Research Article

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Microbial dynamics and dehydrogenase activity in tomato (*Lycopersicon esculentum* Mill.) rhizospheres: Impacts on growth and soil health across different soil types

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Abstract: The dehydrogenase activity (DHA) in the rhizospheres of tomatoes grown in different soil types – Khor Abu-Habil (KA), Bara (B), and Greenhouse (Gr) – in North Kordofan, Sudan, was determined. In addition, the abundance of soil microbes in the tomato rhizospheres during the two growth stages, after 45 and 90 days (short and long term), was analyzed. The KA site (clay soil) showed the highest DHA (81.79 CFUs/g) followed by the B site (63.76 CFUs/g) (sandy loam) after 90 days of sowing, and the Gr site showed the lowest DHA (44.50 CFUs/g) (loamy sand soil) after 45 days. Moreover, the presence of high microbial activity (total density counts, total fungi, phosphate-solubilizing bacteria, *Streptomycetes* sp., *Azotobacter* sp., *Azospirillum* sp., and *Pseudomonas* sp. density counts) after 90 days and minimum microbial abundance after 45 days were identified at all sites. The measured growth parameters of fresh and dry weight, in addition to the root-to-shoot ratio, increased significantly at the same KA site dominated by a higher microbial density after 90 days. During the long term, the growth stage was positively affected by the abundance of adapted microbials that improve and enhance plant growth.

Keywords: dehydrogenase enzyme, phosphate solubilizing bacteria, triphenyl formazan, tomatoes

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1 Introduction

Soil enzyme activity is controlled by soil factors such as nutrient availability, soil microbial activity, and land use management procedures, all of which alter the capacity for soil enzyme-mediated substrate catalysis [1]. The dehydrogenase enzyme is one of the endocellular enzymes present in all living cells, which is essential in catalyzing the biological oxidation of organic compounds [2,3] produced by soil microorganisms, and is a natural catalyst for many important processes that occur in soil, including the formation of organic matter and decomposition of humus [4]. Soil dehydrogenase activity (DHA) is an indication of the soil's microbiological redox system and microbial oxidative activities [5]. It measures microbial activity in semiarid conditions and reflects soil respiratory activity [6,7]. As an active component of organic matter, microbial biomass participates in the transformation and accumulation of nutrients in the soil; it also serves as a good measurement of organic matter turnover and biological activity in forest and agricultural ecosystems [8], and DHA and microbe activity that are positively influenced by organic matter and organic manure [3].

DHA is one of the main components of soil enzymatic activity that participates in biogeochemical cycles and

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ensures the correct sequence of all biochemical pathways [9]; it is also one of the intracellular enzymes in the soil [10] and serves as an indicator of soil quality positively related to microbial activity and soil biomass [9,11]. Soil microbial activity is commonly used to assess disturbed soil [12].

Soil microbial communities play a key role in every ecosystem around the world, establishing feedback processes with plants that influence nutrient cycling [13] and plant growth [14]. Soil microbial activity directly influences ecosystem stability and fertility, and it is widely accepted that a good level of microbiological activity is essential to maintain soil quality and fertility [15,16] and microbial activity, increase water holding capacity, improve soil aeration, and regulate water infiltration rates, as well as to provide important macro and micronutrients [15]. Microbial communities are generally in competition in the rhizosphere of plants, which in turn can be negatively or positively influenced [17,18].

The biological oxidation of organic compounds produced by microorganisms then transfers hydrogen and electrons through a chain of intermediate electron carriers to oxygen as a final electron acceptor [9]. Free radicals and H_2O_2, or acceptors are introduced as triphenyl tetrazolium chloride (TTC), which is reduced to triphenyl formazan (TPF) [19]. This trial attempted to use TTC salt as a substrate and receptor for hydrogen electrons, which expresses microbial activity in the soil.

The most common laboratory procedures used for soil DHA determination are TTC [10] and Indo nitro tetrazolium violet [9], which can specify the flow of electrons. They are useful indicators of electron transport system activity which is carried out with the reduction of colorless water-soluble substrate (TTC) by dehydrogenase present in the soil environment, resulting in the formation of an insoluble product with red color (TPF). Redo-sensitive tetrazolium dye is reduced to insoluble formazan inside the cells as a result of respiratory activity, and then, red TPF salt is formed in microbial cells when TTC irons react with hydrogen atoms; they can be extracted from cells using an organic solvent [20].

TPF can be easily quantified calorimetrically in visible light (485 nm) [10]. The determination of DHA in soil samples provides us with a vast amount of information on the biological characteristics of the soil; it was confirmed that although oxygen and other electron acceptors can be used by dehydrogenase, most of parts of the enzyme are produced by anaerobic microorganisms. In other words, soil DHA significantly increases under anaerobic conditions [10].

Different biotic and abiotic factors, such as incubation time, temperature before incubation, soil aeration, and moisture content, have a significant effect on DHA in soil: the highest DHA was reported in forest soil [9]; considerably low activity was reported in degraded soil from most of the eroded slopes; exponential decreases in enzymatic hydrolytic activities were found in eroded soil [21]. Soil microbial activities over time are influenced by factors including pH, high levels of phenols in acidic soils and water deficit in calcareous soils [8]; changes in nutrient components, interactions, and mechanisms [13]; microbial competition [22]; and soil properties such as pH and nutrients content [23].

A novel approach to understand the underlying principles of increasingly complicated biotrophic interactions is to use bacterial and fungal isolates and their consortia to directly relate the effect to the microorganism species.

This study aimed (i) to assess the effects of microorganisms in two stages of tomato growth under natural non-treatment in different soil conditions and (ii) to identify the action of the association between DHA and soil microbials, as well as soil fertility.

2 Materials and methods

2.1 Experimental design

This study was conducted at three locations with different soils, two in the open fields area and the third site under greenhouse conditions (size of each site: 7 m × 7 m). Plants were grown in a block 7 m × 7 m, 80 cm depth filled with sandy soil, and were normally watered, and the temperature was around 20–27°C in six rows (three replicates for each stage). Soil samples were randomly taken from the plant rhizosphere for each site after 45 and 90 days from the sowing date.

2.2 Study area and sampling

Soil samples were taken from the tomato plant rhizosphere at two growth stages, after 45 and 90 days from the sowing date of the local cultivar in an open field at the Khor Abu-Habil (KA) site, 90 km south east of El Obeid (Longitude 30°38′1 E, Latitude 12°43′18 N); Bara (B) site, 57 km north east of El Obeid (Longitude 30°22′20.55″ E, Latitude 13°41′ 55.13″ N); and Greenhouse (Gr) condition site in El Obeid (Longitude 30°12′59.95″ E, Latitude 13°10′41.57″ N). All soil samples were taken from the surface layer (0–20 cm) in ice-box polyethylene bags and immediately transferred to the laboratory.

KA site is distinguished by cracks and clay soil, which is influenced by seasonal flooded water and is covered with shrubs and grass in summer; B site is characterized by silt, loamy, sand soil, ground water near to the soil surface, and good conditions for growing vegetables and citrus trees. Under Gr conditions, sand soil without any treatment was used with normal irrigation.

2.3 Measurement of DHA

The activity of the dehydrogenase enzyme was measured using the Thalmann method described by [9,20,24]. The dehydrogenase assay is based on the use of TTC to replace atmospheric O_2 with an H acceptor during oxidation.

Briefly, 2 mL of TTC solution (3%) and 0.1% $CaCO_3$, per weight 10 g of soil, were prepared in a 250 mL Erlenmeyer flask and incubated at 37°C for 24 h; it was later extracted with methanol and the contents of the flasks were filtered. The absorbance was read at 485 nm, and the corresponding concentrations were measured from the standard curve.

2.4 Standard curve

The curve was shifted according to 1.0 mg triphenyl formosan, which requires 150.35 mg of H_2. Then, 0.04 g of TPF was dissolved in 50 mL of distilled water and a set of seven solutions (eight concentrations) was prepared. A standard curve was developed from the solutions of TPF and ethyl alcohol with different concentrations. Finally, the optical densities of the prepared solutions were measured (in duplicate) using a spectrophotometer at a wavelength of 485 nm and plotted against the known concentrations of TPF (mg/L), as shown in (Figure 1). A blank sample of ethanol was used to zero the spectrophotometer.

2.5 Microbial determination

Microbial abundance was assessed through culture-based techniques, involving specific methods for different types of microorganisms. Total microbial counts were determined using nutrient agar (Difico) following the approach described by [25]. Total fungi were enumerated using rose Bengal medium as outlined by [26]. Phosphate-dissolving bacteria were identified on modified Bunt and Rovira medium [27]. *Streptomyces* sp. counts were conducted using the method detailed by [28], while *Azotobacter* sp. were identified on modified Ashby medium [29]. In addition,

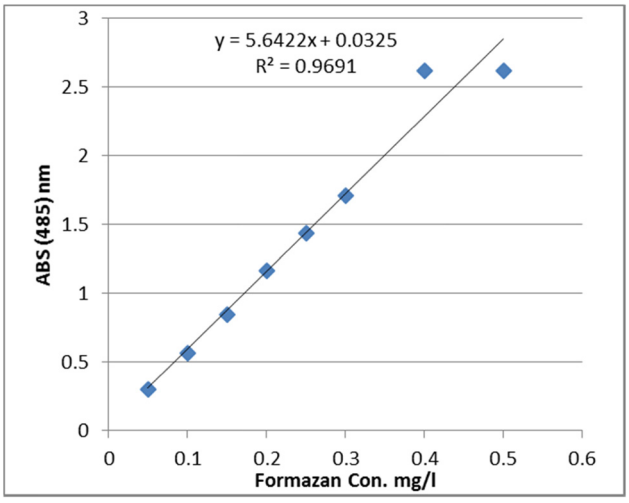

Figure 1: Standard curve.

Azospirillum sp. [29,30] and *Pseudomonas* sp. [27] were determined using their respective specified techniques.

2.6 Growth parameters

The seeds of the local cultivar, tomato (*Lycopersicon esculentum*), were grown at two open field sites with different soil characteristics, KA and B, which were irrigated via rainfall and normal watering, respectively, and a third site under greenhouse conditions (Gr) in El Obeid. All sites were (7 m × 7 m) in size. No treatments were added to the soil at all sites, leaving the plant to grow on original nutrients and organic substrates in the soil. After 45 and 90 days from the sowing date (establishing seedlings and mature stages), fresh weights of shoots (shoot Fr.wt.) and roots (roots Fr.wt.), dry weights of shoots (shoot Dr.wt.), roots (roots Dr.wt.), and roots-to-shoots ratio (RS ratio) per plant were randomly taken.

2.7 Soil analysis

Some soil analyses, such as mechanical analysis [31], total nitrogen, available phosphorus, available potassium, organic carbon, organic matter, pH, and electrical conductivity (EC), were determined according to the standard methods of Sparks et al. [32].

2.8 Statistical analysis

Data from this study were analyzed as a factorial experiment in Complete Randomized Design using the Statistix 8

program. Means were separated using the least significant difference and Tukey's test at ($p < 0.05$).

3 Results and discussion

Measurement of soil enzyme activity may provide significant information about the primary reactions that slow down SOM degradation and nutrient transformation in the soil [33]. Because of their link to soil biology and simplicity of measurement, soil enzymes are widely used to assess soil quality [34]. The measurement of soil enzyme activity is critical for understanding soil microbial activity in relation to cropping system, moisture, and nutrient levels [35]. Since all species adopt different strategies to acquire sufficient water and nutrients for vegetative development and root growth, the root/shoot (R/S) ratio has a significant impact on ecological succession, thereby making it vital to comprehend the entire plant's intricacy at the root and shoot levels. This study provides a method for assessing the biological behaviors of soil to determine the association between DHA and soil microbials, as well as soil fertility, in two stages of tomato growth, which were produced in three different sites under natural non-treatment conditions with diverse soil types.

3.1 Soil analysis

Experts across the world are becoming more interested in developing agricultural strategies that will improve soil quality. Any change in soil management or land use practices may modify the activity of soil enzymes [16]. The qualitative and quantitative content of root exudates is determined by plant species, cultivar, growth stage, and several environmental conditions such as temperature, pH, soil type, and microorganism availability [36,37]. Declining soil fertility is a major concern for agricultural sustainability, and most researchers' attention is focused on the influence of nutrients and irrigation on crop output, with only a few studies conducted to analyze their effect on soil health. The results of the soil analysis, with considerable differences in several characteristics, are shown in Table 1. The soil textures were Gr loamy sand, B sandy loam, and KA clay soil. The results obtained in our study aligned with those of Diekow et al. [38]. Grasslands, fertilizations, and high biomass production have great potential to increase the content of C and N in soil. KA soil is characterized by high values of nitrogen and organic carbon (0.21 and 0.82%, respectively) when compared to the B and Gr sites; furthermore, the contents of organic matter (1.41%) and phosphorus (27.1 mg/kg) in KA soil suggest that it is characterized by an improved soil water holding capacity and nutrients availability in plant rhizosphere [39]. Except for Gr soil, potassium (197 mg/kg) appears to have a low content at both KA and B sites, which refers to the movement and exchangeability of K in clay soil [40]. Regarding soil PH, KA is better because, generally, enzyme activities tend to increase with soil pH.

3.2 Measurement of DHA

Microbial enzymes help with both the transformation and mineralization of these nutrients. Soil or microbial enzymes are also in charge of managing soil toxicity and other pollution biotransformation processes [41,42]. These enzymes could be found either intracellularly or extracellularly in microbial cells. Soil enzymes initiate and maintain nutrient biogeochemical cycles, providing direct support for plant fertility, and healthy growth and development [43]. Dehydrogenase is the most important and vital indicator of microbial activity in soil. This enzyme is present intracellularly in all viable cells as a part of their respiratory system, playing a role in the measurement of the metabolic state of soil microbes [44]. The enzyme activity of dehydrogenase is among the most appropriate, crucial, and responsive soil fertility indicators [10]. Its activity depends on the same factors that affect the abundance and activity of microorganisms. Dehydrogenase enzyme primarily obligates anaerobic microbes in the soil, most abundantly in the genus *Pseudomonas*, particularly in *Pseudomonas entomophila* [45].

Table 1: Selected physicochemical properties of the soil samples

Soil sample	Sand, %	Silt, %	Clay, %	Texture class	Total *N*, %	Available P, mg kg^{-1}	Available K, mg kg^{-1}	O.C., %	O.M., %	pH	EC, ds/m
Gr	86.92	8.05	5.03	LS	0.0003	4.1	197	0.48	0.83	7.3	0.63
B	83.0	3.0	14	SL	0.02	14.4	140	0.72	1.12	6.5	0.2
KA	43.4	7.5	49.1	C	0.21	27.1	159	0.82	1.41	7.5	0.3

Table 2: DHA values obtained from selected soils

Soil types (days)	DHA values ABS (485) (nm)
KA 45	40.2 ± 3.05
KA 90	81.79 ± 6.81
B 45	23.4 ± 2.13
B 90	63.75 ± 5.95
Gr 45	10.5 ± 0.84
Gr 90	44.50 ± 3.76

It participates in oxidation-reduction reactions in the soil by transferring electrons from substrate to acceptors.

The results from the standard curve indicate that higher values were found at all sites after 90 days compared to 45 days; KA site recorded a higher value (81.79), followed by the B site (63.75), and the lowest values were found in Gr site (44.50) mg H_2/g after 24 h; after 45 days, all sites recorded the least values. The obtained results on soil character indicate that fertile soil and organic matter content encourage microbial activity in the plant rhizosphere (root zones) at the KA site, which is similar to that reported by [46]. Poorer soils with less DHA activity (Table 2) contain less organic matter and a low pH status, which leads to a decrease in microbial activity [47].

3.3 Microbial determination

The vast spectrum of soil microorganisms and their activities are critical to the soil's survival as well as fort biogeochemical cycles. Soil microorganisms serve as microbial indices to measure and enhance soil health [48]. Plants only take up specific kinds of nutrients from the soil's nutrient pool. Nutrients that are necessary may exist in inaccessible forms [49]. Soil organic matter is a vast reservoir of nutrients, the majority of which are in inaccessible forms. Soil microorganisms play an important role in the biotransformation of these inaccessible nutrient forms to available forms [50,51].

The KA site was distinguished by an overall high clay percentage and higher microbial activity at two tomato growth stages compared to the other sites; this is attributed to the presence of organic compounds and secretion roots (Figure 2a–c). Dehydrogenase enzymes appear to be associated with microbial activity that is involved in the initial breakdown of organic matter [9]; it is also dependent on the metabolic state of the soil or the biological activity of the microbial population [52].

The results indicate that the highest total density counts (Figure 2a) occurred at the KA site after 90 and 45 days (184.6×10^8, 120.3×10^8 CFU g^{-1}, respectively) followed by Gr after 90 days (107.3×10^8 CFU g^{-1}), while the lowest counts were recorded in Gr site after 45 days (69.3×10^8 CFU g^{-1}). High total density fungi (Figure 2b) were registered at the KA site after 90 days (120.3×10^4 CFU g^{-1}), followed by Gr after 90 days (83.6×10^4 CFU g^{-1}) and minimum density counts were found in Gr after 45 days (15×10^4 CFU g^{-1}). Figure 2c shows that greater *Streptomyces* sp. density counts were observed at the KA site during 90 and 45 days (79.6×10^3 and 67.3×10^3 CFU g^{-1}, respectively), followed by the B site after 90 days (44.3×10^3 CFU g^{-1}), and both the B and Gr sites recorded few cell counts (28×10^3 CFU g^{-1}) after 45 days. Environmental factors such as soil structure, texture, moisture, and nutrients certainly influenced the soil microbial activity and DHA. Soils with organic substrates, plant residues, and suitable soil moisture (fertile soil) appear to have high microbial activities, thus leading to their high values of dehydrogenase enzyme, which is different than non-fertile soil or poor soil.

Figure 3a shows that the highest density counts of phosphorus-solubilizing bacteria (PSB) sp. were observed at the KA site after 90 days (85.6×10^4) followed by the B site at the same stage (74.3×10^4), and the minimum density was recorded in Gr after 45 days (49×10^4 CFU g^{-1}). Maximum counts of *Azotobacter* sp. density counts were shown at the KA site after 90 and 45 days (36.6×10^3 and 35×10^3 CFU g^{-1}, respectively) and minimum bacteria counts were found in the B and Gr sites (12.3×10^3 and 11×10^3 CFU g^{-1}, respectively) after 45 days (Figure 3b). High *Azospirllum* density counts (Figure 3c) were found at the KA and B sites after 90 days (84.6×10^2 and 79.6×10^2, respectively), followed by the KA site after 45 days (66.6×10^2), and lower counts were observed in Gr after 45 days (13×10^2) CFU g^{-1}). *Pseudomonas* density counts (Figure 3d) were slightly higher in the KA site followed by the B site after 90 days (73×10 and 64×10 CFU g^{-1}, respectively) compared to the earlier growth stage in all sites.

Due to the type, structure, and character of the KA site soil, there are greater microbial populations in the two tomato growth stages (after 90 and 45 days). Microbial activity varies mainly due to root and soil types [38,53]. Soil quality negatively affected tomato growth and productivity [54]. Raji and Thangavelu [29] found that potassium-solubilizing bacteria promoted tomato growth in different soil types. The increase in microbial counts in the plant rhizosphere with the development of the plant at certain stages [55], the alleviation of biotic stresses [53], and the impact of agriculture practice in the early stages of growth [56] further improves the growth and productivity [57]. Organic substrates, grasslands, and humus positively enhanced microbial activity and their populations [58,59]; soil pH and substrate

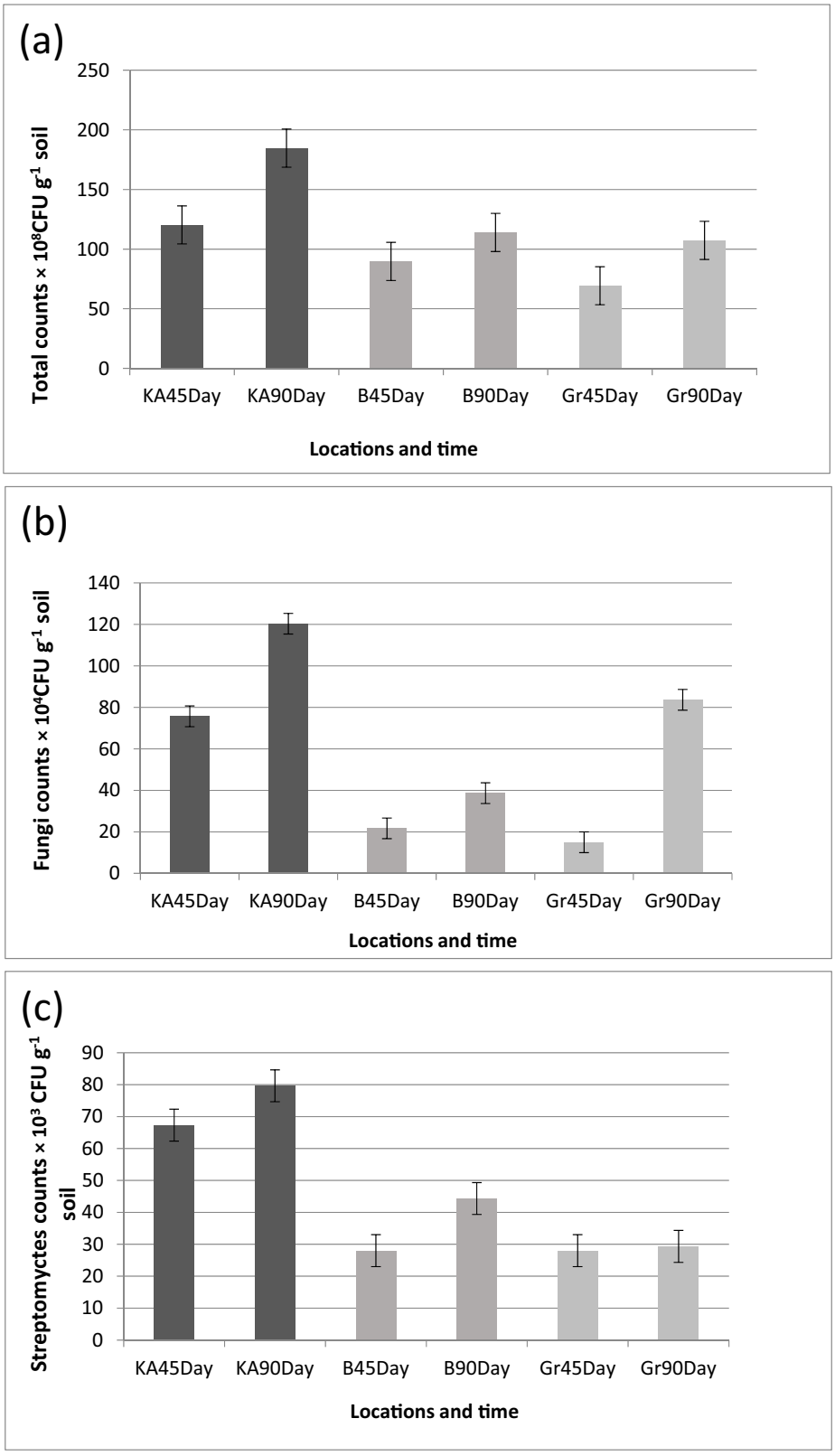

Figure 2: Microbial communities (CFU g^{-1} soil) in tomato rhizosphere. (a) Total density counts, (b) total fungi counts and (c) *Streptomycetes* density counts at three sites, (KA) Khor Abu-Habil, (B) Bara, and (Gr) greenhouse conditions in El Obeid.

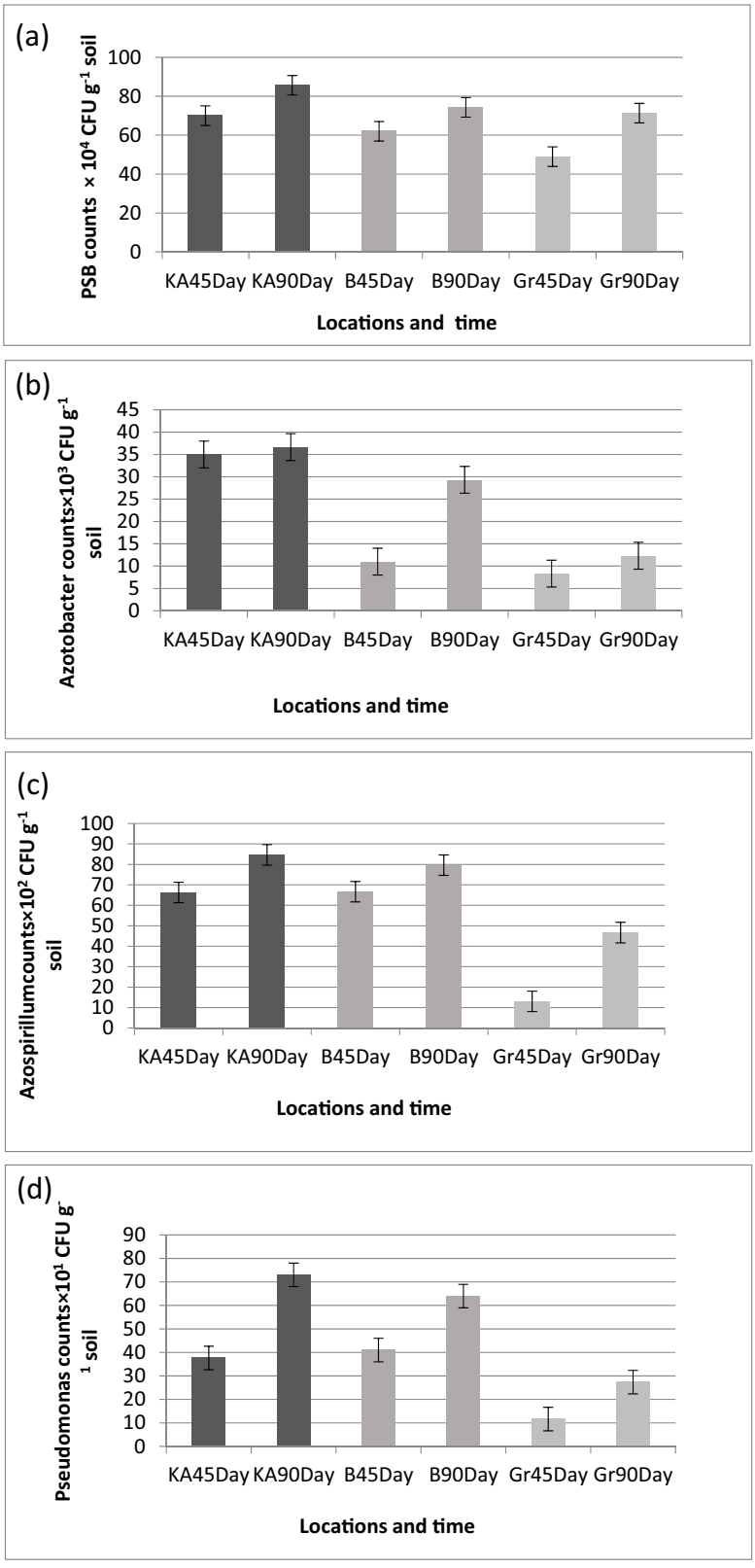

Figure 3: Microbial communities (CFU g^{-1} soil) in the tomato rhizosphere. (a) PSB density counts, (b) *Azotobacter* density counts, (c) *Azospirillum* density counts, and (d) *Pseudomonas* density counts at three sites (KA) Khor Abu-Habil, (B) Bara, and (Gr) greenhouse conditions in El Obeid.

Table 3: Influence of microbial activity on tomato's fresh and dry weights (g) and root to shoots ratio in the two stages (45 and 90 days)

Site × time	Shoots Fr.wt	Roots Fr.wt.	Shoots Dr.wt.	Roots Dr.wt	RS ratio
KA 45 days	127.33^{B}	15.333^{B}	24.367^{B}	7.8333^{B}	0.4900^{B}
KA 90 days	153.00^{A}	19.000^{A}	30.567^{A}	9.8000^{A}	0.8000^{A}
B 45 days	100.67^{C}	11.333^{C}	16.667^{D}	4.7333^{C}	0.4900^{B}
B 90 days	126.33^{B}	14.333^{B}	20.700^{C}	6.8000^{B}	0.4267^{BC}
Gr 45 days	79.000^{D}	8.0000^{D}	11.067F	3.5333^{C}	0.2267^{D}
Gr 90 days	101.67^{C}	11.667^{C}	14.100E	4.5000^{C}	0.3500^{BCD}
LSD at 0.05	4.89	2.09	2.10	1.59	0.141

$^{A, B, C, D}$ – The mean difference is significant at the ($p < 0.05$) according to LSD test.

quality [18]; and microbial communities differ across different habitat types [60].

3.4 Growth parameters

Results in Table 3 indicate significant differences ($p < 0.05$) between the measured parameters during the growth stages of the two plants (45 and 90 days) at the three sites due to different levels of microbial abundance. The abundance in the tomato rhizosphere significantly increased fresh and dry weight and RS after 90 days (mature stage) at three sites. The maximum value was observed for the fresh weight of shoots and roots at the KA site (153 and 19 g/plant, respectively), followed by KA after 45 days (127 and 15 g, respectively) and site B after 90 days (126 and 14 g, respectively), while Gr showed the minimum value for the weight of shoots and roots after 45 days (79 and 8 g/plant, respectively). The abundance positively reflects the increase in dry weight between the shoots and roots and RS ratio. The dry weight of the shoots and roots modified at the KA site after 90 days (30.6 and 9.8 g/plant, respectively) was the highest, while the lowest weight values were found after 45 days at all sites. Similarly, the KA site achieved a higher RS ratio at the mature stage (80%) and the lowest ratio was observed after 45 days at all sites. Generally, the presence of microbial abundance at the KA site positively influenced tomato growth compared to the B and Gr sites. In the long term, microbial activity had a better effect on growth than in the short term, thus enhancing the relationship with beneficial and symbiotic microorganisms. In the initial growth stage, the dominated microbes appear to have achieved a high completion of the search for a suitable host and colonized around the rhizosphere of smaller and finer roots affected by little root turnover and secretion and organic compounds. There is still time to adapt and tolerate new circumstances with high completion [61]. The microbial communities associated with organic amendment would enhance plant growth, promote better nutrient uptake and higher nutrient availability for plants [62], lead to higher microbial abundance in the plant rhizosphere in the long term than in the short term [63,64], increase agricultural productivity [65], and reduce disease severity and incidence [66]; shoot and root of tomato were positively affected by microbial biomass [67,68]. Particularly, in dominated microbes, root dry weight and RS ratio [69] and community structure [62] significantly increased. Our study shows that the abundance, communities, and activity are naturally higher in the long term (90 days) in the tomato root; thus, it reflects the positive effect that microbes have on plant growth by supplying nutrients, increasing absorption, releasing organic substrates, increasing symbiotic relationships and by providing pathogenic protection. Soil and plant microorganisms interacted and influenced one another in terms of soil application and plant productivity. This study discovered a relationship between microbial abundance and plant life stages and soil type. Seedlings and young plants have different root systems, which are less impacted by microbial abundance, compared to mature plants, wherein microbial abundance affects soil stability and plant output.

4 Conclusions

The correlation between DHA and soil microorganism abundance is influenced by various ecological factors and soil characteristics. Soils rich in organic substrates and plant residues, with minimal environmental alterations, create conditions conducive to microbial competition. Microbial abundance and DHA in the tomato rhizosphere exhibit variations depending on soil characteristics. The clay soil at the KA site demonstrated greater microbial abundance and DHA due to its favorable properties, followed by the B site. Conversely, the high percentage of poor sandy soil at the Gr site negatively influenced soil fertility. Our findings

highlight elevated DHA and microbial activity during the long-term growth stage of 90 days in the tomato rhizosphere compared to the short-term stage of 45 days. Regardless of soil types, microbial abundance and dehydrogenase enzyme activity exert a more significant impact on tomato growth during the mature stage (long term) and exhibit lower activity during the establishment stage (short term) of plant life. Our research underscores the effectiveness of microbial activity in tandem with increased plant growth over time, influenced by plant root exudates and soil characteristics. In conclusion, our study suggests that DHA, correlated with the relative abundance of microbes, can enhance tomato growth when cultivated in soils with favorable properties. Future perspectives on microbial abundance and plant growth improvement should involve refining screening techniques, such as quantifying antioxidant enzymes, and conducting tests under diverse conditions, including varying soil moisture, temperatures, nutrient levels, and pH. Furthermore, evaluating the potential of microbial enhancement for various tomato species and other vegetable plants is crucial for advancing agricultural practices.

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Conflict of interest: The authors declare no conflicts of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The data obtained in the present research are available from the corresponding author upon reasonable request.

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