Annals of Plant and Soil Research 27(3): 542-548 (2025)
https://doi.org/10.47815/apsr.2025.10501

Isolation, characterization and application of ammonia - oxidizing bacteria to improve growth of wheat crop (*Triticum aestivum*)

[1]LALEETA, [2]RACHANA CHOUDHARY AND [3]PRAGYA KULKARNI

[1]Research Scholar, [2]Head Department of Microbiology, Dr. Manrakhan Lal Sahu Govt. College Jamul Bhilai,

Received: October, 2025; Revised accepted: November, 2025

ABSTRACT

This research was carried out in the Durg district of Chhattisgarh, India, focusing on agricultural fields where microbes play vital roles in processes like nitrification, denitrification, oxidation, and the assimilation of soil minerals. The study aimed to identify and characterize Ammonia-Oxidizing bacteria (AOB) from paddy fields. These bacteria play a crucial role in biological nitrogen fixation and in regulating ammonia levels in the soil. AOB are Gram-negative and belong to the Betaproteobacteria group, which is known to enhance plant growth, development, and soil fertility. Bacterial isolation was performed using serial dilution and plating techniques, along with specific nitrification media to obtain AOB. Laboratory screening involved Peptone water tests, and after that, the four AOB isolates (AOB-3, AOB-9, AOB-14, and AOB-18) were selected. These isolates were subjected to biochemical tests such as Indole production, Methyl red, Voges-Proskauer, Citrate utilization, Catalase, Urease, TSIA test, starch hydrolysis, and antibiotic sensitivity tests. Following the identification of these efficient AOB strains, their effectiveness was carried out pot experiment on Wheat crop (Triticum aestivum) under natural conditions using three wheat varieties: ***Sihori Sarbati*** *(SW),* ***Nirbhaya*** *(NW), and* ***Keda****r (KW). Inoculants mixed with a carrier material were applied, with each treatment having three replicates and one control. Cocopeat was used as the carrier material, acting as a substrate to maintain microbial viability. All pots, except the control, received the percentage-based amount of inoculant. The results demonstrated improved biological yield of wheat due to the use of ammonia-oxidizing bacteria.*

Keywords: Ammonia-oxidizing bacteria, Nitrification, Oxidation, Assimilation, peptone Water, Wheat crop, Morphological and Biochemical.

INTRODUCTION

The soil is consisting of mineral and organic material themselves as well as from underlying materials in their morphology, chemical composition and biological characteristics (Solanki and Chavda, 2012; Wagh *et al.,* 2014; Uma and Jeevan, 2019). Soil types are a major factor in determining of plants will grow in a certain area as plants use inorganic elements. However, microorganisms like bacteria and other microscopic life forms available within the soil are also vital and hence soil is a dynamic medium made up of minerals, organic matter, water and air. The nature of soil primarily depends upon its continued change under the effect of physical factors like the parent material, the climate and the organic activity in it (Solanki and Chavda, 2012; Uma and Jeevan, 2019)**.** Soil represents the loose upper crust of the earth surface distinctly different from the underlying bed rock. The color and composition very different from place to place, but all soils are common in consisting of inorganic mineral and organic matter, water and gaseous phases. The soil is composed of major components, such as inorganic matter, organic matter, soil air, water and soil organisms. The soil organisms divided in different groups like Bacteria, Fungi, Algae and protozoan. Some microorganisms are very useful like; Bacteria play a beneficial role of the crop development, stimulate their amount and growth for the use of biofertilizer. ammonia-oxidizing bacteria (AOB) are capable of directly converting ammonia into nitrate. AOB share the common trait of using ammonia as their energy source and carbon dioxide as their main carbon source (Hooper *et al.* 1997). These organisms are obligate aerobes, meaning they require oxygen to survive. The biological oxidation of ammonia is the initial stage in removing nitrogen during waste treatment, with the rapid rise in nitrogen-rich waste from animal farming, nitrogen-based industries and other human activities, managing nitrogen waste has become essential for environmental protection.

Corresponding Author: lalitayk89@gmail.com, [3]Head Department of Microbiology, Govt. V.Y.T.PG. Autonomous College Durg Chhattisgarh India

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from a paddy field belonging to Durg district (Chhattisgarh). After collection of samples, they were kept in air-tight polythene bags and used for the isolation of ammonia-oxidizing bacteria. These bacterial isolates were tested for ammonification and observed their characterization.

Isolation and screening of Ammonia – Oxidizing bacteria

Bacterial isolation by using the serial dilution and plating method. The media used to grow bacteria is Ammonium phosphate agar media, and follow the incubation procedure at 370 °C. After incubation, bacteria are isolate to pure culture techniques. A total of 20 AOB isolates were obtained in pure culture and designated as AOB1- AOB20. The entire isolated colony is screened by the peptone test. This is a preliminary test for the analysis and identification of potent ammonia-oxidizing bacteria (Pathak *et al.* 2021), after which four potent AOB were identified: AOB-03, AOB-09, AOB-14, and AOB-18. All the AOB isolates are subject to further analysis.

Morphological and Biochemical Characterization

All potent AOB isolates were characterized by their morphological characterization as Margin, Color, Texture, Elevation, and Gram's reaction. Indole, Methyl-red test, Citrate utilizing test, Voges-Proskaur test, Catalase test, Urease test, TSIA test, Confirmatory test for bacterial identification, antibiotic sensitivity test. Starch hydrolysis (Daniel j.*et al.,* 2007).

16s rRNA for molecular identification and confirmation

Molecular characters and identification of selected bacterial strains were done from SLS Research Pvt. Ltd., Surat 394210, Gujarat.

Growth Parameters for Analysis

- Number of leaves
- Stem height
- Number of Tillers

Statistical Analysis

Through the analysis of variance test, SPSS 27 software statistically analyzed all of the data created during the experiments for this study (ANOVA). All of the experiments for this study were done in triplicate, and the findings were shown as mean ±SE. The results mean values were analyzed and compared using Duncan's Multiple Range Test. A difference of 5% level of significance.

RESULT

In this study, soil samples were collected from paddy fields in Durg district. A total of 20 isolates were obtained (Table 1).

Table 1: Isolation of ammonia-oxidizing bactereia

S. No.	Aob isolates	High potential of ammonia-oxidation
1	AOB-01	-
2	AOB-02	-
3	AOB-03	+
4	AOB-04	-
5	AOB-05	-
6	AOB-06	-
7	AOB-07	-
8	AOB-08	-
9	AOB-09	+
10	AOB-10	-
11	AOB-11	-
12	AOB-12	-
13	AOB-13	-
14	AOB-14	+
15	AOB-15	-
16	AOB-16	-
17	AOB-17	-
18	AOB-18	+
19	AOB-19	-
20	AOB-20	-

After that screened for their higher activity of ammonia-oxidation through the peptone water test. Four potent AOB isolates are obtained. In these four isolates differentiated by their Morphological and biochemical characterization (Table 2 and 3). The result shows different colony morphologies. 16s-rRNA molecular sequencing identified AOB as *Nitrosomonas* sp. This AOB inoculum was used as a plant growth promoting to wheat crops and observed their growth. The AOB perform ammonia oxidation to enhance nitrification in soil, and these organisms play a very important

Table 2: Morphological characterization

S. No.	Characterization	AOB-03	AOB-09	AOB-14	AOB-18
1	Gram's reaction	Gram - ve	Gram - ve	Gram - ve	Gram - ve
2	Margin	irregular	Smooth	irregular	Smooth
3	shape	Rod shaped	Rod shaped	Rod shaped	Rod shaped
4	Color	White and slightly grey	white	slightly grey	white
5	Elevation	Flat and group	Oval and pair	Flat and group	Oval

role in plant growth and development. The isolated bacteria were used for testing the growth parameters of the wheat plant. After a month of sowing, data were collected. Data were collected and summarized with their Mean±S.E. value. This was utilized to compute the analysis of variance (ANOVA). ANOVA was performed to compare all potential pair wise treatment. All Wheat variety shows good growth of biological productivity, but Nirbhaya wheat is showing the highest growth rate. After the experiment, it was clearly indicated that Ammonia-Oxidizing bacteria are useful for wheat plant growth, development, and enhance yield of crop production.

Table 3: Biochemical characterization

S. No.	Test	AOB-03	AOB-09	AOB-14	AOB-18
1	Indole test	+ ve	+ ve	+ ve	+ ve
2	Methyl-red test	- ve	+ ve	+ ve	+ ve
3	Citrate utilizing test	+ ve	+ ve	+ ve	+ ve
4	Voges-Proskaur test	+ ve	+ ve	+ ve	+ ve
5	Catalase test	+ve	+ve	+ve	+ve
6	Urease test	- ve	+ ve	+ ve	+ ve
7	TSIA test	+ ve	+ ve	+ ve	+ ve
8	Antibiotic sensitivity test	- ve	- ve	- ve	- ve
9	Starch hydrolysis	- ve	-ve	- ve	-ve

16s rRNA testing for molecular identification and confirmation

Molecular characters and identification of selected bacterial strains were done from SLS Research Pvt. Ltd., Surat 394210, Gujarat. AOB- as *Nitrosomonas sp.*, using 16s-rRNA PCR amplification, sequencing, and phylogenetic tree structure. 16S r- RNA Gene sequence of Bacterial Strain *Nitrosomonas* AOB- 16S r-RNA Gene Sequence.

GATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTC
AGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGA
GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCG
AAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCC
CCTGGACAAAGACTGACGCTCAAGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAATTTGGAGGTTGT
GCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACT
GTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGG
CCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACA
ATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGG
AATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG

Table 4: Blast search results of *nitrosomonas*

Scription	Scientific Name	Identification %	Accession
Nitrosomonas sp. R7c140 16S	Nitrosomonas sp.	84.78%	AF386753.1
Nitrosomonas marina strain HT22 16S	Nitrosomonas	84.56%	HQ147563.1
itrosomonas sp. R7c187 16S	Nitrosomonas sp.	84.67%	AF386757.1
Nitrosomonas sp. R7c155 16S	Nitrosomonas sp.	84.67%	AF386751.1
Nitrosomonas sp. Is343 16S rRNA gene,	Nitrosomonas sp.	84.56%	AJ621032.1
Nitrosomonas sp. enrichment culture clone Cb9 16S ribosomal RNA gene,	Nitrosomonas sp. enrichment culture	84.44%	KC706457.1
Nitrosomonas marina strain Nm22 16S	Nitrosomonas	84.44%	NR_104815.1
Nitrosomonas marina 16S ribosomal	Nitrosomonas	84.44%	AF272418.1
Nitrosomonas sp. BF16c57 16S	Nitrosomonas sp.	84.33%	AF386746.1

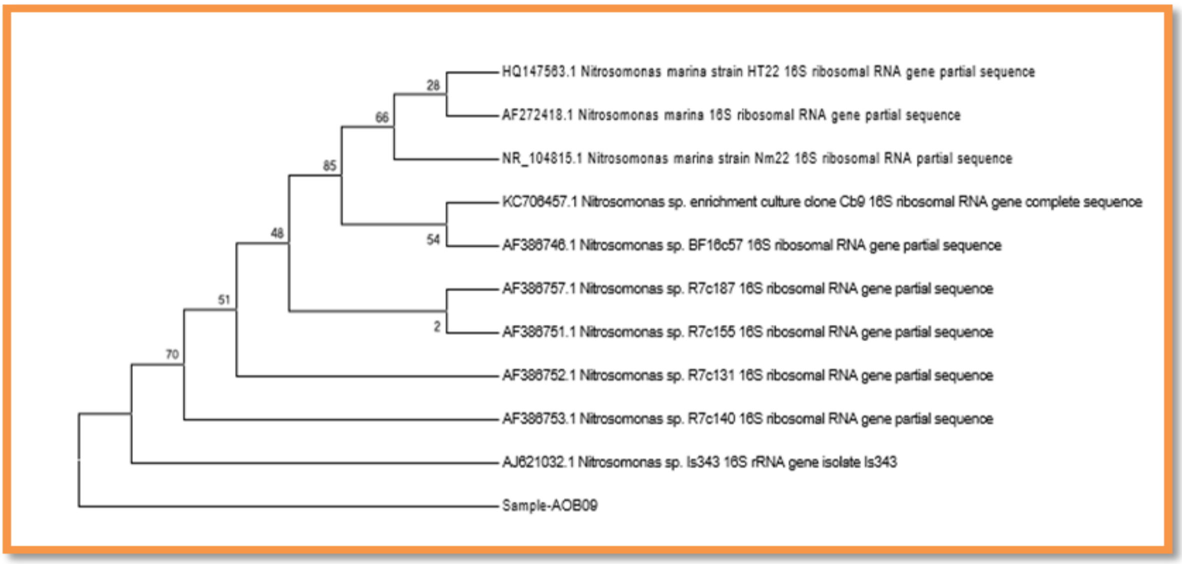

Fig. 1: 16S r- RNA sequencing phylogenetic tree analysis of *Nitrosomonas*

NUMBER OF LEAVES

Table 5: Comparative analysis of the number of leaves in all wheat varieties

Wheat variety and control	Biofertilizer dose (%)				
	100%	80%	60%	40%	20%
Sihori sarbati	13 ± 0	11.6 ± 0.24	11 ± 0.32	10.2 ± 0.2	9 ± 0.32
Sihori sarbati control	8 ± 0.24	8 ± 0.24	7.4 ± 0.2	7.4 ± 0.32	7.4 ± 0.32
Nirbhaya	15 ± 0	13.4 ± 0.24	12.4 ± 0.24	11.4 ± 0.24	10.4 ± 0.24
Nirbhaya control	8.2 ± 0.2	8 ± 0	7.8 ± 0.2	7.8 ± 0	7.8 ± 0.2
Kedar	14 ± 0	12.8 ± 0.2	11.2 ± 0.49	10.2 ± 0.2	09.2 ± 0.2
Kedar control	7.6 ± 0.24	7.4 ± 0.24	7.2 ± 0.2	7.2 ± 0.2	7 ± 0

Wheat plant varieties and their effect on plant growth, as compare of control experimental pot. Measured the growth of the plant in all pots and concluded. Pot experiments are performed through the Randomized Block design (RBD) method. The values are presented in Mean ± S.E., which indicates average growth with standard error. Number of leaves of all wheat varieties, along with their controls. **Nirbhaya wheat** showed the highest Number of leaves in comparison to the two other wheat varieties.

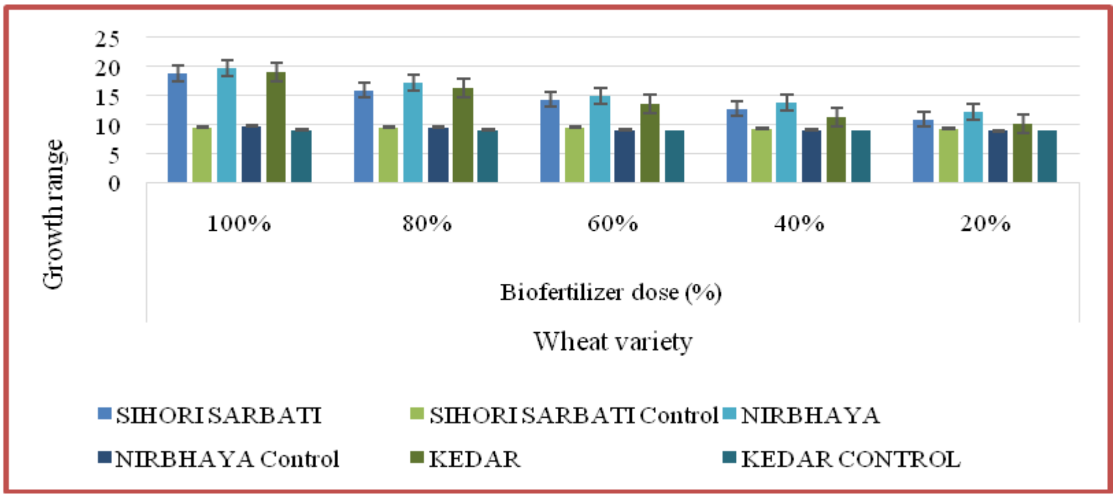

Fig. 2: Comparative analysis of the number of leaves in all wheat varieties

Stem height

Table 6: Comparative analysis of the stem height in all wheat varieties

Wheat variety and control	Biofertilizer dose (%)				
	100%	80%	60%	40%	20%
Sihori sarbati	2.5 ± 0[e]	2.3 ± 0.03[d]	2.18 ± 0.04[c]	2.06 ± 0.04[b]	1.78 ± 0.06[a]
Sihori sarbati control	1.46 ± 0.02[a]	1.46 ± 0.02[a]	1.44 ± 0.02[a]	1.44 ± 0.02[a]	1.42 ± 0.02[a]
Nirbhaya	3.5 ± 0[d]	3.26 ± 0.02[d]	3.12 ± 0.04[c]	2.72 ± 0.14[b]	2.38 ± 0.19[a]
Nirbhaya control	1.44 ± 0.02[a]	1.42 ± 0[a]	1.4 ± 0.02[c]	1.05 ± 0.02[b]	1 ± 0[a]
Kedar	3 ± 0[e]	2.5 ± 0[d]	2.22 ± 0.05[c]	2.01 ± 0.07[b]	1.75 ± 0.08[a]
Kedar control	1.4 ± 0[a]	1.4 ± 0[a]	1.4 ± 0[a]	1.4 ± 0[a]	1.36 ± 0.02[a]

Wheat plant varieties and their effect on plant growth, as compare of control experimental pot. Measured the growth of the plant in all pots and concluded. Pot experiments are performed through the Randomized Block design (RBD) method. The values are presented in Mean ± S.E., which indicates average growth with standard error. Stem height of all wheat varieties, along with their controls. **Nirbhaya wheat** showed the highest Stem height in comparison to the two other wheat varieties.

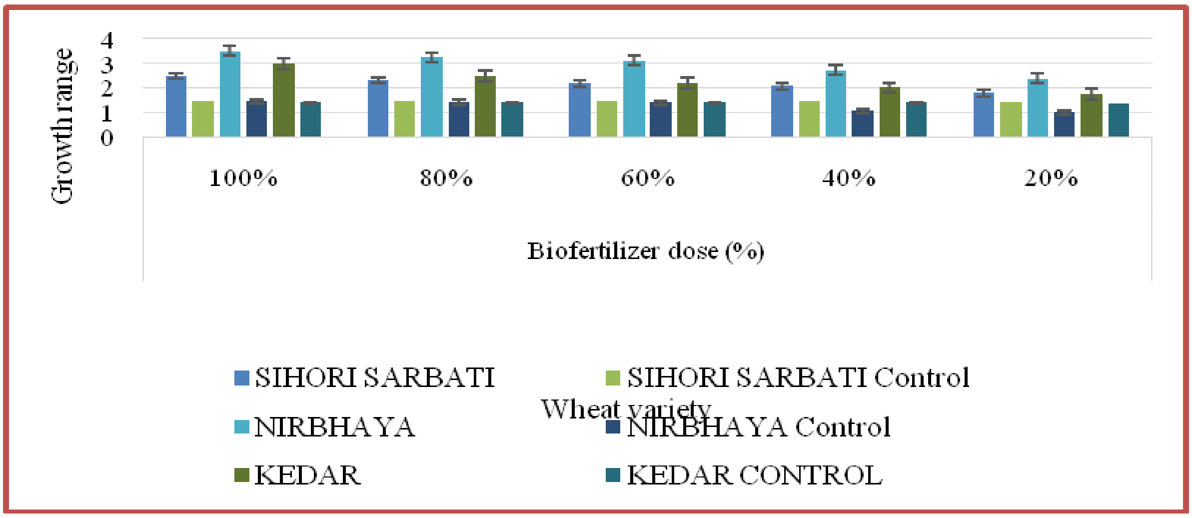

Fig. 3: Comparative analysis of the stem height in all wheat varieties

Number of Tillers

Table 7: Comparative analysis of the number of tillers in all wheat varieties

Wheat variety and control	Biofertilizer dose (%)				
	100%	80%	60%	40%	20%
Sihori sarbati	13 ± 0[d]	11.6 ± 0.24[c]	11 ± 0.32[c]	10.2 ± 0.2[b]	9 ± 0.32[a]
Sihori sarbati control	8 ± 0.24[a]	8 ± 0.24[a]	7.4 ± 0.2[a]	7.4 ± 0.32[a]	7.4 ± 0.32[a]
Nirbhaya	15 ± 0[e]	13.4 ± 0.24[d]	12.4 ± 0.24[c]	11.4 ± 0.24[b]	10.4 ± 0.24[a]
Nirbhaya control	8.2 ± 0.2[a]	8 ± 0[a]	7.8 ± 0.2[a]	7.8 ± 0[a]	7.8 ± 0.2[a]
Kedar	14 ± 0[d]	12.8 ± 0.2[d]	11.2 ± 0.49[b]	10.2 ± 0.2[c]	09.2 ± 0.2[a]
Kedar control	7.6 ± 0.24[a]	7.4 ± 0.24[a]	7.2 ± 0.2[a]	7.2 ± 0.2[a]	7 ± 0[a]

Wheat plant varieties and their effect on plant growth, as compare of control experimental pot. Measured the growth of the plant in all pots and concluded. Pot experiments are performed through the Randomized Block design (RBD) method. The values are presented in Mean ± S.E., which indicates average growth with standard error. Number of tillers of all wheat varieties, along with their controls. **Nirbhaya wheat** showed the highest Number of tillers in comparison to the two other wheat varieties.

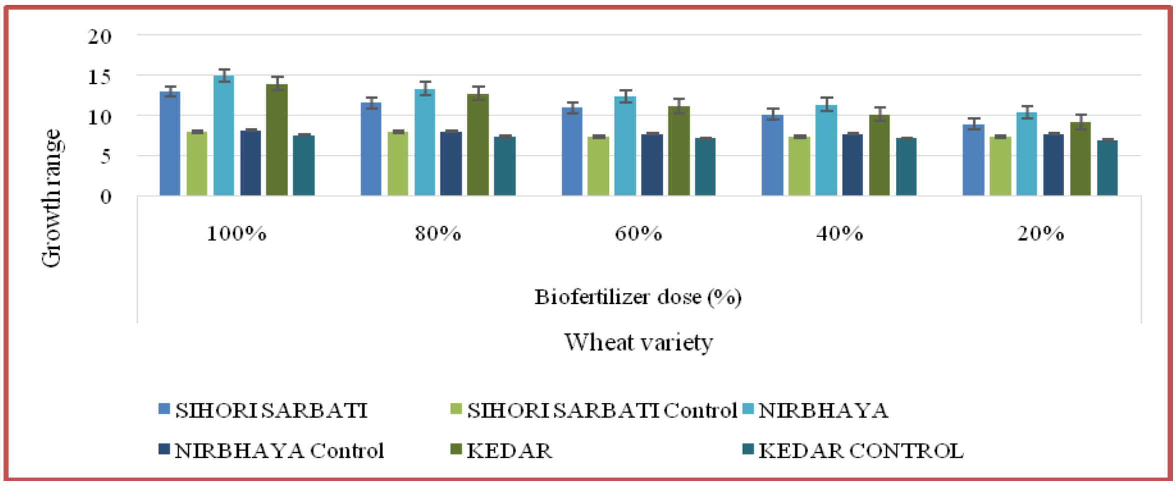

Fig. 4: Comparative analysis of the number of tillers in all wheat varieties

DISCUSSION

The present study offers valuable insights into the potential of ammonia-oxidizing bacteria isolated from paddy field soils. The successful isolation of Nitrosomonas from paddy soil samples demonstrates the adaptability and resilience of these microorganisms under challenging environmental conditions. The distinct morphological and biochemical characteristics of Nitrosomonas spp. highlight their ecological significance. These bacteria play a vital role in the nitrogen cycle, thereby supporting plant growth and development. Furthermore, their contribution to plant growth promotion and reduction in dependence on chemical fertilizers underscores their importance in sustainable agriculture and their considerable benefits to society.

CONCLUSION

The present study concludes that ammonia-oxidizing bacteria (AOB) were successfully isolated from soil samples and characterized based on their morphological and biochemical properties. The results clearly indicate that AOB exhibit significant biological activity that contributes to the promotion and enhancement of plant growth, development, and overall crop productivity. These findings highlight the potential application of AOB as effective biofertilizers, offering a sustainable and environmentally friendly alternative to chemical fertilizers in agricultural practices.

ACKNOWLEDGEMENT

The author thanks the Departments of Microbiology, Govt. V. Y. T. PG, Autonomous College, Durg, for providing the research facility.

REFERENCES

Akao S, Ishii K, and konno T. (1983) Method of measurement of nitrogen fixation by intact soybean plants. JPNJ. Soil science. *Plant Natural*: **54**.58-60.

Bhardwaj D, Wahid Ansari M, Kumar Sahoo R, and Tuteja N. (2014) Biofertilizers function as key players in sustainable agriculture by improving soil fertility, plant tolerance, and crop productivity. *Microbial cell factories*:**13**.01-10.

French E, Kozlowski J, and Bollmanna. (2021) Compiton between ammonia-oxidizing archaea and bacteria from environments. *Appl Environ Microbiol:*87.1021-38.

Fujikake H, Yashima H, Sato T, Ohtake N, Sueyoshi K and Ohyama T. (2002). Rapid and reversible nitrate inhibition of nodule growth and N_2 fixation activity in soybean. *Soil Sci. and plant nutrition*: **48**.211-217.

George A and John R, Stephen (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology, maximizing the use of biological nitrogen fixation in agriculture. *Annu Rev Microbial*: **55**. 485-529.

Harms G, Alice C, Hebe M, Igrid R, Victoria M, Shawn A, Kevin G. and Gary S. (2003) Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. *Environ. Science Technology*: **37**.343–351.

Hanks, J.H., & Weintraub, R.L. (1936) The Pure Culture Isolation of Ammonia-Oxidizing Bacteria. *Journal of Bacteriology*, **32**(6), 653-670.

Hellinga C., Schellen A.A.J.C., Mulder J.W., van Loosdrechet M.C.M., Heijnen J.J. (1998)The SHARON process: an innovative method for nitrogen removal from ammonia-rich wastewater. *Wat Science*.**20**. 255-263.

Harms G and Layton A. (2003) Real time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. *Environ.Sci. Technology*: **37**. 343–351.

Hooper A.B., Vannelli T., Bergmann D.J., Arciero D. (1997) Enzymology of the oxidation of ammonia to nitrite by bacteria. Antoine van Leeuwenhoek *J. Gen. Mol. Microbial*. 71:59–67.

Kowalchuk G, Naoumenko Z, Derikx P, Felske A, Stephen J and Arkhipchenko I.(1999) Molecular analysis of ammonia-oxidizing bacteria of the beta subdivision of the class *proteobacteria* compost and composted materials. *AEM*: **65**. 396-403.

Laleeta, Rachana Chaudhary, and Pragya Kulkarni. (2025) Isolation, Identification and Efficacy testing of Ammonia - Oxidizing Bacteria on Rice plant *(Oryza sativa) Journal of Emerging Technologies and Innovative Research* (JETIR)

Malik S, Kishore S, Shradha A. and Kumar V. (2022) Anaerobic ammonia oxidation: From key physiology to full-scale applications. *Microbial Ecology*: **20**. 393-414.

Mandal N, Datta S.C., Manjaiah K.M. (2015) Synthesis, Characterization and Controlled Release Study of ZN From Zincated Nanoclay Polymer Composites (ZNCPCS) In Relation to Equilibrium Water Absorbency Under Zinc Deficient Typic Haplustept. *Annals of Plant and Soil Research*.**17** (2):187-195.

Nademo Z.M., Shibeshi N.T., Gemeda M.T. (2023) Isolation and screening of low-density polyethylene (LDPE) bags degrading bacteria from Addis Ababa municipal solid waste disposal site “koshe”. *Annals of Microbiology*.**73**:6.

Rodriguez A, Mau S, piedra Castrol L, Jimenez Montealgre R and Herrera Vargas J. (2016).Isolation of ammonium and nitrite-oxidizing bacterial strains from soil, and their potential use in the reduction of nitrogen in household waste water. *Revista biologia tropical*: **65.** 1527-1539.

Spasov E, Jackson M, Laura A, Andrew C, Parker W, Josh d. and Neufeld. (2020) High functional diversity among *nitrospira* populations that dominate rotating biological contactor microbial communities in a municipal wastewater treatment plant. *ISME Jour.*: **14**.1857-1872.

Sun B., yang Y., Jin B., Zhuang G. and Zhuang X. (2021) Efficiency and mechanism of reducing ammonia volatilization alkaline soil using *bacillus amvloliqu efaciens* biofertilizers. *Environ. Res*: **11**. 111-123.