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Effect of NaCL and $CaSO_4$ on Activty of *Rhizobium* Bacteria and Testing its Efficincy of Produceing IAA, Enzymes in Gypsiferous Soil

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ABSTRACT: Rhizobium bacteria are of significant environmental and agricultural importance. However, the impact of salts on the growth of these bacteria is a crucial topic that warrants research, particularly in areas with high soil salinity levels. Therefore, this study was conducted to investigate the effects of sodium chloride (NaCl) and calcium sulfate (CaSO4) on the activity of Rhizobium bacteria and to evaluate their efficiency in producing amino acids and enzymes in gypsum soils. A laboratory experiment was conducted at the Soil Science and Water Resources Department, College of Agriculture, Tikrit University, during the academic year 2021-2022. Rhizobium bacteria were isolated from gypsum soil, and the best isolates were selected based on their activity and spread. These isolates were then morphologically characterized. A total of 10 isolates were obtained and identified according to their agricultural and microscopic characteristics. The growth rates of the isolated bacteria on YEMA medium varied from one isolate to another. Six isolates (BR1, BR3, BR5, BR6, BR9, BR10) grew after being incubated for 7 days at 28°C, and were classified as slow-growing Bradyrhizobium bacteria. Meanwhile, four isolates (BR2, BR4, BR7, BR8) grew on the same medium and were classified as fast-growing Rhizobium bacteria. All isolates exhibited the ability to produce indole-3-acetic acid (IAA) at varying levels, with the highest production recorded in isolate BR5 (13.9 $\mu g \cdot mL^{-1}$), followed by isolate BR4 (12.6 $\mu g \cdot mL^{-1}$). The lowest IAA production was observed in isolate BR2 (6.5 $\mu g \cdot mL^{-1}$).

All isolates were able to produce catalase, except for isolate BR10. Six isolates successfully produced the oxidase enzyme, while four isolates did not. Seven isolates produced urease, while three isolates failed to do so. Additionally, five isolates produced nitrate reductase enzyme.

The best isolate of Rhizobium was selected for further testing using four levels of calcium sulfate (0, 0.5, 1.0, 2.0 $g \cdot L^{-1}$) and four levels of sodium chloride (0, 0.2, 0.4, 0.6 $g \cdot L^{-1}$) to determine the optimal level of each salt that the bacteria could tolerate and grow in. The results showed that the bacterial colony growth exceeded 60% at all levels above 0.5 $g \cdot L^{-1}$ of CaSO4, with continued bacterial growth at higher levels of CaSO4. The control treatment (0 $g \cdot L^{-1}$) exhibited only 10% growth, as the bacteria require gypsum for growth. As for the sodium chloride levels, the 0.2 $g \cdot L^{-1}$ level showed the best bacterial growth (50%), while growth decreased to 10% at the 0.6 $g \cdot L^{-1}$ level due to the bacteria's inability to tolerate high levels of sodium chloride. The 0 $g \cdot L^{-1}$ level exhibited the lowest growth, indicating the bacteria's requirement for elemental sodium and chloride during growth, but only at a specific threshold.

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INTRODUCTION

Worldwide, salinity severely affects agricultural production, mainly in arid to semi-arid regions. Over 800 million/ha area has been estimated to be under salt stress with an annual increase of nearly 1–2% (Hernández,2019). About 33% of irrigated areas worldwide and 20% of cultivated areas were under deteriorated and still under salt stress. Iraq falls under arid and semi-arid regions were shortage and irregular Rainfall patterns cause sodicity and salinity in fertile lands.

These problems are related to the physical, chemical, and biological properties of these soils, so researchers are seeking ways to increase the agricultural area by reclaiming these lands which suffer from many problems (Al-Jumaily et al,.2022).

Rhizobia bacteria help to fix atmospheric nitrogen. Herridge et al. (2008) estimated that about 21 Tg of nitrogen is fixed annually through the crop legume-rhizobia symbiosis. However, a number of factors affect the rhizobium-legume symbiotic relationship. These include the host symbiont compatibility and the physicochemical conditions of the soil, especially salinity and soil pH, nutrient deficiency, mineral and heavy metal toxicity, soil moisture. (Brockwell et al., 1995). Salinity adversely affects the survival and proliferation of Rhizobium spp. in soil and rhizosphere, in addition to reducing plant growth, photosynthesis and demand for nitrogen from host plant. However, Singleton et al. (1982) showed that rhizobium strains can grow and survive at salt concentrations which are inhibitory to most agricultural legumes.

Furthermore, saline soil was also shown to improve when they were inoculated with salt-tolerant strains of rhizobia (Shamseldin and Werner, 2005).

Because of rhizobia's environmental and economic benefits, its application may be useful to achieve sustainability, Rhizobia improves soil fertility under salinity stress and helps reintroduce crops specifically to nitrogen-deficient areas (Hussain et al,2009; Ali et al, 2023).

Also, it has been shown to boost legume growth and production under salt stress conditions (Sindhu etal, 2020).

Gypsum soil contain high levels of gypsum, which leads to the formation of hard gypsum layers within the soil, significantly affecting its physical, chemical, and biological properties. Gypsum reduces the permeability of the soil to water, which increases water retention and effectively hinders root movement. Additionally, the presence of soluble salts in the soil can lead to soil degradation and increased salinity, limiting the plant's ability to absorb water and essential nutrients (Sharma and Dahiya, 2019). Furthermore, the soluble salts in the soil may reduce the soil's ability to retain minerals and nutrients due to the high concentration of soluble cations and anions. Therefore, gypsum soils require careful management to control salt content in order to improve their fertility and agricultural effectiveness (Hassan, 2017).

Regarding soil microorganisms, salinity and alkalinity affect the composition and diversity of these organisms, limiting their ability to fix nitrogen or produce essential organic matter. Studies indicate that high concentrations of gypsum prevent microorganisms from interacting with nutrients such as nitrogen and phosphorus, resulting in decreased natural fertilization of the soil (Rashid et al., 2020).

There is a dire need to find eco-friendly methods to enhance the growth and productivity of crops under salt stress conditions. So, current work was done to find out conducted to explore rhizobia isolated from gypsum soil potential in growth under salinity stress.

MATERIALS AND METHODS

Collection of root nodule

The study samples were collected randomly on 30.03.2022 from seven different agricultural fields in the University of Tikrit and taken from root of some cultivated transported to the laboratory in plastic bags for proceed with the isolation and laboratory identification steps. The plant samples taken in this study are (Alfalfa, Cowpea, Bean, Rosa davidii).

Isolation and Screening of Rhizobium

The nodules were separated from the roots and washing in distilled water several times, surface sterilization with 70% ethanol for 10 seconds. These nodules were crushed with the help of a sterilized glass rod to obtain a milky suspension of bacteroid. These were streaked on YEMA plates containing Congo red and incubated at 28°C for 24 - 72 hours.

The pure colonies were picked up and transferred to the YEMA plate for purification. Pure isolates were streaked on YEMA slants for preservation and further analysis.

Identification of Rhizobium

The bacterial isolates were examined by following standard protocols viz. for Gram-staining reaction and other morphological characteristics as described by Somasegaran and Hoben (1994).

Test the efficiency of isolates in the production of indole acetic acid (IAA): The test was carried out according to the method by (Chhaya Datta, 2000).

Tests for oxidase, catalase, urease: These tests were performed according to Collee et al. (1996).

Nitrate Reductase test: the test was carried out according to **Kaur et al** (**2012**) .

In vitro test for salinity tolerance

tolerate salinity was studied under in vitro conditions. For this, the isolates were grown in Petri plates containing YEMA medium supplemented with

different concentrations of sodium chloride (NaCl) (0,0.2,0.4,0.6) and different concentrations of $CaSo_4$ (0, 0.5, 0.10, 0.20).

RESULTS AND DISCUSSION

Morphological characterization of rhizobial isolates

Isolation of Rhizobium from root nodule: The preserved nodules were surface sterilized, crushed and spread over the YEMAC medium. After 4 to 7 days of incubation of plates the colonies formed were selected according to their morphology. A total of ten translucent, convex, and circular colonies were selected for Cultural and microscopic characterization (Table 1). The isolates from nodules were coded as BR. Culture characterization of isolates show obtaining 10 isolates belonging to the Rhizobiaceae family from gypsum soil, diagnosed based on cultural and microscopic characteristics.

The table demonstrates that the growth rates of isolated bacteria on YEMA medium varied from one isolated to another. Six isolates (BR1, BR3, BR5, BR6, BR9, BR10) grew after being incubated for 7 days at 28°C, which are attributed to slow-growing Bradyrhizobium bacteria. At the same time, four isolates (BR2, BR4, BR7, BR8) grew on the same medium, classified as fast-growing Rhizobium bacteria. All isolates were characterized by colonies with spherical, convex, and round shapes, with smooth gummy, and complete edges, exhibiting colors ranging from white to creamy and yellow. These results are consistent with the study conducted by Kumar et al. (2017a), which also observed convex and spherical shapes with smooth surfaces and white, cream, and yellow colors when grown on the same medium. Additionally, this study revealed that slow-growing Bradyrhizobium, forming root nodules on the bean plant (Alkurtany et al., 2022), is more common than fast-growing Rhizobium in dry and semi-dry lands. This is because Bradyrhizobium in these areas could proliferate under stressful and low rainfall conditions compared to fast-growing strains, which is one of the survival strategies in the samples used in this study (Altai et al., 2022).

The result presented in the same table showed Gram's reaction, cell shape and Motility, The Gram's reaction indicated them to be gram negative rods. They were motile, non-endospore forming capsulated microbes. These findings are consistent with those reported by Alkurtany (2022), Altai, et al (2022), as well as Kaur et al (2012), in their studies on leguminous plants such as beans and peas, which yielded positive results for all isolates.) Verma et al, (2022).

Table 1. Morphological and Microscope characterization of rhizobial strains

Rhizobial Isolates	Colony colour	Growth	Shape	Texture	Motility	convex	gummy	Gram's reaction	Endospore staining
BR1	White	Slow	Cocci	Slimy	+	convex	gummy	_	_
BR2	White	Fast	Cocci	Slimy	+	convex	gummy	_	_
BR3	White	Slow	Cocci	Slimy	+	convex	gummy	_	_
BR4	Yellow	Slow	Cocci	Slimy	+	convex	gummy	_	_
BR5	Cream	Fast	Cocci	Slimy	+	convex	gummy	_	_
BR6	Cream	Slow	Cocci	Slimy	+	convex	gummy	_	_
BR7	White	Fast	Cocci	Slimy	+	convex	gummy	_	_
BR8	White	Fast	Cocci	Slimy	+	convex	gummy	_	_
BR9	Cream	Slow	Cocci	Slimy	+	convex	gummy	_	_
BR10	Cream	Slow	Cocci	Slimy	+	convex	gummy	_	_

Indole-3-Acetic Acid and Enzyme Production in Rhizobial Strains

The table (2) illustrates the ability of different rhizobial strains to produce Indol-3-acetic acid and Some enzymes. According to the table, all isolates showed the ability to produce Indol-3-acetic acid at varying levels, with the highest production recorded in isolate BR5 13.9 $\mu g.ml^{-1}$, followed by isolate BR4 12.6 $\mu g.ml^{-1}$. The lowest production of indole was observed in isolate BR2 with a value of 6.5 $\mu g.ml^{-1}$.

The same table reveals that all isolates, except for isolate BR10, were capable of producing the catalase enzyme. For the oxidase enzyme, six isolates succeeded in production, while four isolates were unable to produce it.

The table also shows that seven isolates produced the urease enzyme, while three isolates (BR3, BR9, and BR10) failed to produce it. Furthermore, the table highlights that five isolates (BR3, BR4, BR5, BR9, and BR10) produced the nitrate reductase enzyme.

The *Rhizobium* bacteria exhibit a remarkable ability to produce a wide range of enzymes that contribute to their vital activities in the soil and their relationship with plants. Among these enzymes, oxidases, catalases, and ureases are the most important, as they play a fundamental role in protecting bacterial cells from oxidative stress and enhancing the bacteria's ability to decompose organic matter and fix nitrogen. Indole-3-acetic acid (IAA) is considered one of the key phytohormones that stimulates root growth and enhances the plant's response to various environmental factors. Research has shown that *Rhizobium* bacteria producing IAA can stimulate root growth in leguminous plants, improving the absorption of nutrients such as nitrogen and phosphorus, which ultimately enhances plant health and increases yield (Yildirim et al., 2009; Schwender et al., 2021; Liu et al., 2020).

Urease is a critically important enzyme that aids in the conversion of urea into ammonia, a vital component in the interaction between *Rhizobium* bacteria and plants. *Rhizobium* bacteria require this enzyme to supply plants with the nitrogen essential for growth. Urease also contributes to the removal of toxic nitrogen compounds, such as urea. Therefore, the presence of this enzyme in *Rhizobium* bacteria is vital for stimulating and facilitating nitrogen fixation in the plant's rhizosphere (Sharma and Kumar, 2016).

Studies have shown that *Rhizobium* bacteria produce large quantities of oxidases in oxygen-poor environments, where oxidases help protect bacterial cells from damage caused by oxygen deficiency and harmful oxidative effects. Additionally, some *Rhizobium* species utilize oxidases to stimulate metabolic processes within bacterial cells, helping them survive in unfavorable environments (Park et al., 2007).

Nitrate reductase, an enzyme produced by *Rhizobium* bacteria, plays a pivotal role in the conversion of nitrate to nitrite in the microbial environment. It is a crucial part of the chemical processes involved in the nitrogen cycle in nature. This enzyme is integral to biological processes that contribute to nitrogen fixation in the soil and enhance the plant's ability to absorb nitrogen (Van Hees and Meije, 2017).

These results are consistent with what researchers have concluded regarding the ability of *Rhizobium* bacteria to produce enzymes. (Singha et al., 2018; Gopalakrishnan et al., 2018; Igiehon et al., 2019; Khalid et al., 2020, Mir et al., 2022)

Table 2. Indole acetic acid and Enzymes production of rhizobial strains

Rhizobial Isolates	IAA $\mu g.ml^{-1}$	Catalase	Oxidase	Urease	Nitrate reductase
BR1	11.2	+	-	+	-
BR2	6.5	+	+	+	-
BR3	8.2	+	+	-	+
BR4	12.6	+	-	+	+
BR5	13.9	+	+	+	+
BR6	11.8	+	+	+	-
BR7	12.3	+	-	+	-
BR8	7.5	+	-	+	-
BR9	12.8	+	+	-	+
BR10	7.3	-	+	-	+

Test for salinity tolerance

From Tables (3 and 4), it is noticeable that the initial reading after 3 days of inoculation showed a 5% increase in the percentage of growth of Rhizobium bacteria colonies at the zero level of $CaSo_4$. However, it increased at levels 0.05 and 0.10 by 100% because the bacteria require Caso4 during their growth stages. The percentage decreased to 5% with an increase in the level, which may be attributed to an increase in osmotic pressure in the growth medium and an increase in the pH value above 7.5 (PAUL, 2011).

The percentage of growth in the petri dishes ranged between 30% to 60% for days 3 to 9, then decreased after 12 days due to the accumulation of metabolic by-products, as well as depletion of nutrients, in addition to an increase in osmotic pressure for moisture penetration into the medium. The continued growth of bacteria in the medium up to 12 days, even with high levels of $CaSo_4$, is evidence of Rhizobium bacteria's tolerance to high levels of gypsum.

When CaSO4 salts are added to the soil, their physical and chemical properties change, which contributes to modifying the environment in which microorganisms, such as bacteria, live. Research indicates that the interaction between sulfates and calcium in the soil can lead to increased bacterial activity in some cases. This is due to the fact that sulfates are an important source of sulfur,

which is an essential nutrient for bacteria involved in the breakdown of organic matter in the soil (Chen et al., 2018). Additionally, calcium can improve the stability of bacterial cells by regulating cell membranes, thus enhancing bacterial growth in the soil (Bertin et al., 2003).

Chemically, CaSO4 salts help modify the soil pH, which affects the availability of nutrients and organic matter. The interaction with sulfates typically results in a decrease in soil acidity, making the soil environment more suitable for certain bacterial species that prefer acidic or neutral conditions (Zhang et al., 2017).

However, not all concentrations of CaSO4 are beneficial for bacterial growth. At high concentrations of calcium salts, this may lead to salt accumulation in the soil, which increases the osmotic pressure in the soil medium. This pressure can hinder the absorption of water by microorganisms, thereby reducing bacterial activity (Yao et al., 2016). On the other hand, moderate concentrations of CaSO4 may help enhance bacterial growth by providing optimal conditions for microbial activities.

Table 3. Percentage growth of Rhizobium bacteria colonies with levels of gypsum

Treatments	growth% after 3 days	growth% after 6 days	growth% after 9 days	growth% after 12 days
0	5	30	30	20
0.05	10	60	60	10
0.10	10	50	40	5
0.20	5	40	30	1

Table 4. Numbers of Rhizobium bacteria growing in dishes containing gypsum levels.

Treatments	Growth rate after 3 days CFU=X^{10-5}	Growth rate after 6 days CFU=X^{10-5}	Growth rate after 9 days CFU=X^{10-5}	Growth rate after 12 days CFU=X^{10-5}
0	15	30	44	12
0.05	34	38	65	7
0.10	26	28	41	3
0.20	22	32	38	2

From tables (5 and 6), it is evident that the percentage of colony growth in petri dishes was 10% for the zero level of NaCl salt, and the percentage increased to 20% and 25% for the levels of 0.2 and 0.4 grams each, respectively, due to the bacteria's need for sodium and chlorine elements during growth. The increase continued up to level 0.6 after 3 days of incubation. After 6 days, the highest growth percentage for colonies was reached due to the suitability of conditions for bacterial growth, and it began to decrease after 9 days, especially at high levels, due to increased osmotic pressure and low humidity in the environment. The presence of sodium chloride salt during growth leads to an increase in osmotic pressure, causing water molecules to be drawn out of bacterial cells, leading to their death.

By the 12th day, the colonies reached the death stage. The optimal level of $NaCl_2$ is 0.2, suitable for preparing a medium for the growth of Rhizobium bacteria.

Table 5. Percentage growth of Rhizobium bacteria colonies with levels of $NaCl_2$

Treatments	growth% after 3 days	growth% after 6 days	growth%after 9 days	growth% after 12 days
0	10	50	30	5
0.2	20	55	30	5
0.4	25	40	20	0
0.6	25	30	10	0

Table 6. Numbers of Rhizobium bacteria growing in dishes containing $NaCl_2$ levels.

Treatments	Growth rate after 3 days CFU=X^{10-5}	Growth rate after 6 days CFU=X^{10-5}	Growth rate after 9 days CFU=X^{10-5}	Growth rate after 12 days CFU=X^{10-5}
0	5	44	22	10
0.2	3	65	31	7
0.4	3	41	19	0
0.6	2	38	8	0

Salts, such as sodium chloride (NaCl), are factors that can significantly influence bacterial activity in soil, as their effects can be complex depending on their concentrations and the surrounding environmental conditions. The addition of NaCl salts alters the

physical and chemical properties of the soil, which in turn affects microbial activity. It is known that saline soils may provide an environment rich in ions like sodium and chloride, which are important sources for many microbial processes (Oren, 2011).

The addition of NaCl can increase the soil's water retention capacity, providing a favorable environment for certain bacterial species that require high humidity. This effect enhances the ability of microorganisms to continue their metabolic activities and decompose organic compounds in the soil (Zhou et al., 2018).

Despite the potential benefits in increasing bacterial growth in saline environments, high concentrations of NaCl can have negative effects on microbial activity. Studies indicate that excessively high concentrations of NaCl can be toxic to some bacterial species, leading to inhibited growth (Shao et al., 2008). Increased salt accumulation in the soil can raise osmotic pressure to a level that prevents water absorption by bacterial cells, thereby reducing their activity.

CONCLUSIONS

We can be concluded from the current study that the nitrogen-fixing bacteria Rhizobium can grow in media containing Nacl and CaSo4 salts, whether in the laboratory or in the field soil, provided that the concentrations of these salts do not exceed the permissible limit, because it affects the osmotic pressure of the bacterial cell. The importance of this study is coming from Rhizobium bacteria promoting plant growth, fix nitrogen, and are environmentally friendly. We can prepare an inoculum from this bacteria that is effective in improving plant growth standards and can reduce the use of chemical fertilizers that are harmful to the environment, and dependence on environmentally friendly inputs.

REFERENCES

1. Ali, Q., Shabaan, M., Ashraf, S., Kamran, M., Zulfiqar, U., Ahmad, M., ... & Arslan, M. 2023. Comparative efficacy of different salt tolerant rhizobial inoculants in improving growth and productivity of Vigna radiata L. under salt stress. Scientific Reports, 13(1), 17442.
2. Al-Jumaily, M. M., Al-Hamandi, H. M., Al-Obaidi, M. A., & Al-Zidan, R. R. 2022. Quantity-intensity ratio of potassium in gypsiferous soils in Iraq. Pesquisa Agropecuária Tropical, 52, e71620.
3. Alkurtany, A. E. S., Altai, S. H. M., & Alsamarrai, N. S. H. 2022. Phenotypic and molecular diagnosis of Rhizobium bacteria isolated from Vigna unguiculata plants grown in gypsiferous soils and testing their efficiency in producing indole acetic acid, chelating compounds and dissolving phosphates. Biochemical & Cellular Archives, 22(1).
4. Altai, S. H. M., Khalefah, K. M., & Amin, E. A. M. 2022. Isolation and identification of Rhizobium bacteria from Faba bean (Vicia faba L.) roots grown in gypsiferous soil and testing their efficiency in production growth regulate (IAA) and siderophorses. Tikrit Journal for Agricultural Sciences, 22(4), 96-105.
5. Bertin, L., Laurent, F., & Calvaruso, C. (2003). Sulfur cycle in soils: Impact on the sulfur cycling and sulfur bacterial populations. Soil Biology and Biochemistry, 35(12), 1789–1795.
6. Brockwell, J., Bottomley, P. J., & Thies, J. E. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. In Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems: Extended versions of papers presented at the Symposium on Biological Nitrogen Fixation for Sustainable Agriculture at the 15th Congress of Soil Science, Acapulco, Mexico, 1994 (pp. 143-180). Springer Netherlands.
7. Chen, H., Zhang, C., & Li, Y. (2018). The role of sulfate-reducing bacteria in sulfur metabolism in soil. Applied Microbiology and Biotechnology, 102(18), 7415–7425.
8. Chhaya Datta, P.S. Basu, 2000, Indole acetic acid production by a Rhizobium species from root nodules of a leguminous shrub, Cajanus cajan, Microbiological Research, Volume 155, Issue 2,P 123-127,ISSN 0944-5013.
9. Collee, J. G.; Miles, R. S. and Watt, B. 1996.Tests for the identification of bacteria. In: *Mackie and McCartney's practical Medical Microbiology* , 14th Edition,(Eds.),**14** :131-149.
10. Gopalakrishnan, S., Srinivas, V., Vemula, A., Samineni, S., & Rathore, A. (2018). Influence of diazotrophic bacteria on nodulation, nitrogen fixation, growth promotion and yield traits in five cultivars of chickpea. *Biocatalysis and agricultural biotechnology*, *15*, 35-42.
11. Hassan, G. (2017). Soil salinity and its management in arid and semi-arid regions: A case study of gypsum-rich soils. Journal of Soil Science and Plant Nutrition, 17(4), 933-942.
12. Hernández, J. A. 2019. Salinity tolerance in plants: trends and perspectives. International Journal of Molecular Sciences, 20(10), 2408.
13. Herridge, D. F., Peoples, M. B., & Boddey, R. M. 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant and soil, 311, 1-18.

14. Hussain, M. B., Mehboob, I., Zahir, Z. A., Naveed, M., & Asghar, H. N. 2009. Potential of Rhizobium spp. for improving growth and yield of rice (Oryza sativa L.). Soil Environ, 28(1), 49-55.
15. Igiehon, N. O., Babalola, O. O., & Aremu, B. R. (2019). Genomic insights into plant growth promoting rhizobia capable of enhancing soybean germination under drought stress. *BMC microbiology*, *19*, 1-22.
16. Kaur, K., Sharma, P., Kaur, N. and Gill, B. S. 2012. Phenotypic and Biochemical Characterization of *Bradyrhizobium* and *Ensifer* spp. Isolated from Soybean Rhizosphere. *Bioscience Discovery*, **3(1):** 40-46.
17. Khalid, R., Zhang, X. X., Hayat, R., & Ahmed, M. (2020). Molecular characteristics of rhizobia isolated from Arachis hypogaea grown under stress environment. Sustainability, 12(15), 6259.
18. Liu, Y., Zhang, Q., & Liu, X. (2020). Indole-3-acetic acid production and its impact on plant growth. *Plant Growth Regulation*, 90(2), 147-155. https://doi.org/10.1007/s10725-020-00559-1
19. Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., ... & Dotaniya, M. L. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. *Ecological Engineering*, *107*, 8-32.
20. Mir, M. I., Hameeda, B., Quadriya, H., Kumar, B. K., Ilyas, N., Kee Zuan, A. T., ... & Sayyed, R. Z. (2022). Multifarious indigenous diazotrophic rhizobacteria of rice (Oryza sativa L.) rhizosphere and their effect on plant growth promotion. Frontiers in Nutrition, 8, 781764.
21. Oren, A. (2011). The ecology of halophilic microorganisms. Environmental Microbiology, 13(9), 1909–1923.
22. Park, K. H., et al. (2007). Effect of oxygen availability on oxidative stress and enzyme production in rhizobia. Microbial Ecology, 53(3), 427–434.
23. Rashid, A., et al. (2020). The impact of gypsum on soil fertility and its potential for sustainable agriculture. Environmental Sustainability, 8(1), 45-56.
24. Schimel, J. P., & Bennett, J. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology, 85(3), 591-602.
25. Schwender, J., et al. (2021). The role of indole-3-acetic acid in the relationship between Rhizobium and leguminous plants. *Frontiers in Plant Science*, 12, 623209. https://doi.org/10.3389/fpls.2021.623209
26. Shamseldin, A., & Werner, D. 2005. High salt and high pH tolerance of new isolated Rhizobium etli strains from Egyptian soils. Current microbiology, 50, 11-16.
27. Shao, H., Chu, L. M., & Yu, L. (2008). Impact of NaCl on microbial diversity in soils: A review. Environmental Microbiology Reports, 1(6), 428–438.
28. Sharma, A., & Kumar, A. (2016). Urease production by Rhizobium species and its role in nitrogen fixation. Microbial Ecology, 12(1), 34–45.
29. Sharma, M., & Dahiya, R. (2019). Impact of gypsum on soil salinity and crop growth in saline soils. Journal of Agricultural and Food Chemistry, 67(5), 1370-1379.
30. Sindhu, S., Dahiya, A., Gera, R., & Sindhu, S. S. 2020. Mitigation of abiotic stress in legume-nodulating rhizobia for sustainable crop production. Agricultural Research, 9(4), 444-459.
31. Singha, B. S., Mazumder, P. B., & Piyush Pandey, P. P. (2018). Characterization of plant growth promoting rhizobia from root nodule of two legume species cultivated in Assam, India.
32. Singleton, P. W., El Swaify, S. A., & Bohlool, B. B. 1982. Effect of salinity on Rhizobium growth and survival. Applied and Environmental microbiology, 44(4), 884-890.
33. Somasegaran, P., Hoben, H. J., (1994). Collecting nodules and isolating rhizobia. *Handbook for rhizobia: methods in legume-Rhizobium technology*, 7-23.
34. Van Hees, A., & Meijer, H. (2017). The role of nitrate reductase in nitrogen fixation and crop productivity. *Journal of Agricultural Chemistry*, 65(2), 321-335. https://doi.org/10.1002/jac.381
35. Verma, H., Patra, R. K., Sethi, D., & Pattanayak, S. K. 2022. Isolation and characterization of native Rhizobium from root nodules of raikia french bean growing area of Odisha. Indian Journal of Biochemistry and Biophysics (IJBB), 59(9), 918-926.
36. Yao, H., Wei, L., & Xie, X. (2016). Effects of soil salinity on the microbial community composition and activity in a saline-alkali soil. Soil Science Society of America Journal, 80(2), 418–428.
37. Yildirim, E., et al. (2009). Effect of Rhizobium inoculation and indole-3-acetic acid production on the growth of beans. *Journal of Plant Growth Regulation*, 28(4), 376-381.
38. Zhang, Z., Li, F., & Zheng, M. (2017). Impact of sulfate addition on soil microbial diversity and enzyme activity. Soil Science and Plant Nutrition, 63(3), 278–288.
39. Zhou, J., Liu, X., & Zhao, J. (2018). Soil salinity alters microbial communities and enzyme activity. Journal of Soil Science and Plant Nutrition, 18(2), 343–352.