



## Developing Practical Strategies for Long-Term Storage of Compost Tea: The Role of Molybdate and Nitrate in Maintaining Microbial Activity and Plant Benefits



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**T**HIS study aimed to get a simple and reliable method to enhance the shelf life of compost tea. Freshly prepared compost tea was dispensed into transparent 1.5 L plastic bottles, enabling visual monitoring, with each bottle containing 1.3 L of compost tea. The treatments included T1 (3 mM sodium nitrate), T2 (6 mM sodium nitrate), T3 (0.2 mM sodium molybdate), T4 (0.4 mM sodium molybdate), and T5 (control without additives). The experiment was conducted in triplicate, and the bottles were stored in darkness at room temperature. Samples were withdrawn from each bottle at intervals of 2, 5, 10, 15, 25, and 30 days for analysis. At the end of the storage period, compost tea preparations underwent FT-IR analysis, revealing critical molecular features. A controlled pot experiment was conducted to evaluate the *in vivo* efficacy of the stored compost tea by assessing its impact on the growth and developmental progress of *Corchorus Olitorius* plants. Additionally, the enumeration of distinct microbial communities within the rhizosphere of the growing plants was performed. After 30 days, the most effective treatments compared to the control (T5) were T2 with increased bacterial Colony Forming Unit (CFU) (7.21) and Total Fungal colony (4.23), while T1 and T2 showed higher *Azotobacter* counts (4.95 and 5.00) respectively. However, T5 consistently displayed the lowest counts for Spore Formers Bacterial Unit (3.84). After extended storage, a control treatment exhibited new peaks at 1792 cm<sup>-1</sup> and 1175 cm<sup>-1</sup> suggested chemical changes, potentially carbonyl-containing compounds. These findings may have significant implications for sustainable agricultural practices and the utilization of compost tea as an environmentally friendly soil amendment.

**Keywords:** Molybdate, Nitrate, *Corchorus olitorius*, FT-IR.

### 1. Introduction

Compost tea has emerged as a sustainable and effective organic fertilizer and disease-management tool in agriculture (De Corato, 2020; Elbaalawy et al., 2020; Abou Hussien et al., 2021; Hegazi et al., 2023; Nada et al., 2023; Hafez et al., 2024). This nutrient-rich liquid, derived from the leachate of compost, contains a diverse array of beneficial microorganisms, enzymes, and plant growth-promoting compounds (Arancon et al., 2020; Khattiyaphutthimet et al., 2020). Its popularity stems from its ability to improve soil health, enhance plant growth, and suppress pathogenic organisms (Kim et al., 2015; Fouda & Ali, 2016; Sabagh, 2016). However, one persistent challenge associated with compost tea is its limited shelf life,

which hampers its widespread application and commercial viability (Debnath et al., 2019).

The shelf life of compost tea refers to the duration during which the product retains its efficacy and biological activity. Typically, compost tea experiences a rapid deterioration in quality and viability, primarily due to microbial activity, aeration limitations, and nutrient depletion (Islam et al., 2016). As a result, farmers and growers often face difficulties in preserving the integrity and effectiveness of the compost tea they produce.

In recent years, researchers and practitioners have focused on exploring strategies to extend the shelf life of compost tea. Among these, the addition of specific additives has shown promising results (Pane et al., 2012; Hegazi & Algharib, 2014). In

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Received: 28/03/2024; Accepted: 26/04/2024

DOI: 10.21608/EJSS.2024.279867.1742

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this article, we delve into the potential of molybdate and nitrate as additives to improve the shelf life of compost tea.

Molybdate, a compound containing molybdenum, has been known for its role in plant nutrition and growth regulation (Elattar et al., 2022). Additionally, it exhibits antimicrobial properties, making it a potential candidate for inhibiting the growth of spoilage organisms in compost tea (Mardare et al., 2016; Moura et al., 2019). Nitrate, on the other hand, acts as a supplemental nutrient source and electron acceptor for beneficial microorganisms, potentially enhancing their survival and activity during storage (Xu et al., 2014).

H<sub>2</sub>S production is primarily mediated by sulfate-reducing bacteria (SRB) during anaerobic conditions. SRB utilize sulfate as an electron acceptor, producing H<sub>2</sub>S as a metabolic byproduct (Qigen et al., 2018). The addition of molybdate, a compound containing molybdenum, has shown promise in inhibiting H<sub>2</sub>S production by interfering with the enzymatic processes involved in sulfate reduction (Barajas et al., 2011; De Jesus et al., 2015).

Molybdate acts as a competitive inhibitor by binding to the active site of enzymes involved in sulfate reduction, such as sulfite reductase and nitrate reductase. By blocking these enzymatic pathways, molybdate effectively reduces the availability of sulfate as an electron acceptor, impeding the metabolic pathway that leads to H<sub>2</sub>S production. Furthermore, molybdate also stimulates the growth of alternative microorganisms that do not produce H<sub>2</sub>S, thus shifting the microbial community composition towards less sulfur-reducing species (de Jesus et al., 2015; Stoeva & Coates, 2019).

The potential of molybdate as a preventive agent against H<sub>2</sub>S production offers a promising solution to address these challenges. By incorporating molybdate into composting processes or industrial systems, it is possible to limit H<sub>2</sub>S production, mitigate health risks, minimize odorous emissions, and protect infrastructure (Pudi et al., 2022).

In closed microbial systems, such as anaerobic environments or oxygen-depleted conditions, the availability of oxygen is limited, which can hamper the growth and metabolic activities of microorganisms. However, nitrate (NO<sub>3</sub><sup>-</sup>) has been recognized as an alternative electron acceptor that can serve as a substitute for oxygen in microbial respiration (Song et al., 2023). Nitrate respiration occurs when certain microorganisms utilize nitrate as a terminal electron acceptor during anaerobic respiration (Kartal et al., 2012; Park et al., 2020). The enzymatic reduction of nitrate to nitrite (NO<sub>2</sub><sup>-</sup>) and further to nitrogen gas (N<sub>2</sub>) provides a source of oxygen-independent respiration (Thamdrup, 2012; Kamp et al., 2015). By utilizing nitrate,

microorganisms can maintain their energy metabolism and sustain their growth even in oxygen-depleted environments. This ability of microorganisms to use nitrate as an alternative source of oxygen not only contributes to their survival but also affects various biogeochemical processes and nutrient cycling in ecosystems (Currie et al., 2017; Hicks et al., 2018). Understanding the role of nitrate as an electron acceptor in closed microbial systems can shed light on the metabolic flexibility of microorganisms and their adaptation strategies to diverse environmental conditions.

Molybdate holds significant promise as a preventative agent against H<sub>2</sub>S production in both agricultural and industrial settings. It achieves this by inhibiting sulfate-reducing bacteria and promoting the growth of alternative microorganisms (Pudi et al., 2022).

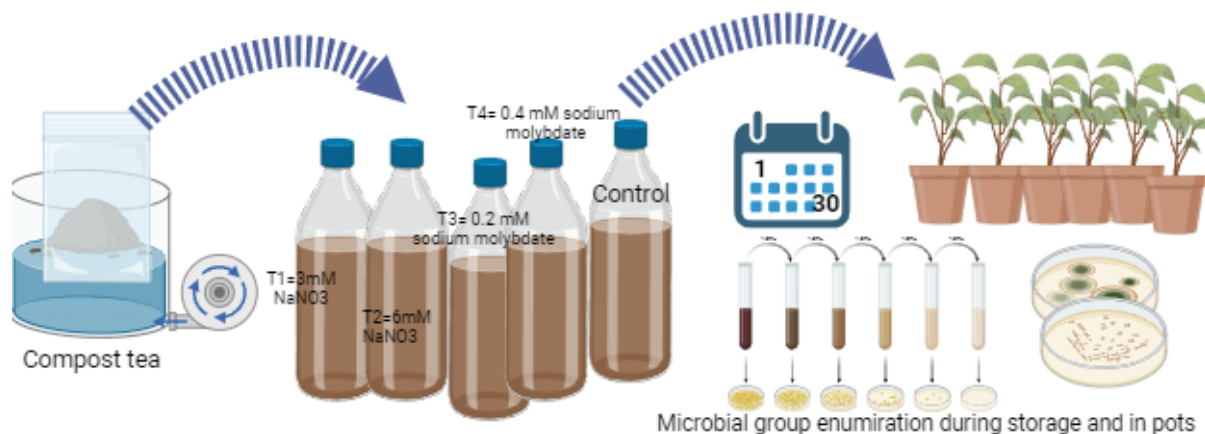
The objective of this article is to review recent studies and findings that explore the effects of molybdate and nitrate addition on compost tea shelf life. By understanding the potential of molybdate and nitrate as shelf life-enhancing agents, we can develop strategies that improve the long-term effectiveness and application of compost tea in agriculture.

## 2. Materials and methods

### *Compost and extraction of compost tea*

Composts were produced in the composting facility of the Agricultural Microbiological Research Department, Soils Water and Environment Research Institute, Sakha Agriculture Research station, Egypt. Composts were formed mainly from rice straw and the initial C: N ratio was adjusted at 25 by cow manure. Composting lasted for 100 days including thermophilic and mesophilic phases and a final curing period. The mature compost was air-dried, milled, and sieved at 2 mm and stored at 4 °C for the subsequent analysis. The mature compost has the following composition: 50% organic matter. Moisture content 50%. Nutrient-wise, it contains 2% nitrogen, 0.5% phosphorus, and 0.7% potassium. The pH of the compost is slightly acidic at 6.5. The electrical conductivity is 1.7 dS/m. Additionally, the carbon to nitrogen (C/N) ratio is 16:1.

To obtain compost tea, 1000 g of compost was weighed into a gauze bag and then placed in a plastic container containing 10 L of distilled water (w/v 1/10). The extraction of compost tea was achieved by actively insufflating air into the compost-containing gauze bag at regular intervals (5 min every 3 h) with an automatic aeration pump device for 3 days. The final product possessed the following composition: Compost tea brewed from the previous compost has the following composition: (N 0.05%, P 0.01%, K 0.03%). The pH of the tea 7.1, EC 9.0 dS/m.



**Fig. 1. Illustrates a comprehensive graphical flowchart detailing the sequential steps undertaken throughout the experiment.**

#### ***Storage experiment***

Freshly prepared compost tea was distributed in 1.5 L capacity transparent plastic bottles to permit the observation of the contents, each bottle received 1.3 L compost tea. The treatments were T1= 3mM sodium nitrate, T2= 6mM sodium nitrate, T3= 0.2 mM sodium molybdate, T4=0.4mM sodium molybdate, T5= control without additives. The experiment was conducted in triplicates. The bottles were stored in the dark at room temperature and samples from each bottle were withdrawn after 2, 5, 10, 15, 25, and 30 days respectively for analysis.

#### ***Pot experiment***

A controlled pot experiment was undertaken to assess the *in vivo* efficacy of stored compost tea by examining its impact on the growth and developmental progression of *Corchorus olitorius* plants. Furthermore, the enumeration of distinct microbial cohorts within the rhizosphere of the burgeoning plants was performed. Each plastic pot, with a capacity of 0.75 kg, was laden with 0.5 kg of previously sterilized fertile clayey soil sourced from the Agricultural Microbiological Research Department's Garden at the Soils, Water and Environment Research Institute, Sakha Agriculture Research Station. Sterilization of the soil material involved subjecting it to autoclaving at 121°C for 60 minutes, a procedure iterated thrice over 5 days to ensure thorough and complete sterilization. To each pot, 0.2 g of *Corchorus olitorius* seeds and 10 ml of one of the five compost tea treatments (T1:T5) were administered. This was augmented by two control treatments: freshly prepared compost tea and water (T6, T7) respectively. Adequate irrigation was maintained using tap water. After a cultivation period of 20 days, the plants were uprooted for the assessment of vegetative parameters. Simultaneously, the rhizosphere region associated with each treatment was isolated for subsequent microbiological scrutiny.

#### ***Microbiological enumeration in stored compost tea treatment and plant rhizosphere***

For the quantification of the overall microbial population, soil samples originating from the rhizosphere were meticulously gathered. Enumeration of total fungi and bacteria was achieved through the implementation of the plate count technique, using potato dextrose agar medium (PDA) for fungi and nutrient agar medium (Difco Agar Medium, 2009) for bacteria. The identification and enumeration of free-living nitrogen-fixing microorganisms, and phosphate-solubilizing bacteria, involved a systematic process of serial dilution coupled with standard counting methods. Specifically, media tailored to their respective nutritional needs were utilized: N-free media for free-living nitrogen fixers, N-deficient medium according to Day and Döbereiner (1976) for *Azospirillum* spp., Modified Ashby's broth medium for *Azotobacter* spp. as outlined by Abd-El-Malek and Ishac (1968). To ascertain the count of spore formers, the dilutions underwent pasteurization for 15 minutes, at a temperature of 80°C, before being planted. The spore formers bacteria were introduced onto plates and subjected to incubation at a temperature of 30°C, for 7 days, employing a nutrient agar medium and Pikovskaya's agar medium for phosphate solubilizers following the method by Pikovskaya (1984).

#### ***Chemical analytical methods***

pH and electrical conductivity (EC) values for the compost's tea preparations were measured using JENWAY 3510 pH Meter and JENWAY 4510 Conductivity Meter. Sulfide concentration was assayed as described by Baird and Bridgewater, 2017.

#### ***FT-IR analysis***

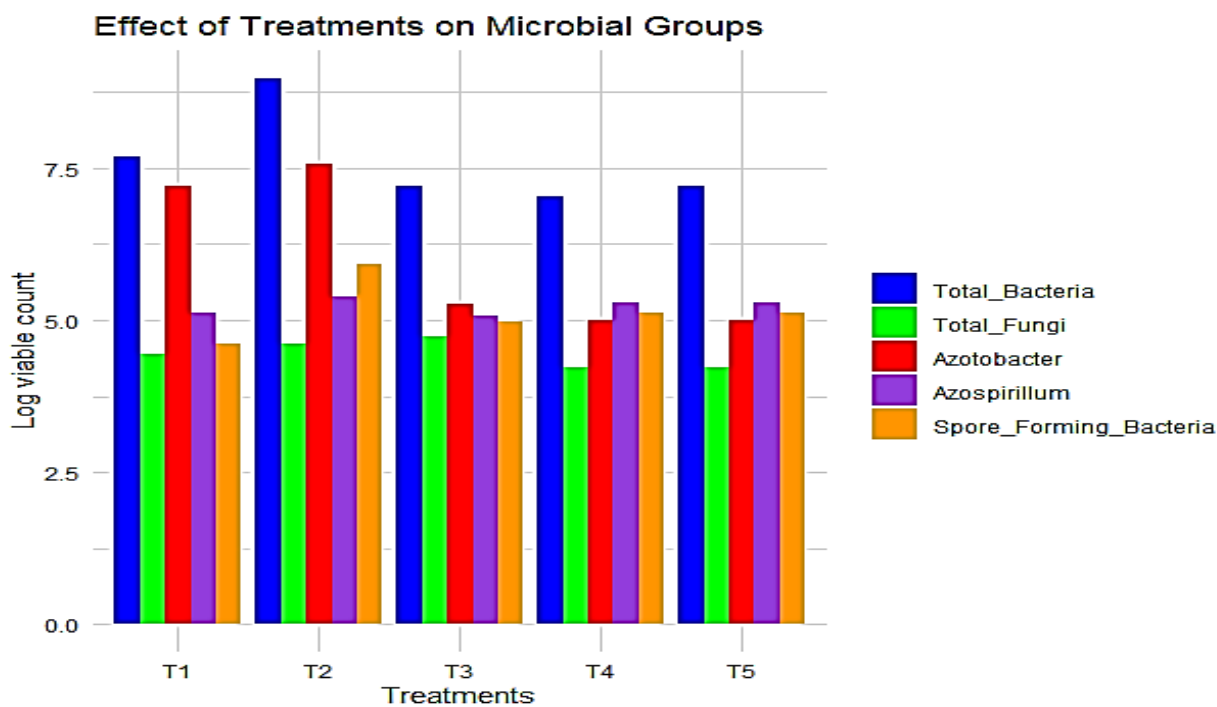
At the end of storage time, the compost tea preparations were lyophilized and subjected to Fourier Transform Infrared (FT-IR) analysis using

a JASCO FT/IR-6800typeA spectrometer (Serial Number: A005261794) with standard settings, including a TGS detector, 32 accumulations, 2  $\text{cm}^{-1}$  resolution, cosine apodization, and automatic gain and aperture adjustments. The sample, identified as "Sample name," was subjected to FT-IR spectroscopy in the wavenumber range of 399.675  $\text{cm}^{-1}$  to 4000.12  $\text{cm}^{-1}$  with a data interval of 0.482117  $\text{cm}^{-1}$ , resulting in a dataset comprising 7469 data points. Data analysis involved peak identification, spectral subtraction, and relevant processing steps, with the extended information section providing additional sample details and instrument parameters. The obtained FT-IR spectrum was then interpreted in the context of our research objectives, and data quality was assessed for accuracy and reliability. This comprehensive FT-IR methodology allowed for the characterization and elucidation of critical spectral

features, contributing to the outcomes presented in this manuscript.

### 3. Results

The findings depicted in Figure (2) illustrate the average counts of the microbial groups under investigation. The survival and dynamic fluctuations in these counts serve as pivotal indicators of the efficacy of the applied treatments. It is reasonable to observe that the total bacterial count demonstrated the highest numerical value among the various groups, while total fungi exhibited the lowest count. Notably, treatment T2, characterized by the addition of 6mM sodium nitrate, yielded the highest viable count across all targeted microbial groups. This observation underscores the significance of sodium nitrate in influencing microbial community dynamics and emphasizes its potential role in enhancing treatment effectiveness.



**Fig. 2. Mean of viable count of each microbial group during the storage period (30 days).**

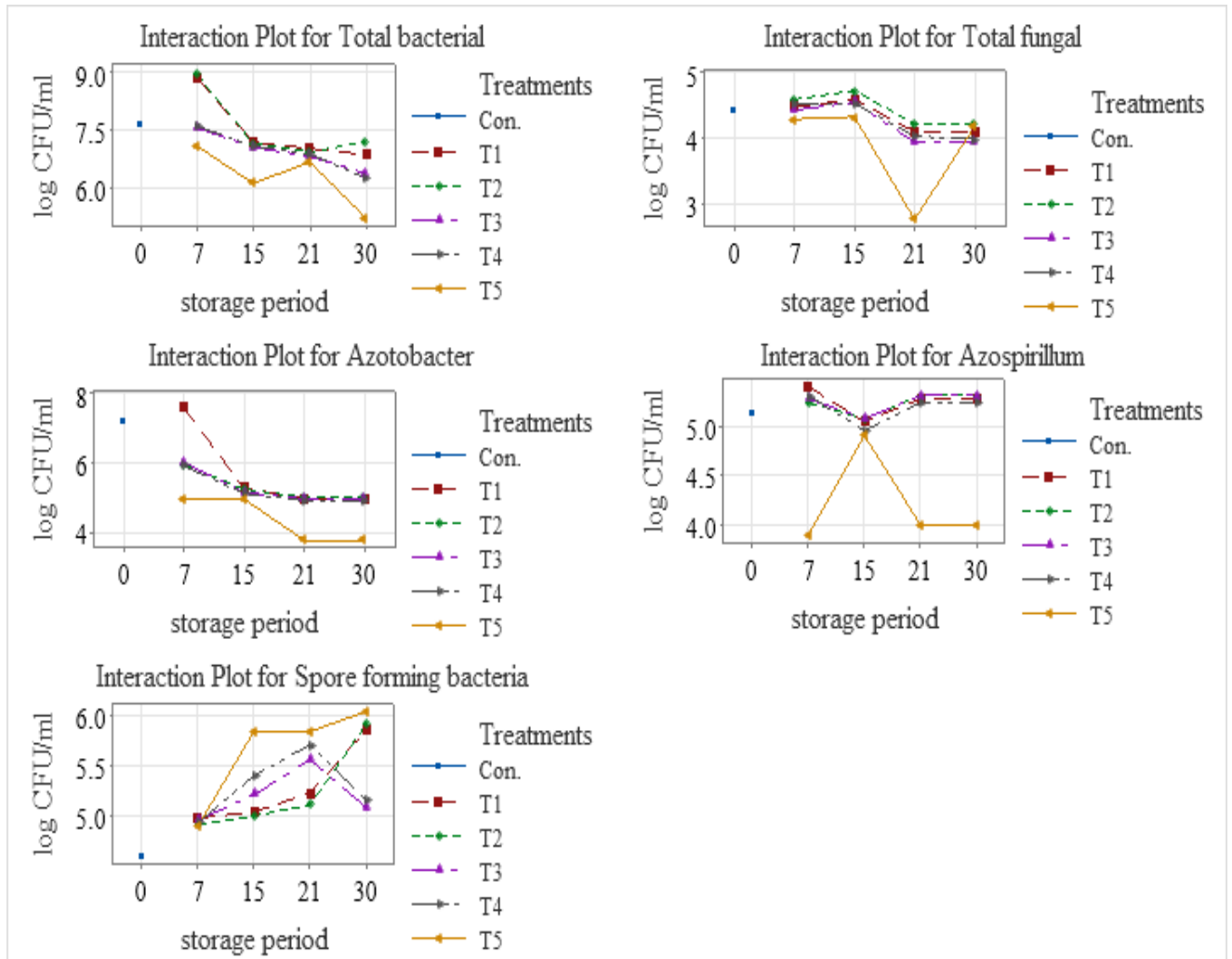
In this study, we aimed to investigate the dynamics of various microbial groups over a period (Zero time, 7 days, 15 days, 21 days, and 30 days) under different treatment conditions (T1 to T5). The microbial groups studied included Total Bacteria, Total Fungi, *Azotobacter*, *Azospirillum*, and Spore Formers Bacteria. The data showed distinct patterns of microbial abundance throughout the experiment. At the initial "Zero time" point, all treatments (T1 to T5) exhibited similar microbial counts across the studied groups, with values approximately around 7.69 for Total Bacteria, 4.45 for Total Fungi, 7.21 for *Azotobacter*, 5.13 for *Azospirillum*, and 4.6 for Spore Formers Bacteria.

As the experiment progressed, significant variations emerged in response to the different treatments. For instance, after 7 days, treatment T2 showed a notable increase in Total Bacteria and Fungi counts compared to other treatments. However, there was a decrease in the viability of Spore Formers Bacteria in all treatments., with the most substantial increase observed in T5.

Moving to the 15-day time point, T1 exhibited a slight increase in *Azotobacter* counts, while T2 displayed elevated Total Fungi counts. Meanwhile, Spore Formers Bacteria counts decreased in all treatments, with the highest counts in T5.

The trends continued into the subsequent time points, with distinct variations in microbial counts observed among the treatments. For instance, at the 21-day time point, T2 had higher *Azotobacter* counts compared to other treatments, while T5 displayed the lowest counts. Additionally, T5

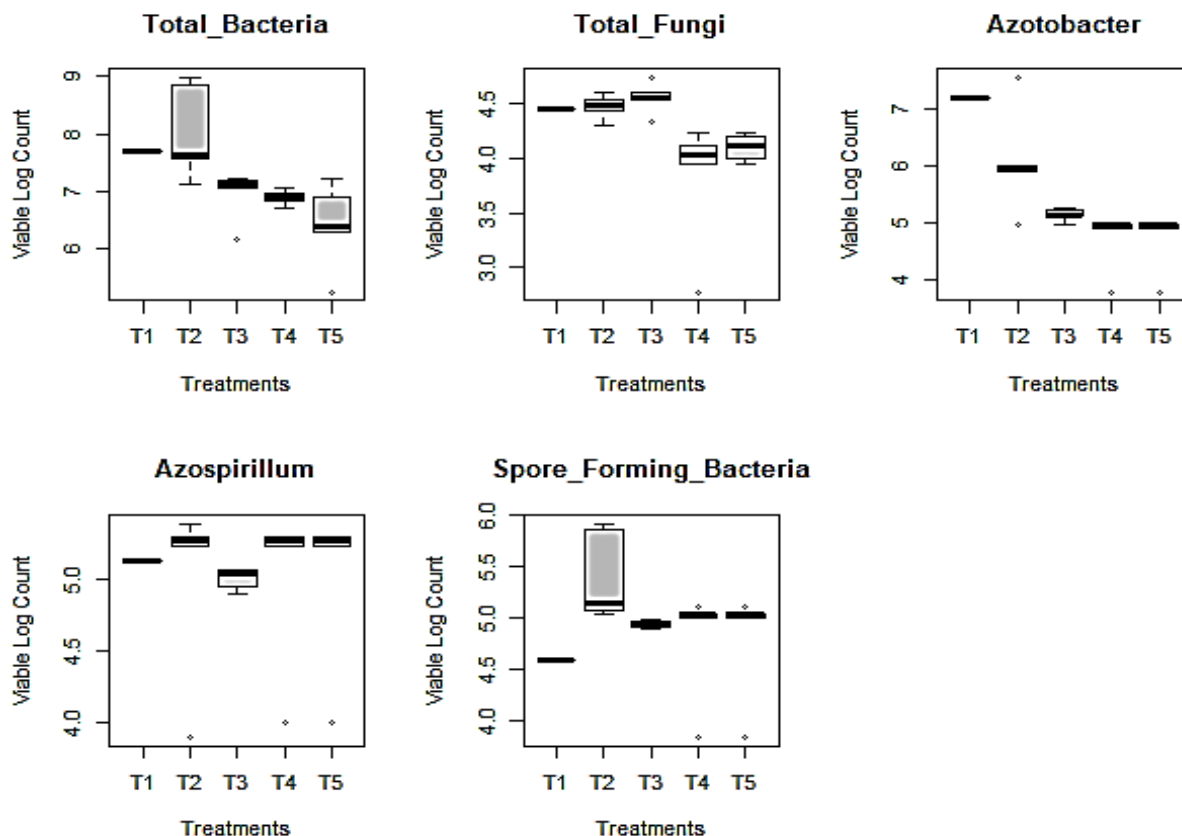
consistently had the highest counts of Spore Formers Bacteria throughout the experiment. Overall, the data illustrates the dynamic nature of microbial populations in response to different treatments and over time Fig (3).



**Fig. 3.** the interaction plot between enumerated microbial species and storage period.

The microbial dynamics over time, with different treatments (T1 to T5), were assessed for their impact on various microbial groups. Analysis of variance (ANOVA) was conducted to investigate treatment effects on microbial groups under study, revealing significant differences among treatments (Total Bacteria:  $F = 7.317$ ,  $p = 0.000843$ ; Total Fungi:  $F = 6.001$ ,  $p = 0.00243$ ). These findings indicate that the treatments significantly influenced the abundance of Total Bacteria and Total Fungi. Similarly, ANOVA results for *Azotobacter* demonstrated a highly significant treatment effect

( $F = 19.72$ ,  $p < 0.001$ ), highlighting the treatments' substantial impact on *Azotobacter* populations. Conversely, *Azospirillum* was not significantly affected by treatments ( $F = 0.063$ ,  $p = 0.992$ ). The population of Spore Formers Bacteria displayed a moderate treatment effect ( $F = 2.946$ ,  $p = 0.0458$ ). Collectively, these results underscore the significance of treatment conditions in shaping microbial populations, emphasizing the need for further post-hoc analyses to elucidate specific treatment differences (Fig. 3).



**Fig. 4.** Analysis of variance (ANOVA) of microbial counts over time intervals for different treatments.

The slight increase in pH at the 7<sup>th</sup> day may be attributed to the release of ammonia specially in treatments received nitrate while it remained in acceptable levels in all treatments during the study. The variation in EC at the start up point of the study is attributed to the addition of sodium nitrate. These findings align with the original dataset, which includes EC and pH measurements for different treatments across time intervals. The lack of considerable variations between measurements of the same treatment during study period suggests that

the storage conditions did not induce evident variations in either EC or pH levels at any of the assessed time intervals, highlighting the stability of these measurements throughout the experimental study. These results underscore the reliability and consistency of both EC and pH values across treatments and time intervals, providing valuable insights into their stability within the experimental context table (1).

**Table 1.** Change in pH and EC of compost tea stored under different treatments during 30 days.

	After 3 days		After 7 days		After 15 days		After 21 days		After 30 days	
	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC
T1	7.2±0.10	10.5±0.13	7.96±0.05	9.51±0.08	7.09±0.07	10.53±0.09	7.54±0.11	10.7±0.1	7.61±0.11	11.3±0.15
T2	7.2±0.11	11.3±0.11	7.94±0.09	10.7±0.09	7.52±0.1	11.32±0.2	7.85±0.07	11.76±0.1	7.91±0.13	12.31±0.12
T3	7.2±0.04	8.6±0.1	7.62±0.11	8.62±0.06	7.6±0.15	8.83±0.13	7.58±0.05	9.33±0.1	7.63±0.10	9.95±0.08
T4	7.2±0.03	9.2±0.11	7.7±0.09	8.66±0.1	7.69±0.08	9.19±0.11	7.73±0.13	9.45±0.1	7.82±0.09	10.11±0.11
T5	7.2±0.1	9.1±0.1	7.9±0.10	9.17±0.1	7.68±0.1	9.09±0.11	7.66±0.10	9.37±0.1	7.75±0.11	9.65±0.11

T1= 3mM sodium nitrate, T2= 6mM sodium nitrate, T3= 0.2 mM sodium molybdate, T4=0.4mM sodium molybdate, T5= control without additives

#### **Sulfide assay in stored compost tea during storage intervals**

The sulfide assay results presented in the table hold significant importance as they serve as a crucial indicator for unfavorable changes in the stored

compost tea samples. Sulfide measurements provide valuable insights into the dynamic microbial activity and the potential development of undesirable conditions. Elevated sulfide concentrations often correlate with anaerobic



conditions and microbial metabolism, signaling potential shifts in the compost tea environment. Given the pivotal role of compost tea in agricultural practices and soil health enhancement, understanding and monitoring sulfide levels become imperative. The variations observed in sulfide concentrations under different treatments, including sodium nitrate and sodium molybdate interventions, offer a comprehensive assessment of their efficacy in mitigating sulfide accumulation, thereby contributing to informed strategies for optimizing compost tea quality and stability.

The presented table delineates the outcomes of a sulfide assay conducted on stored compost tea samples subjected to distinct treatments: T1:T5, over discrete temporal intervals (3, 7, 15, 21, and 30 days), quantifying sulfide concentrations in milligrams per liter (mg/L). Clear temporal trends

emerge, with sulfide concentrations generally escalating over the study duration. Treatment-specific variations are evident, notably with T5 (control) consistently exhibiting higher sulfide levels across all time points. Conversely, T4 (0.4mM sodium molybdate) demonstrates a comparatively mitigating effect on sulfide accumulation. This data underscores the dynamic influence of treatments on sulfide concentrations, enabling a nuanced understanding of their temporal and treatment-specific impacts on compost tea quality and stability. The inclusion of a control group is instrumental in establishing baseline levels, emphasizing the necessity of interventions, such as sodium nitrate and sodium molybdate, in controlling sulfide accumulation.

**Table 2. Sulphide concentration mg/l in stored compost tea under different treatments (T1:T5).**

Treatments	After 3 days	After 7days	After 15 days	After 21 days	After 30days
T1	2±0.03	3.5±0.02	7±0.08	9±0.1	11±0.14
T2	1.3±0.03	2.7±0.02	5±0.02	7.4±0.07	9±0.11
T3	0.9±0.02	1.1±0.01	1.8±0.03	1.8±0.02	2.3±0.01
T4	0.6±0.01	0.9±0.01	1.3±0.04	1.3±0.01	1.9±0.02
T5 (control)	3.5±0.04	5.2±0.04	8.1±0.04	11.3±0.12	13.6±0.12
LSD 0.01	0.26**	0.25**	0.33**	0.35**	0.32**

T1= 3mM sodium nitrate, T2= 6mM sodium nitrate, T3= 0.2 mM sodium molybdate, T4=0.4mM sodium molybdate, T5= Control without additives.

### **Pot experiment**

The presented results in table (3) reveals the log-transformed bacterial counts obtained from a controlled pot experiment assessing the in vivo efficacy of stored compost tea on the growth of *Corchorus olitorius* plants and the enumeration of distinct microbial cohorts within the rhizosphere. The Total Bacterial Count (TBC), Total Fungal Count (TFC), and specific microbial groups, including *Azotobacter*, *Azospirillum*, and Phosphorus Solubilizers, were quantified for each treatment. Noteworthy trends emerge in the numerical data: Treatment T1 exhibits the highest log values for TBC, TFC, and most microbial groups, indicating a substantial microbial presence in the rhizosphere. Comparatively, T7 (water control) displays lower log values, particularly for P solubilizers. Percentagewise, T1 demonstrates a higher TBC count by approximately 31.7% compared to T5. Additionally, T6 (freshly concocted compost tea) shows a TBC increase of around 20.5% compared to T2. These percentage differentials underscore the treatment-specific effects on microbial abundance, providing a more nuanced understanding of the observed trends in both plant growth and rhizospheric microbial

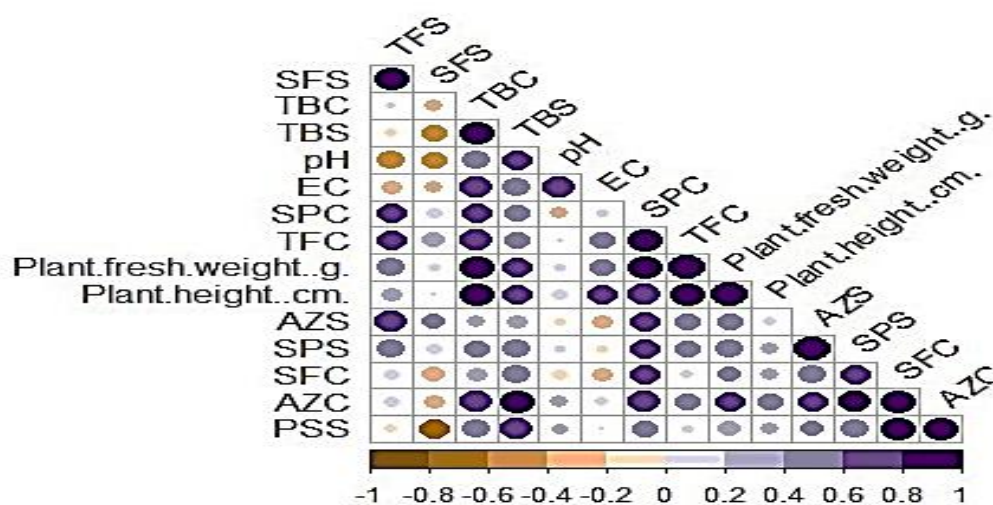
dynamics. The plant fresh weight and height data exhibit notable variations among the different treatments. Treatment T2 stands out with the highest values, boasting a plant fresh weight of 45g and a height of 23.5 cm, indicating a positive influence on both plant biomass and stature. In contrast, T5, the control treatment without additives, displays the lowest plant fresh weight (32 g) and height (17 cm), suggesting a potential growth-promoting effect of the applied treatments, particularly T2, on the *Corchorus olitorius* plants. These findings underscore the significance of the treatments in influencing the growth parameters of the plants.

Pearson correlation analysis unveiled a plethora of significant correlations among the assessed traits, illustrated in Figure (5). These correlations, whether positive or negative, denote statistical significance. Particularly striking are the robust positive correlations observed between the total bacterial counts in compost tea and soil, as well as various growth parameters. Conversely, a negative correlation is evident between total bacterial counts and spore-formers bacteria.

**Table 3. *In vivo* Assessment of stored compost tea treatments in a pot experiment cultivated by *Corchorus olitorius* plants. Values of microbial count represented by log number per gram soil.**

Treatments	TBC	TFC	<i>Azotobacter</i>	<i>Azospirillum</i>	Spore formers	P solubilizers	Plant fresh weight (g)	Plant height (cm)
T1	8.09	5.76	3.98	6.54	6.54	3.75	43	21
T2	7.24	4.82	4.13	5.06	4.94	3.19	45	23.5
T3	6.28	4.84	4.07	5.07	4.92	3.41	40	20.3
T4	6.24	5.18	4.37	5.28	5.16	3.22	41	19.8
T5	6.11	4.56	4.06	5.02	4.82	3.1	32	17
T6	8.4	6.24	4.43	5.59	5.14	3.75	44	22.8
T7	6.08	4.57	3.8	4.68	4.59	3	32	16.3
LSD 0.01	0.045**	0.055**	0.029**	0.035**	0.04**	0.14**	0.21**	0.17**

T1= 3mM sodium nitrate, T2= 6mM sodium nitrate, T3= 0.2 mM sodium molybdate, T4=0.4mM sodium molybdate, T5= compost tea without additives, T6= fresh compost tea, T7= water

**Fig. 5. Pearson correlation analysis among the assessed traits.****IR spectroscopy**

The infrared (IR) spectrum of compost tea displays several characteristic peaks at specific wavenumbers. The peak at 3392  $\text{cm}^{-1}$  is associated with O-H stretching, suggesting the presence of hydroxyl groups in organic compounds. Two peaks at 2915  $\text{cm}^{-1}$  and 2846  $\text{cm}^{-1}$  correspond to C-H stretching in aliphatic compounds, indicating the presence of hydrocarbons. The peak at 1625  $\text{cm}^{-1}$  is indicative of C=O stretching, potentially pointing to carbonyl-containing compounds like ketones or aldehydes. The 1398  $\text{cm}^{-1}$  peak could be linked to C-H bending in alkane groups or C-N stretching in amines. At 1115  $\text{cm}^{-1}$ , the spectrum reveals C-O stretching, which may be related to ethers, esters, or alcohols. The 988  $\text{cm}^{-1}$  peak suggests C-H bending in aromatic compounds, such as benzene rings. Additionally, two peaks at 777  $\text{cm}^{-1}$  and 613  $\text{cm}^{-1}$  fall in the fingerprint region, making precise identification challenging without additional context but may be related to various bending and wagging vibrations.

The appearance of new peaks in the IR analysis of compost tea after extended storage, at wavenumbers 1792  $\text{cm}^{-1}$  and 1175  $\text{cm}^{-1}$ , suggests chemical changes within the sample. The 1792  $\text{cm}^{-1}$  peak in the carbonyl region implies the presence of carbonyl-containing compounds, possibly ketones or aldehydes, indicating oxidation or degradation of organic matter during storage. The 1175  $\text{cm}^{-1}$  peak, located in the fingerprint region, is less specific but could indicate the formation of new compounds or changes in the compost tea's chemical composition during storage. Further investigations or chemical testing may be needed to identify the specific compounds associated with these peaks and assess their potential impact on the compost tea's properties and quality.

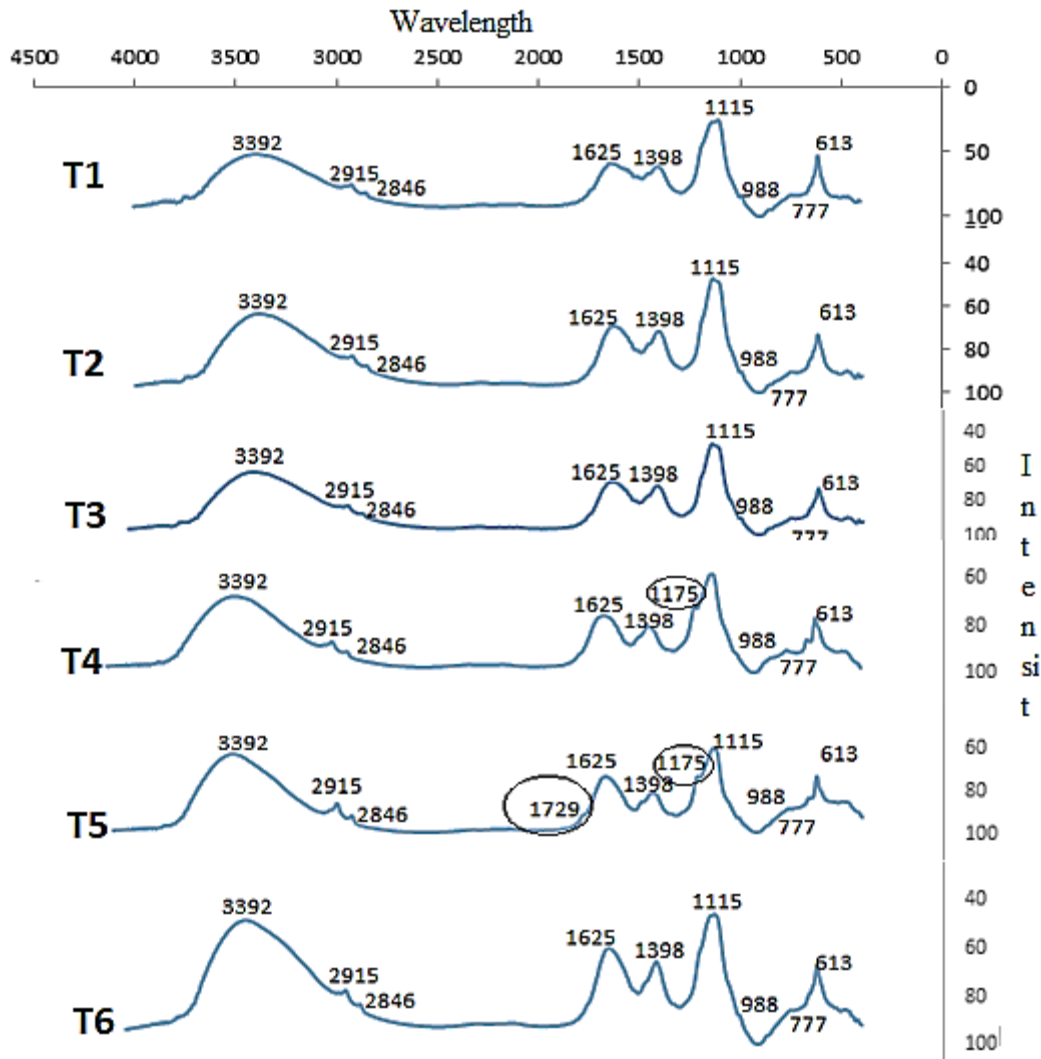
carbonyl groups can emerge after storage under anaerobic (oxygen-deprived) conditions, though the processes and compounds involved may differ from those in aerobic conditions. In anaerobic environments, organic matter can undergo various chemical transformations, including reduction reactions. Reduction reactions can convert



functional groups like carboxylic acids into carbonyl groups (e.g., ketones or aldehydes) or produce other organic compounds with carbonyl moieties.

The specific compounds and reactions involved in anaerobic conditions will depend on the composition of the organic matter and the specific

environmental conditions. The emergence of carbonyl groups in the IR spectrum after storage under anaerobic conditions may indicate the formation of these functional groups because of chemical reactions in the absence of oxygen.



**Fig. 6.** FT-IR spectroscopy for the stored compost tea after storage for 30 days. T1= 3mM sodium nitrate, T2= 6mM sodium nitrate, T3= 0.2 mM sodium molybdate, T4=0.4mM sodium molybdate, T5= compost tea without additives, T6= fresh compost tea.

#### 4. Discussion

Compost teas (CTs) are natural solutions created through the infusion of mature compost with tap water in carefully controlled settings. The utilization of these solutions presents a sustainable and environmentally friendly option as a bio-stimulant in agriculture (Isabel et al., 2022). In addition to incorporating all the soluble nutrients extracted from the compost, compost tea encompasses a diverse array of microorganisms, including bacteria, fungi, protozoa, and nematodes found in the original

compost. It is noteworthy that the final compost tea brew contains representatives of all species present in the compost, rather than every individual organism (Samet et al., 2022; Pilla et al., 2023).

Preserving the efficacy of compost tea and averting contamination or degradation of beneficial microorganisms and nutrients necessitates proper storage. The presence of aerobic organisms in compost tea is particularly advantageous, as they facilitate plant growth, minimize stress, and enhance disease resistance. Maintaining an aerobic

environment with oxygen levels surpassing 5.5 ppm is crucial for fostering this community of beneficial organisms. Conversely, anaerobic conditions during brewing, characterized by oxygen levels below 2 to 4 mg per liter, can foster the growth of detrimental microbes and lead to the production of harmful metabolites. It is important to acknowledge the marginal augmentation in electrical conductivity (EC) observed in treatments T1 and T2. This phenomenon can be attributed to the abundance of microorganisms present in these treatments, as stated by Vehniwal *et al.* (2020). The researchers suggested that the microbial and enzymatic activities could have been facilitated during the storage process, thereby contributing to the enhanced solubilization of water-soluble substances and subsequently resulting in an escalated EC.

Given that compost tea contains sulfur-containing compounds, anaerobic conditions can result in the reduction of these compounds and the generation of malodorous hydrogen sulfide (H<sub>2</sub>S) (Gemici & Wallace, 2015; Bohacz, 2019). Considering this, our current study hypothesizes that inducing anoxic conditions in compost tea during storage, either through the addition of nitrate or by inhibiting the activity of sulfur-reducing bacteria with molybdenum ions, will contribute to extending the shelf life of compost tea.

Nitrates have been observed to function as the ultimate electron acceptor when molecular oxygen is absent (Zedelius *et al.*, 2011). Consequently, they can support the survival of aerobic microbes present in compost tea. Conversely, nitrate is employed to regulate the production of hydrogen sulfide (H<sub>2</sub>S) in compounds containing sulfur under anaerobic conditions (Londry & Suflita, 1999). In the current study, the addition of nitrate to stored compost tea reflected on reduction of H<sub>2</sub>S production and the maintaining the effective number of microbial groups under study as mentioned earlier in result part. Molybdate is a structural analog of sulfate and inhibits sulfate respiration of sulphate reducing bacteria (SRB) Biswas *et al.*, (2009). The proposed mechanisms in which nitrate may hinder sulfate reduction involve nitrate ions prompting the dominant of native microorganisms. These microorganisms perform the following functions: i) they compete with sulphate reducing bacteria for the available carbon source in the medium; ii) they biologically oxidize the sulfides produced by SRB; and iii) they generate intermediates, like nitrite, which elevate the redox potential (Hubert *et al.* 2005).

The molybdate ion is commonly utilized to hinder sulfate reduction in various sectors such as wastewater treatment (Isa & Anderson 2005; De Jesus & de Andrade Lima 2016), biogas production

(Banat *et al.*, 1983), and the petroleum industry (De Jesus & de Andrade Lima 2021). Molybdate functions as a functional analogue of sulfate and, during cellular respiration, can be transported into bacteria, leading to the depletion of sulfur-reducing compounds (Patidar & Tare, 2005; Barajas *et al.*, 2011). Consequently, it serves as an ion-specific metabolic inhibitor, restricting sulfate reduction and proving toxic to these microorganisms. In general, the influence of storage duration on the chemical properties of compost tea was found to be insignificant. However, the populations of fungi and bacteria were observed to be more affected by the storage duration. This can be attributed to the decrease in populations with increasing storage duration, which may be due to competition for nutrients and oxygen among microorganisms and the release of metabolic toxic molecules. Notably, the populations of spore formers were observed to be more significantly impacted. Prolonged storage times hurt the diversity of microorganisms present in compost tea, as well as the nutrients it carries for plant utilization (Bess, 2000). The number and activity of organisms decrease significantly during storage, and while this reduction is acceptable for soil application of compost tea, it is deemed unacceptable for foliar application (Ingham, 2005). Furthermore, it was discovered by McQuilken *et al.*, (1994) that the age of compost tea affects its subsequent effectiveness against the germination and mycelial growth of *B. cinerea*.

## 5. Conclusion

In conclusion, this study introduces a novel approach to extend the shelf life of compost tea, employing additives like sodium nitrate and sodium molybdate. The findings, as demonstrated by the log-transformed bacterial counts and microbial community assessments, shed light on the potential of these treatments, particularly T1 with 3 mM sodium nitrate, to influence microbial dynamics and enhance rhizospheric diversity. The *in vivo* efficacy of stored compost tea in promoting the growth of *Corchorus olitorius* plants underscores the practical utility of this method in sustainable agriculture. The unique combination of shelf-life extension and positive impacts on plant growth and microbial diversity marks a significant contribution to the field. The outcomes not only advance our understanding of compost tea but also hold promise for its broader application as an environmentally friendly soil amendment. The importance of this work is underscored by its potential implications for sustainable agricultural practices. However, further research is warranted to refine and expand upon these results, exploring additional parameters and conditions to optimize the method's efficacy. This work sets the stage for future investigations that can build upon its findings, contributing to the ongoing

quest for innovative and sustainable agricultural solutions.

### Declarations

#### Ethics approval and consent to participate

**Consent for publication:** The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

**Availability of data and material:** Not applicable.

**Competing interests:** The authors declare that they have no conflict of interest in the publication.

**Funding:** Not applicable.

**Authors' contributions:** Authors TE, SE, FM collectively write the original draft and edit and finalize the manuscript. All authors read and agree for submission of manuscript to the journal.

**Acknowledgments:** We extend our sincere gratitude to our colleagues for their invaluable support during this study

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