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Effect of the Rhizosphere of Date Palm (Phoenix dactylifera L.)" "on Some Biological Properties of Soils in Basrah Governorate

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Abstract

This study investigated the effect of the rhizosphere of date palm (Phoenix dactylifera L.) on some biological properties of soils in four different locations in Basrah Governorate (Kteeban, Al-Tanuma, Abu Al-Khasib, and Nahr Hassan). Soil samples were collected at two depths (0–30 cm and 30–60 cm) from both rhizospheric and non-rhizospheric soils. biological indicators were analyzed, including total bacterial and fungal counts, *Azotobacter spp*., and some enzymes(urease and dehydrogenase) .

The results demonstrated a significant increase in microbial populations and enzymatic activities in rhizospheric soils, particularly in surface layers (0-30 cm) . The Kteeban site showed the highest values for microbial abundance and enzyme activity, which is attributed to leguminous cropping and organic matter inputs. Conversely, the Nahr Hassan site, which retains natural vegetation, exhibited the lowest values for same criteria .

Overall, the rhizosphere was found to be a biologically active zone that enhances soil fertility through microbial colonization and enzymatic activity. The findings highlight the potential of utilizing native *Azotobacter spp.* as a biofertilizer to support sustainable agriculture in arid and seme arid regions.

Keywords: Rhizosphere, Phoenix dactylifera, Azotobacter spp., biological soil properties, soil enzymes, Basrah soils, nitrogen fixation, microbial activity.

I. Introduction

Soil harbors Large diversity of microorganisms, including bacteria, fungi, protozoa, and algae. Among these, bacteria are plenty , and can be found freely in the soil, attached to soil particles, or interacting with plant roots (ICAR, 2019). The part of the soil that is most affected by plant roots, their exudates, and associated microbial activity is called the rhizosphere. The rhizosphere is defined as the zone of soil surrounding the root that is directly influenced by root secretions and the associated microorganisms (Bais, 2006). It represents a dynamic environment where complex interactions occur between plants, soil, and root-associated organisms (Deshmukh et al., 2017). Soil microorganisms are essential components of soil fertility, plant growth, and crop productivity. Plants release a range of root exudates that attract and stimulate the colonization of beneficial microorganisms in the rhizosphere. These microorganisms Participation in biological processes such as nutrient solubilization, suppression of plant pathogens, and enhancement of soil structure and fertility (Bais, 2006).
Among these beneficial microbes are Plant Growth-Promoting Rhizobacteria (PGPRs), which support plant development through both direct and indirect mechanisms. Direct mechanisms include nutrient mobilization (nitrogen, phosphorus, potassium, zinc), ACC deaminase activity, and the modulation of phytohormone (such as auxins, cytokinins, gibberellins, ethylene, and abscisic acid). Indirect mechanisms include biocontrol activities such as antibiotic production, secretion of lytic enzymes, hydrogen cyanide (HCN), siderophores, and antifungal exopolysaccharides (Patel & Minocheherhomji, 2018).
These microbial strategies also enhance plant tolerance to abiotic stresses such as drought, salinity, and high temperatures. As a result, various PGPR have been developed for commercial agriculture to reduce dependency on chemical fertilizers and pesticides (Baker, 2018).
Date palm (Phoenix dactylifera), a key fruit crop in arid and semi-arid regions, has played a central role in providing carbohydrates to local populations for over 5,000 years. In addition to its agricultural value, the date palm contributes

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to improving soil biological and environmental properties. Belonging to the family Arecaceae, the genus Phoenix includes approximately 15 species, with P. dactylifera being the most economically important (Abdul Wahid, 2016). Despite the importance of rhizodeposition in shaping the soil microbiome, challenges remain in quantitatively and qualitatively assessing root exudates and understanding their ecological functions (Ricani, 2018). Given that the rhizosphere is the primary site of root–microbe–soil interactions, the present study aims to quantify the total populations of bacteria, fungi, and nitrogen-fixing Azotobacter spp., along with their production of key enzymes such as dehydrogenase and urease, which are stimulated by root exudates. These microbial isolates will be characterized and assessed for their use as targeted bio-inoculants for date palm cultivation.
The outcomes of this research will contribute to a deeper understanding of the role of date palm rhizospheres in enhancing soil biological quality and support the development of sustainable soil management practices tailored for hot and arid environments.

II. Materials and methods

Soil samples close to the roots (rhizosphere) and far from the roots of date palms at depths of (0-30) (30-60) cm were brought from the study areas (Al-Tanuma, Kutaiban, Abu Al-Khaseeb and Nahr Hassan) to the laboratory, and the percentage of soil moisture was directly estimated by the gravimetric method to be used in estimating the dry weight of soil when used in laboratory experiments. Soil samples were kept frozen at -20°C until biological properties were analyzed. A portion of these samples was taken, air-dried, ground and sifted through a sieve with a diameter of 2 mm to study the primary physical and chemical properties of the studied soil.

Samples were collected from the areas below during the winter (January) of 2018. Samples were taken from the area surrounding the palm roots (rhizosphere) and far from the roots from depths of (0-30) (30-60) cm from the soil surface.

Table (1): Soil sampling locations and agricultural condition

Agricultural situation	sample
Planted vegetables	**Basra / Tanomah**
planted with leguminous and contains Organic matter	**Basra/Katiban**
Planted vegetables	**Basra/Abi Al-Khasib**
Contains natural plants	**Basra/ Hassan River**

The electrical conductivity of salts in the saturated soil paste extract was measured using an Ec-meter. The soil reaction rate was measured in a 1:1 soil:water suspension using a pH-meter. Total nitrogen was determined by soil digestion with concentrated sulfuric acid, and total nitrogen was determined by steam distillation followed by titration with hydrochloric acid. Soil organic carbon was determined using the Walkley-Black method, while soil organic matter was calculated by multiplying the organic carbon value by a conversion factor of 1.725 (Salem and Ali 2017). Soil fractions were determined by the sorbent method (Table 2).

For microbial testing, 10 grams of soil were weighed and 90 ml of sterile distilled water was added to it to make a 10-1 dilution, from which the remaining ten-fold dilutions were prepared. The pour plate method was used for all tests, as the test was conducted under sterile conditions. The number of growing microbes was expressed in CFU g-1 (Colony Forming Unit g-1) (Dioxen and Tilston, 2010). These tests included the following:

Total bacteria

Sterile Nutrient Agar culture medium was used and incubated at 30°C for 24–48 hours. The number of colonies grown was calculated by multiplying by the reciprocal of the dilution (Black, 1965; Mastora and Roy, 2008).

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Total fungi

Sterile potato dextrose agar was used as a culture medium and incubated at 25°C for 2–4 days. Colony counts were calculated by multiplying by the reciprocal of the dilution (Black, 1965; Mastora and Roy, 2008).

Azotobacter bacteria

The liquid medium (Sucrose Mineral Salts) was inoculated. 1 cm3 of the above-prepared soil dilutions was taken to inoculate test tubes containing 9 cm3 of the above-prepared specialized medium. The prepared medium was autoclaved for 20 minutes at 121°C and 1.5 kg/cm-2 pressure, with five replicates for each dilution. The tubes were incubated at 28°C for 5 to 7 days. The tubes were examined for the brown film formed on the surface, which is a positive indicator of Azotobacter growth. The bacterial count was then calculated using the most probable count method.

To purify Azotobacter bacteria, 0.1 cm3 was taken from the tubes that gave a positive growth indicator and spread on the surface of a Petri dish containing solid medium (Sucrose Mineral Salts Agar). The plates were incubated at 28°C for 2-3 days until colonies appeared. Then, the planing was repeated four consecutive times in order to obtain pure isolates of the bacteria. Using this method, pure isolates of Azotobacter.spp. were obtained. Slant agar tubes were prepared from the specialized medium for the purpose of preserving the isolates in the refrigerator. This was done by taking a portion of the pure culture growth using a loop, inoculating the slant agar tubes and placing them in an incubator at 28°C for one day, then transferring them to the refrigerator. In order to test the efficiency of the isolates in fixing atmospheric nitrogen, liquid media free of nitrogen were prepared, as (50) cm3 of the liquid media were placed in (250) cm3 bottles, and (1%) of mannitol solution was added to each of them. The bottles were inoculated by adding (1) cm3 of the liquid culture of the different isolates and incubated with shaking for (21) days at a temperature of (28°C). The amount of ammonia formed in the medium was estimated by taking (10) cm3 of it and estimating it with the Kjeldahl device (Sharma, 2003; Black, 1965).

Dehydrogenase and Urease Enzyme Measurement

Dehydrogenase Enzyme

Dehydrogenase enzyme was measured using the TTC method, according to Tabatabai (1994). This was accomplished by adding a 1% Triphenyltetrazolium-2,3,5 Chloride solution to 10 g of air-dried soil with a buffer solution. The samples were incubated at 37°C for 24 hours, where Triphenylformazan (TPF) was formed, which was extracted with acetone. Absorbance was measured at a wavelength of 490 nm using a spectrophotometer, after which the amount of TPF produced was calculated.

Urease enzyme

Enzyme activity was measured according to the method of Tabatabai and Bremner (1972). 5 g of soil was incubated with 0.2 cm3 of toluene, 9 cm3 of Tris (hydroxymethyl) aminomethane (THAM) buffer solution (pH = 9), and 1 cm3 of 0.2 M urea solution as a substrate at 37°C for 2 hours. 35 cm3 of 2.5 M KCl solution containing 100 ppm Ag2SO4 was then added as an enzyme inhibitor. The volume was made up to 50 cm3 with the same solution. The ammonium ion produced by enzyme activity was then determined using steam distillation, as described in Bremner and Edwards (1965), using heavy magnesium oxide (MgO), and the ammonia was received with boric acid, followed by titration with hydrochloric acid.

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Table (2) Some physical, chemical and biological properties of the study soils

creteria								soil
Azotobacter CFU.g^{-}	**Total fungi CFU.g^{-}**	**Total bacteria CFU.g^{-1}**	**texture**	**Total N g kg^{-}**	**OM g kg^{-}**	**EC**	**pH**	
1.2*10^{2}	2.1*10^{3}	0.13 10^{5}*	غرينية طينية	3.3	49.40	4.50	7.50	**Tanuma**
0.28*10^{2}	0.43*10^{3}	0.10 * 10^{5}	غرينية طينية مزيجة	2.0	25.00	12.0	7.18	**Kteeban**
0.03*10^{2}	0.02*10^{3}	0.001 *10^{5}	طينية	1.8	23.80	9.72	7.26	**Abu Alkaseb**
0.001*10^{2}	0.17*10^{3}	0.02*10^{5}	طينية	1.7	19.00	18.00	7.11	**Naher hassan**

The results were analyzed statistically using a completely randomized design (RCD). The data were analyzed using SPSS version 11 and the means were compared at a probability level of 0.01 (Al-Rawi and Khalaf Allah, 2000).

III. Results and Discussion

The variation in vegetation cover and salinity and alkalinity values in the studied areas (Tables 1, 2) play a significant role in the variation in microbial populations (Table 3) and enzyme secretion (Table 4). This is due to differences in root secretions and the type of organic matter left behind by cultivated plants. Therefore, planting the Kitaban soil with legumes, along with organic matter, improved its physical, chemical, and biological properties. This may be the reason for the increased microbial population compared to the Hassan River soil (which retains its natural vegetation cover), the Tanuma soil (planted only in winter with vegetable plants), and the Abu Al-Khaseeb soil, which is planted with vegetable plants. Microbial populations are related to the type of vegetation present and the type of organic matter it leaves behind. The variation in the chemical composition of different plant residues leads to changes in the microbial environment and, consequently, to differences in the dominant types of microorganisms. This may be a reason for changes in microbial activity.

The results of Table (3) showed clear differences in the numbers of microorganisms (total bacteria, total fungi, and Azotobacter spp.) and the amount of nitrogen fixed between different soils, whether in terms of depth or proximity or distance from the rhizosphere. The results showed a noticeable superiority of soils located within the rhizosphere, especially at surface depths (0–30 cm). The rhizosphere soil of the Tanuma area, at a depth of 0–30 cm, recorded the highest values for total bacteria (6.2 × 10^{6} CFU), total fungi (5.5 × 10^{6} CFU), and Azotobacter spp. (0.12) compared to the rest of the sites and depths. Meanwhile, the same soil, but far from the rhizosphere and at the same depth, recorded a significant decrease in the numbers of microorganisms, as the number density of total bacteria reached 0.2 × 10^{6} CFU, and total fungi (1.01 × 10^{6} CFU, and Azotobacter (0.004). This significant variation is attributed to the unique biological activity of the rhizosphere, where plant root exudates serve as a rich and easily assimilated food source for microorganisms, contributing to their activation and increase in number (Ricani, 2018).

The results indicate that depth influences the distribution of microbial communities, with numbers decreasing as depth increased from 0–30 cm to 30–60 cm in all soil types. Organic matter, aeration, and temperature are available in the upper layers, compared to the lower layers, which are often less porous and less aerated. The results reflect the interrelationship between soil environmental components (depth, texture, proximity and distance to roots) and beneficial microbial activity, highlighting the importance of the rhizosphere in enhancing soil fertility and supporting biological processes.

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Table (3) Total bacterial and fungal numbers and percentage of nitrogen fixed by Azotobacter isolates and their numbers in date palm rhizosphere soils and soils far from the rhizosphere in the study areas.

Creteria				**Depth **	
Total nitrogen	**Azotobacter spp.** Cfu **10^4**	**Total fungi 10^3 Cfu**	**Total bacteria** Cfu **10^7**		
0.12	6.2	0.01	5.5	**30-0 cm**	**Tanuma rizospher**
0.02	3.5	0.04	5.1	**60-30cm**	
0.004	0.02	0.001	1.01	**30-0**	**Tanuma Non rizospher**
0.0002	0.001	0.011	0.001	**60-30**	
0.18	7.9	0.06	7.3	**30-0**	**Kteeban Rizospher**
0.05	5.8	0.10	5.7	**60-30**	
0.08	0.07	0.01	2.04	**30-0**	**Kteeban Non rizospher**
0.0005	0.001	0.011	0.01	60-30	
0.15	7.1	0.01	6.8	30-0	**Abu alkaseeb rizospher**
0.02	4.2	0.02	5.2	60-30	
0.04	0.02	0.002	1.01	30-0	**Abu alkaseeb Non rizospher**
0.0001	0.002	0.021	0.001	60-30	
0.08	2.2	0.0001	3.1	30-0	**\nahar hassan Rizospher**
0.005	0.21	0.01	2.4	60-30	
0.0002	0.002	0.0001	0.01	30-0	**Nahar hassan Non rizospher**
0.00001	0.0001	0.001	0.0001	60-30	

$LSD_{Bacteria}$ (0.55) , LSD_{Fungi} (0.001) , $LSD_{Azotobacter}$ (1.00) , $LSD_{Nitrogen}$ (0.01)

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The values of enzyme activity (dehydrogenase and urease) in soil samples taken from different areas of Basra Governorate (Tanuma, Kutaiban, Abu Al-Khaseeb, and Nahr Hassan) varied according to the proximity or distance of the sample from the rhizosphere of palm trees, as well as the depth of the soil taken (0-30 cm and 30-60 cm). The results indicate that the highest values of enzyme activity were for rhizosphere soil samples and surface depths, as the value of dehydrogenase enzyme in the surface rhizosphere of Tanuma (0-30 cm) reached 22 µg TPF g^{-1}, and the value of urease was 45 µg NH_4^+-N g^{-1} soil $2hr^{-1}$, compared to the rest of the soils and different depths. While the results showed that the soils far from the rhizosphere and at a depth of 30-60 cm showed a decrease in values, as they were around 0.002 µg TPF g^{-1} for dehydrogenase and 0 µg NH_4^+-N for urease in the Abu Al-Khaseeb soil far from the rhizosphere. This decrease is attributed to the low microbial activity in the deep soil layers due to the lack of ventilation and light and its distance from the effects of roots (Rikani, 2018). The values of the date palm rhizosphere soil in the Hassan River at a depth of 0–30 cm for the activity of the two enzymes (dehydrogenase 27, urease 19), while the rhizosphere soil of the Kitaiban area gave the highest values for dehydrogenase 32 µg TPF g^{-1} and urease 55 µg NH_4^+-N, compared to the rest of the soils. The variation in the enzyme valuesbetween the soils of the studied areas may be due to the difference in the physicochemical properties of the soil such as organic content, pH, and aeration. The type of agriculture and local practices in each area may also contribute to influencing the biological and enzymatic activity of the soil (Dobereiner, 1974).

Table (4) Dehydrogenase and urease enzymes in the rhizosphere of date palms and non- rhizosphere soil of the study areas.

crteria		**Depth**	
Dehydrogenase µg TPF g^{-1}	**Urease µg NH^+-N g^{-1} soil $2h^{-1}$**		
22	45	**-0 30cm**	**tanoma Rizospher**
12	15	**-30 60cm**	
3	12	**30-0cm**	**Tanuma Non rizospher**
0.1	2	**-30 60cm**	
32	55	**30-0**	**Kteeban Rizospher**
9	19	**60-30**	
14	16	**30-0**	**Kteeban Nonrizosher**
4	7	60-30	
21	46	30-0	**Abu alkaseeb Rizospher**
3	13	60-30	

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10	9	30-0	**Abu alkasseeb Non rizospher**
0.001	3	60-30	
27	19	30-0	**Naher hassan Rizospher**
13	2	60-30	
6	2	30-0	**Naher hassan Non rizospher**
0.002	0	60-30	

LSD_{Urease} (7.33) , $LSD_{Dehidrogenase}$ (5.9)

Based on the current study, the soil in the Kitaban area showed the highest values in microbial counts and enzyme activity, indicating the quality of the cultivated crop (legumes), as well as the presence of animal organic matter, which supports soil fertility and stimulates microbial activity. Enzymatic activity and microbial counts decreased with depth at all study sites, demonstrating the lack of aeration and organic matter in these layers. In general, the rhizosphere can be managed and sustained through inoculation with beneficial microorganisms, particularly Azotobacter bacteria, due to their role in fixing atmospheric nitrogen, improving soil health, and reducing excessive chemical fertilizer use. This can also be achieved through the use of appropriate crop rotations, the addition of bio-enrichers, and reducing intensive agricultural practices.

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