicin, which make up nearly 90% of all capsaicinoids (Li *et al.*, 2024). Potential uses for capsaicinoids include food flavorings and therapeutics; for example, they have antimicrobial qualities and may be effective as biopesticides; they have been shown to protect *Capsicum chacoense* seeds from *Fusarium*, a major cause of predispersal seeds; and they have biological properties that include antitumor, antioxidant, and anti-obesity; they are used in food, pharmaceutical, medical, cosmetic, and dietary industries (Wang *et al.*, 2022, Li *et al.*, 2024). Another key vegetable crops mostly planted in Nigeria is garden egg; this crop is eaten virtually every day by rural and

urban households and is a significant revenue source for majority of rural families (Adjei *et al.*, 2023).

The rhizosphere, or the soil next to roots, provides a niche for the interaction between plant roots and microorganisms, and the rhizosphere microbiomes offer a number of beneficial functions for plant host, including growth promotion, nutrient uptake, stress tolerance, and resistance to pathogens (Li *et al.*, 2024). The interaction between plant roots and rhizosphere microbiota is crucial for plants to grow and function (Lu *et al.*, 2018; Kwak *et al.*, 2018). This is because, in addition to driving plant growth (Qu *et al.*, 2020), root microbiota are important for disease prevention (Meng *et al.*, 2018), nutrient acquisition, and soil nutrient dynamics (Hou *et al.*, 2018).

The interaction between plants and microorganisms are linked with plant secondary metabolites which can protect plants from microbial pathogens, and recent research has demonstrated that plant secondary metabolites influence microbiome composition and function (Qu *et al.*, 2020; Ren *et al.*, 2020; Kudjorjdie *et al.*, 2021; Li *et al.*, 2024). Among these metabolites, the most prominent are glucosinolates (Siebers *et al.*, 2018), triterpenes (Huang *et al.*, 2019), coumarins (Stringlis *et al.*, 2018; Voges *et al.*, 2019), flavonoids (Kudjorjdie *et al.*, 2021), and benzoxazinoids (Hu *et al.*, 2018; Cotton *et al.*, 2019) which according to de Bruijn *et al.* (2018), are emitted from the roots and are generated from indole. Inversely, Korenblum *et al.* (2020) demonstrated that

different microbial populations cause systemic variations in tomato root metabolite exudation. Also, plant rhizosphere microbiomes have been found to be significantly influenced by the quantities of nutrients that are available in the soil (Ren *et al.*, 2020) soil type and variations in plant genotype (Yeoh *et al.*, 2017; Veach *et al.*, 2021) which affects the makeup and functionality of the rhizosphere microbiomes in a variety of plants, including soybean, rice, corn, wheat, and potatoes (Zhong *et al.*, 2019; Li *et al.*, 2024). While studies have explored the impacts of these factors on microbial diversity in the rhizosphere of different plants, there seems to be a dearth of information on microbial diversity in the rhizosphere of garden eggs and pepper at various growth stages especially in Nigeria, highlighting the necessity of this study.

Materials and Methods

Study Area

The study was carried out from May to September 2024 at the University of Port Harcourt's Faculty of Agriculture Teaching and Research Farm in Rivers State, Nigeria. Port Harcourt is located in Nigeria's subequatorial zone, 18 meters above sea level between latitudes 40, 07" and 5050N and longitudes 600, 56'04" and 703'20"E. The average annual temperature in Port Harcourt is between 22°C and 31°C, with a mean annual relative humidity of 85%. The soil type of Port Harcourt is alluvial soil, with low-lying coastal plains that are structurally part of the sedimentary formation of the current Niger Delta (Eludoyin *et al.*, 2015).

Experimental Design and Layout

In this work, a 15m × 15m experimental layout using a Randomized Complete Block Design (RCBD) with four (4) replications were used. The experimental layout was marked using tape, pegs, and ropes after the trial area was manually cleared, and every plot had a 3 × 3m bed raised with a 0.5 m alley way.

Planting Materials and Planting

Pepper and garden egg seeds were bought from the Aluu village local market in Port Harcourt, Rivers State, Nigeria. Before being moved into the field, pepper and garden egg seeds were first raised in the nursery for four weeks.

Soil Sampling

Prior to planting, soil samples were taken at a depth of 0–20 cm using a soil auger. At each growth stage, rhizosphere soil samples were taken from two plant stands in each plot by carefully uprooting the plants and shaking the dirt that adhered to the roots into a polybag with a label. For microbiological analysis, the soil samples that were gathered from the two plant stands in

each plot were combined into a composite sample. Three repetitions of each treatment were used to gather samples, and six samples in all were taken at each sampling period. After being put in clearly marked poly bags, they were taken to the laboratory for examination.

Sample Collection for Nematode

Two plants in each plot had their roots and rhizosphere dirt randomly taken, and the roots and soil were bulked into a composite sample. This process was done at the three (3) chosen growth stages (seedling, flowering, and maturity). Using a hand trowel, soil samples were taken from two plant stands in each plot at a depth of 0–20 cm in the rhizosphere of each plant at the various stages of growth. The soil samples were bulked into a composite sample and appropriately labelled in polythene bags with the crop type, replicate, and the stage of growth at which the sample was taken. Each plant's roots were taken from the same plant stand where the soil samples were taken, and after the earth was removed, the plants' roots were cut out with a knife to reveal them. The roots were also bulked into composite samples and were appropriately labeled in polythene bags as the soil samples, so as to prevent moisture loss and desiccation of roots prior to laboratory examination.

Bacteria count and isolation

Bacteria communities in soils were determined using viable plate count for counting colony from bacterial and propagules. The serial dilution and spread plate methods in nutrient agar, as outlined by Fawole *et al.* (2001), were used to count and isolate the bacteria. Following a 24-hour inoculation period, biochemical tests were performed to identify the isolates.

Fungal count and isolation

The spread plate method on potato dextrose agar (PDA) was used to determine the fungal count according to Monica (2001). Soil samples were prepared by serially diluting them ten times with one gram of soil sample, and after four days of inoculation, the isolated colonies were counted, identified using lactophenol, and examined under a microscope.

Extraction of Plant Parasitic Nematodes from Soil Samples

Plant parasitic nematodes were collected from soil samples using extraction tray method as described by Coyne *et al.* (2007). After mixing each composite sample, 250 ml of soil was measured in a 25 ml beaker and transferred into a lined plastic sieve with face tissue on a plastic flat plate that had been properly labelled. To progressively moisten the soil, 200 milliliters of water

were added to the plastic sieve's side. Before identifying and counting the nematode species, the setup was left undisturbed in the laboratory for 48 hours. Then, the sieve was carefully removed from the plastic plate and the nematode suspension was poured into a small, disposable cup that was clearly labelled. The plate was then rinsed into the cup and allowed to settle. The nematode suspension was then decanted, and the supernatant was transferred into a labelled 30-ml vial and stored in the refrigerator at 4°C.

Extraction of Plant Parasitic Nematodes from Root Samples

Using the extraction tray approach as outlined by Coyne *et al.* (2007), plant parasitic nematodes were extracted from root samples. To get rid of soil particles, distilled water was used to rinse each composite sample. Using a sharp knife, the roots were carefully cut into 1-2 cm pieces, a sensitive balance was used to weigh 10g, which was then transferred to a lined plastic sieve with face tissue that was set on a plastic flat plate with a clear label. To progressively moisten the root, 200 milliliters of water were added to the side of the plastic filter, and after 48 hours of the setup remaining undisturbed in the laboratory, the sieve was carefully removed from the plastic plate and the nematode suspension was transferred into a small, disposable cup with a clear label. The plate was then rinsed into the suspension in the cup and given time to settle. Before the nematode species were identified and counted, the nematode suspension was decanted, the supernatant was transferred into

labelled 30-milliliter vials, and the vials were refrigerated at 40 degrees Celsius.

Nematode Identification and Counting

As explained by Doncaster (1962), a pipette was used to transfer a 2ml aliquot of nematode suspension into a Doncaster counting plate. To identify and count plant parasitic nematodes using Bell's Key, the Doncaster counting dish was alternatively examined with a dissecting microscope and a compound microscope (Bell, 2004). The total number of plant parasitic nematodes in each sample was determined by multiplying the mean number of nematodes determined from the suspension by the total volume of the suspension.

Statistical Analysis

Using Genstart Discovery 3rd edition, 2009, analysis of variance (ANOVA) was performed on the obtained data, and the least significant difference at 5% was used to separate the mean difference.

RESULTS

Soil Microbial Count and Diversity before Planting

The result of soil microbial count and diversity before planting is shown in Table 1. Nematode and fungi count (450 and 11 respectively) were the same in both pepper and garden egg plots, while bacteria count was higher in garden egg plot (50) compared with pepper plot (30). Bacteria species include: *Staphylococcus spp*, *Bacillus spp*, *streptococcus spp*, Fungi species include: *Aspergillus spp*, *Rhizopus*, *Yeast*, while nematode species include: *Pratylenchus spp*.

Table 1: Soil Microbial Counts and Diversity before Planting

Microbial Diversity	Pepper Plot	Garden Egg Plot	Microbial Species
Bacteria (x10 ³ Cfu/g of soil)	30	50	<i>Staphylococcus spp</i> , <i>Bacillus spp</i> , <i>streptococcus spp</i>
Fungi (x10 ³ Cfu/g of soil)	11	11	<i>Aspergillus spp</i> , <i>Rhizopus</i> , <i>Yeast</i> .
Nematode	450	450	<i>Pratylenchus spp</i> .

Soil Microbial Diversity at Seedling Stage

The result of soil microbial diversity at seedling stage is shown in Table 2. Bacteria species were the same in the rhizosphere of pepper and garden egg and include: *Staphylococcus spp*, *Streptococcus spp*, and *Bacillus spp*. For fungi species, *Aspergillus*, *Rhizopus*, and *Yeast* were isolated in the rhizosphere of pepper while

Aspergillus, *Rhizopus*, *Yeast*, and *Fusarium* were isolated in the rhizosphere of garden egg. For nematode, *Helicotylenchus spp*, and *Pratylenchus spp* were isolated in the rhizosphere of pepper while *Meloidogyne spp*, *Helicotylenchus spp*, and *Pratylenchus spp* were isolated in the rhizosphere of garden egg.

Table 2: Soil Microbial Diversity at Seedling Stage

Seedling Stage			
Crops	Bacteria Isolates	Fungi Isolates	Nematode Isolates
Pepper	<i>Staphylococcus spp</i> , <i>streptococcus spp</i> , <i>Bacillus spp</i> .	<i>Aspergillus</i> , <i>Rhizopus</i> , Yeast.	<i>Helicotylenchus spp</i> , <i>Pratylenchus spp</i> .
Garden Egg	<i>Staphylococcus spp</i> , <i>streptococcus spp</i> , <i>Bacillus spp</i> .	<i>Aspergillus</i> , <i>Rhizopus</i> , Yeast, <i>Fusarium</i> .	<i>Meloidogyne spp</i> , <i>Helicotylenchus spp</i> , <i>Pratylenchus spp</i> .

Soil Microbial Diversity at Flowering Stage

The result for soil microbial diversity at flowering stage is shown in Table 3. Bacteria species were the same in the rhizosphere of pepper and garden egg and include: *Staphylococcus spp*, *streptococcus spp*, and *Bacillus spp*. For fungi species, *Aspergillus*, *Rhizopus*, and Yeast were isolated in the rhizosphere of pepper while *Aspergillus*,

Rhizopus, Yeast, and *Fusarium* were isolated in the rhizosphere of garden egg. For nematode, *Helicotylenchus spp*, and *Pratylenchus spp* were isolated in the rhizosphere of pepper while *Meloidogyne spp*, *Helicotylenchus spp*, and *Pratylenchus spp* were isolated in the rhizosphere of garden egg

Table 3: Soil Microbial Diversity at Flowering Stage

Crops	Bacteria Isolates	Fungi Isolates	Nematode Isolates
Pepper	<i>Staphylococcus spp</i> , <i>streptococcus spp</i> , <i>Bacillus spp</i> , <i>Pseudomonas</i> .	<i>Aspergillus</i> , <i>Rhizopus</i> , <i>Mucor</i> , <i>Fusarium</i> .	<i>Helicotylenchus spp</i> , <i>Pratylenchus spp</i> .
Garden Egg	<i>Bacillus spp</i> , <i>Lactobacillus</i> , <i>Staphylococcus spp</i> , <i>E. coli</i>	<i>Aspergillus</i> , <i>Rhizopus</i> , <i>Fusarium</i> , <i>Mucor</i> .	<i>Helicotylenchus spp</i> , <i>Pratylenchus spp</i> .

Soil Microbial Diversity at Maturity Stage

The result for soil microbial diversity at maturity stage is shown in Table 4. Bacteria species were the same in the rhizosphere of pepper and garden egg and include: *Staphylococcus spp*, *streptococcus spp*, and *Bacillus spp*. Fungi species were also the same in the rhizosphere of pepper and garden egg and include: *Aspergillus*,

Rhizopus, and *Fusarium*. For nematode, *Meloidogyne spp*, and *Helicotylenchus spp* were isolated in the rhizosphere of pepper while *Meloidogyne spp*, *Helicotylenchus spp*, and *Tylenchus spp* were isolated in the rhizosphere of garden egg.

Table 4: Soil Microbial Diversity at Maturity Stage

Crops	Bacteria Isolates	Fungi Isolates	Nematode Isolates
Pepper	<i>Staphylococcus spp</i> , <i>streptococcus spp</i> , <i>Bacillus spp</i> .	<i>Aspergillus</i> , <i>Rhizopus</i> , <i>Fusarium</i> .	<i>Meloidogyne spp</i> , <i>Helicotylenchus spp</i> .
Garden Egg	<i>Staphylococcus spp</i> , <i>streptococcus spp</i> , <i>Bacillus spp</i> .	<i>Aspergillus</i> , <i>Rhizopus</i> , <i>Fusarium</i> .	<i>Meloidogyne spp</i> , <i>Helicotylenchus spp</i> , <i>Tylenchus spp</i> .

Percentage Occurrence of Bacteria Species at Different Stages of Growth

The result of percentage occurrence of bacteria species at different stages of growth is shown in Table 5. *Bacillus spp* occurrence was highest (60%) in the rhizosphere of pepper during maturity stage, *Staphylococcus spp* occurrence was highest (38%) in the

rhizosphere of pepper during seedling stage, *Streptococcus spp* occurrence was highest (15%) in the rhizosphere of garden egg during seedling stage, *Lactobacillus* was highest (5%) in the rhizosphere of garden egg during flowering stage, among others.

Table 5: Percentage Occurrence of Bacteria Species at Different Stages of Growth

Crops	Seedling Stage						
	<i>Bacillus</i> spp	<i>Staphylococcus</i> spp	<i>Streptococcus</i> spp	<i>Lactobacillus</i>	<i>Pseudomonas</i> spp	<i>Cyanobacterium</i>	<i>E. coli</i>
Pepper	52	38	14	0	0	0	0
Garden Egg	55	33	15	0	0	0	0
Crops	Flowering Stage						
	<i>Bacillus</i> spp	<i>Staphylococcus</i> spp	<i>Streptococcus</i> spp	<i>Lactobacillus</i>	<i>Pseudomonas</i> spp	<i>Cyanobacterium</i>	<i>E. coli</i>
Pepper	55	29	11	0	4	0	0
Garden Egg	53	32	7	5	0	0	3
Crops	Maturity Stage						
	<i>Bacillus</i> spp	<i>Staphylococcus</i> spp	<i>Streptococcus</i> spp	<i>Lactobacillus</i>	<i>Pseudomonas</i> spp	<i>Cyanobacterium</i>	<i>E. coli</i>
Pepper	60	29	11	0	0	0	0
Garden Egg	58	31	11	0	0	0	0

Percentage Occurrence of Fungi Species at Different Stages of Growth

The result of percentage occurrence of fungi species at different stages of growth is shown in Table 6. *Rhizopus* spp occurrence was highest (58%) in the rhizosphere of garden egg during maturity stage, *Aspergillus* spp occurrence was highest (44%) in the rhizosphere of pepper during seedling stage, *Yeast* spp occurrence was

highest (10%) in the rhizosphere of pepper and garden egg during seedling stage, *Fusarium* spp occurrence was highest (18%) in the rhizosphere of garden egg during seedling stage, and *Mucor* spp occurrence was highest (8%) in the rhizosphere of pepper during flowering stage.

Table 6: Percentage Occurrence of Fungi Species at Different Stages of Growth

Crops	Seedling Stage				
	<i>Rhizopus</i> spp	<i>Aspergillus</i> spp	<i>Yeast</i> spp	<i>Fusarium</i> spp	<i>Mucor</i> spp
Pepper	46	44	10	0	0
Garden Egg	36	36	10	18	0
Crops	Flowering Stage				
	<i>Rhizopus</i> spp	<i>Aspergillus</i> spp	<i>Yeast</i> spp	<i>Fusarium</i> spp	<i>Mucor</i> spp
Pepper	45	31	0	15	8
Garden Egg	52	34	0	8	7
Crops	Maturity Stage				
	<i>Rhizopus</i> spp	<i>Aspergillus</i> spp	<i>Yeast</i> spp	<i>Fusarium</i> spp	<i>Mucor</i> spp
Pepper	48	32	0	16	0
Garden Egg	58	34	0	9	0

Percentage Occurrence of Nematode Species at Different Stages of Growth

The result of percentage occurrence of nematode species at different stages of growth is shown in Table 7. *Meloidogyne* spp occurrence was highest (40%) in the rhizosphere of garden egg during seedling stage, *Pratylenchus* spp occurrence was highest (67%) in the

rhizosphere of garden egg during flowering stage, *Helicotylenchus* spp occurrence was highest (67%) in the rhizosphere of pepper during seedling stage, and *Tylenchus* spp occurrence was highest (33%) in the rhizosphere of garden egg during maturity stage.

Table 7: Percentage Occurrence of Nematode Species at Different Stages of Growth

Crops	<i>Meloidogyne spp</i>	<i>Pratylenchus spp</i>	<i>Helicotylenchus spp</i>	<i>Tylenchus spp</i>
Seedling Stage				
Pepper	0	33	67	0
Garden Egg	40	20	40	0
Flowering Stage				
Pepper	0	50	50	0
Garden Egg	0	67	33	0
Maturity Stage				
Pepper	0	0	33	0
Garden Egg	33	0	33	33

DISCUSSION

The interaction between plant roots and rhizosphere microbiota is crucial for plants growth and functionality (Lu *et al.*, 2018; Kwak *et al.*, 2018); because, in addition to driving plant growth (Qu *et al.*, 2020), root microbiota are important for disease prevention (Meng *et al.*, 2018), nutrient acquisition, and soil nutrient dynamics (Hou *et al.*, 2018). However, the diversity of rhizosphere microbiota is affected by a lot of biotic and abiotic factors (Li *et al.*, 2019; Li *et al.*, 2024) and in this study, we evaluated the diversity of rhizosphere microbiota at seedling, flowering and maturity stages of plant development in order to determine variations in rhizosphere microbial diversity at these stages.

In this study, before planting, nematode and fungi count were the same in both pepper and garden egg plots, while bacteria count was higher in garden egg plot than in pepper plot. Bacteria species include: *Staphylococcus spp*, *Bacillus spp*, *Streptococcus spp*, Fungi species include: *Aspergillus spp*, *Rhizopus*, *Yeast*, while nematode species include: *Pratylenchus spp* (Table 1). At the seedling stage, bacteria species were the same in the rhizosphere of pepper and garden egg, fungi species isolated include: *Aspergillus*, *Rhizopus*, and *Yeast* and *Fusarium* while isolated nematode species include: *Meloidogyne spp*, *Helicotylenchus spp*, and *Pratylenchus spp* (Table 2). At the flowering stage, bacterial, fungal and nematode species were the same with those isolated at the seedling stage (Table 3), while at the maturity stage, bacterial and fungal species were the same with those isolated at the seedling stage, and in addition to the nematode species isolated at the seedling stage, *Tylenchus spp* was isolated at the maturity stage (Table 4).

These results (Tables 1, 2, 3, and 4) showed variations in rhizosphere microbial diversity and agrees with the findings in the study by Li *et al.* (2019) who compared the diversity of phosphate-solubilizing bacteria in the aubergine rhizosphere at four developmental stages among plants grown with three different cultivation methods and found that phosphate-solubilizing bacteria were enriched during the aubergine fruiting stage, indicating that plants can selectively recruit microbes at various developmental stages depending on their current needs. Also, in agreement with findings in this study, Li *et al.* (2024) found that pepper varieties had a significant impact on the structure of rhizosphere microorganisms, which is consistent with other studies that found significant differences in rhizosphere microbial communities among different potato, tea, and alfalfa varieties (Zhang *et al.*, 2022; Qi *et al.*, 2023).

Furthermore, the bacterial and fungal isolates reported in this study (Tables 1, 2, 3, and 4) were previously found in the studies by Swift *et al.* (2021), Zahid *et al.* (2021), Darriaut *et al.* (2022), Marasco *et al.* (2022), Qi *et al.* (2023), Li *et al.* (2024), and Zhang *et al.* (2024). According to the study by Li *et al.* (2024), the predominant bacterial phyla in the pepper rhizosphere were Proteobacteria, Acidobacteria, and Actinobacteria. This was in line with earlier research on maize (Kong *et al.*, 2019; Li *et al.*, 2022) and soils cultivated with Vaccinium (Yurgel *et al.*, 2017; Li *et al.*, 2020; Ramirez-Villacis *et al.*, 2023). These bacterial species according to Zhang *et al.* (2024), are essential for elemental cycling and organic matter decomposition, and they also help plants become more resilient to stress and fight off soil-borne diseases. In the study conducted by Onyia *et al.*

(2015), the fungi *Aspergillus niger* and *Penicillium spp.* were isolated from the rhizosphere of pepper and garden-egg plants while the fungal community was primarily composed of Ascomycota in previous studies by Swift *et al.* (2021), Zahid *et al.* (2021), Darriaut *et al.* (2022), Marasco *et al.* (2022), Qi *et al.* (2023), and Li *et al.* (2024). These results were expected because Proteobacteria members are well adapted to plant rhizosphere across diverse plant species (Yang *et al.*, 2023), and Acidobacteria is one of the most abundant bacterial phyla in the rhizosphere soil (Cheng *et al.*, 2020).

Additionally, in this study, variations in percentage occurrence was reported for the different bacterial, fungal, and nematode species. Highest percentage occurrence include: *Bacillus spp* (60%) in the rhizosphere of pepper during maturity stage, *Staphylococcus spp* (38%) in the rhizosphere of pepper during seedling stage, *Streptococcus spp* (15%) in the rhizosphere of garden egg during seedling stage, *Lactobacillus* (5%) in the rhizosphere of garden egg during flowering stage (Table 5), *Rhizopus spp* (58%) in the rhizosphere of garden egg during maturity stage, *Aspergillus spp* (44%) in the rhizosphere of pepper during seedling stage, *Yeast spp* (10%) in the rhizosphere of pepper and garden egg during seedling stage, *Fusarium spp* (18%) in the rhizosphere of garden egg during seedling stage, and *Mucor spp* (8%) in the rhizosphere of pepper during flowering stage (Table 6), *Meloidogyne spp* (40%) in the rhizosphere of garden egg during seedling stage, *Pratylenchus spp* occurrence was highest (67%) in the rhizosphere of garden egg during flowering stage, *Helicotylenchus spp* (67%) in the rhizosphere of pepper during seedling stage, and *Tylenchus spp* (33%) in the rhizosphere of garden egg during maturity stage (Table 7). These variations clearly demonstrated microbial diversity at various stages of pepper and garden egg development and can be explored in intercropping system to boost productivity and sustainable agriculture.

Finally, apart from the impacts of different plant developmental phases on microbial diversity, microbial organic fertilizer (MOF) have been found to increase microbial biomass and microbial diversity (Dasgupta *et al.*, 2021; Singh *et al.*, 2021; Bamdad *et al.*, 2022; Zhang *et al.*, 2024). Also, microbial inoculant (MI) have been reported to increase soil quality, fertility, and

rhizosphere microbial equilibrium (Gong *et al.*, 2018; Zhang *et al.*, 2024), MI can decrease pathogenic fungal invasion by increasing the number of possibly advantageous bacteria and fungi in the rhizosphere soil of continuously planted peanuts (Ahsan *et al.*, 2023), and Quicklime (Q) was discovered to prevent the growth of harmful bacteria and rearrange soil microbial communities (Yang *et al.*, 2024). These factors either as single or interactive factors may be investigated further in subsequent studies.

CONCLUSION

Pepper and garden egg promoted bacterial, fungal and nematode population and diversity as there were variations in percentage occurrence of the various microorganisms in response to plant growth and development, indicating that plant growth stages influence rhizosphere microbial diversity. This microbial diversity was obvious between seedling and matured stages and may reflect changes in the root exudates pattern of young plants to that of matured plants. These variations in microbial diversity at various stages of pepper and garden egg development can be explored in intercropping system to boost productivity and sustainable agriculture.

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