**Enumeration of native rhizobial population nodulating *Phaseolus vulgaris* of North-Western Himalaya.**

**Abstract**

Optimal native rhizobial population required for efficient nodulation of *Phaseolus vulgaris*, leading to maximum dry matter production and nitrogen accumulation in legume crops. Notably, there has been limited investigation into, especially in the NWH region of Uttarakhand. To address this, the study employed the Most Probable Number technique across 12 sites in 5 districts of Uttarakhand. The results revealed a wide range of rhizobia populations, ranging from less than 1 x 102/g of soil to 3.1 x 104/g of soil. These varying number of rhizobia were found in perfect corelation with the organic carbon content, microbial biomass carbon and dehydrogenase activity of soils. Two locations, "Seli sama" (100/g of soil) and "Sui" (1000/g of soil), displayed MPN values below the threshold population required for optimal nodulation. Furthermore, locations with a high number of native rhizobia but relatively low biological nitrogen fixation, specifically "Daranti" (MPN: 31000/g of soil, BNF: 4.92 mg plant /g) and "Gwaldam" (MPN: 31000/g of soil, BNF: 4.88 mg plant /g), have shown the potential to enhance biological nitrogen fixation through the introduction of efficient rhizobial inoculants or by maintaining a high number of effective native rhizobia in the soil.

**Key words**: Biological Nitrogen Fixation, Most Probable Number, Native rhizobia, Rajmash,

## Introduction

French bean has long been considered to contribute minimally to biological nitrogen fixation (BNF), necessitating the use of nitrogen fertilizers. This issue is attributed to the plant's promiscuity, which leads to interactions with native rhizobial strains that are abundant in soil but have low BNF efficiency **(Hungria et al., 2000).** Inoculation often fails due to competition with native strains or environmental factors, limiting yield potential and allowing less efficient rhizobia to dominate. Under non-limiting conditions, soil rhizobia may be insufficient to meet the plant's N2 requirements **(Raverkar, 2017; Yadav and Raverkar, 2021; Wani et al., 1995; Thies et al., 1991).** The rhizosphere microbiome plays a key role in plant resilience, and the diversity and number of microorganisms must be optimal for effective nitrogen fixation.

The number of nodulating rhizobia varies based on the legume, cropping system, and abiotic/biotic factors, as well as soil properties. **Slattery et al. (2004)** state that a rhizobial population below 50/g soil can limit legume symbiotic growth. Conversely, Naziah and Weaver (1994) suggest a threshold of 1000 CFU/g soil is required for maximum dry matter production and nitrogen accumulation in legumes.

Numerous studies have enumerated French bean rhizobia in the rhizosphere. Rhizobium leguminosarum phaseoli populations were measured by researchers using the most probable number (MPN) method, which is crucial for estimating microorganisms within a heterogeneous population **(Alexander, 1982).** The MPN method relies on statistical dilution rather than direct counts. In Himachal Pradesh soils, Dubey et al. (2007) reported Rhizobium populations ranging from 2.5 × 10² to 218 × 10²/g soil. In acidic **Assam soils, Nath et al. (2015)** found pea and lentil rhizobia populations of 9 to 14,700/g soil. **Ansari and Rao (2014)** observed soybean rhizobia in Madhya Pradesh Vertisols, ranging from 0.5-3.3 × 10³ cells/g soil in summer, improving to 3.6-9.6 × 10³ cells/g soil during chickpea season. In Ethiopia, **Argaw (2013)** reported populations of 30 to 5.8 × 10³ cells/g dry soil. Chickpea rhizobia in India varied from <10 to >10⁴ rhizobia/g soil, with higher counts in research stations compared to farmers' fields **(Rupela et al., 1987).** Poor nodulation persisted despite high rhizobia levels, indicating other critical factors influence nodulation

**Diaz-Alcantara et al. (2013)** explored the diversity of rhizobia nodulating French bean, while **Sanchez et al. (2014)** identified 20 plant growth-promoting bacteria in the Phaseolus vulgaris rhizosphere. Rhizobial communities vary significantly, with different species and strains exhibiting diverse symbiotic efficiencies. Due to the low number of nitrogen-fixing rhizobia, inoculation with selected rhizobia strains is commonly practiced to improve nitrogen fixation and crop yield **(Das et al., 2018; Mishra et al., 2014).** However, competitive native, inefficient rhizobia often limit inoculation success. Literature suggests that for improved French bean production, quality, and BNF, enumerating native rhizobial populations is essential. This study aimed to determine the native rhizobial population, nitrogen fixation efficiency, and soil properties in WIH soils to develop inoculation strategies or enhance native rhizobia.

## Material and methods:

### Sampling site and sample collection

The sampling sites were located at varying altitudes of N-W Himalayas of Uttarakhand ranging from approximately 1200 m to 2500 m MSL (Figure 1). Rajmash (*P. vulgaris* L.) rhizospheric soil samples (and/or soil sample from field where Rajmash were grown in previous season) (not deeper than 15 cm) were collected using sterile spatula and spade in sterile polythene bags and transported to laboratory under sterile and cold conditions. Each soil sample was collected in triplicates, which were later mixed to make a single composed sample per site. For each sample one part is air dried and passed through 2 mm sieve and other is stored at 20ºC for microbiological analysis.

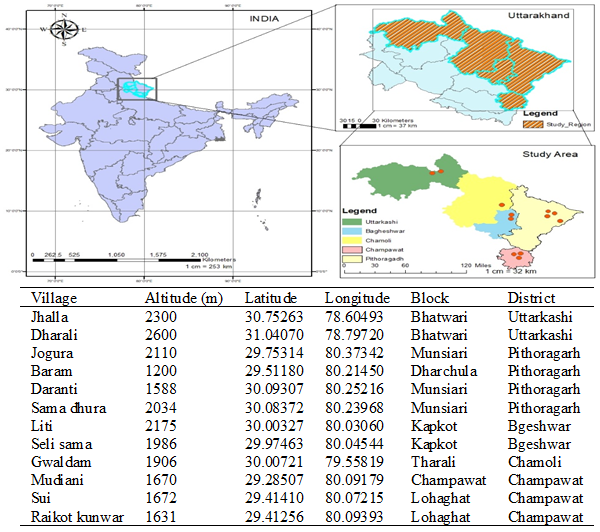


Figure 1: Sampling location from NWH region of Uttarakhand and MPN count of native French bean rhizobia

Population of French bean rhizobia in the soil was estimated by using MPN technique **(Vincent, 1970**) using French bean as a host (Figure 2). A 10-fold dilution series with **four replications** was adopted with an initial dilution of 1:10 (soil: water) and rhizobia was calculated using MPN table. One gram (Dry weight equivalent soil) was mixed in 9 ml water blank in test tube and shaken for vigorously using vortex shaker. This suspension was further diluted up to 108 dilutions under aseptic conditions. Uniform, undamaged seeds [variety BASPA (KRC-8)] were first immersed in 0.1per cent mercuric chloride solution for 3 minutes followed by drained off excess bleach rinsing with five changes of sterile distilled water and imbibed by soaking for overnight and then rinsed twice with sterile water. Firstly, French bean seeds was sown at a depth of 2-3 cm in respective pots filled with the sterilized sand (wet intermittent sterilization) and allow to germinate. After germination of seeds (3-4 days), 1 ml aliquot of appropriate dilution of soil suspension was inoculated at the base of seedling. Plants was allowed to grow in the growth chamber (light intensity 2400 Lux, temperature min: not less than 13 and maximum up to 24-25ºC) for 45 days/till flowering. Plants was watered every day alternatively with sterile N free solution (Hoagland Solution). Uninoculated seeds was also be maintained as checks at the rate of 10% of the total pots. The roots system was analysed for the presence/absence of nodules after 45 days of germination and MPN tables were used to estimate the rhizobium population. **Dry shoot, root, and total dry weight, total nitrogen uptake, total Chl, Chl a and b. Nitrogen fixation was calculated by comparing the nitrogen uptake of pots with and without soil suspension (Figure 2).**

Soil samples were analysed for pH in a soil: solution of 1:2.5, using a glass electrode (**Jackson 1973**); oxidizable soil organic C (SOC) following Walkley and Black (**Walkley and Black 1934)**, available N following Subbiah and Asija (**Subbiah and Asija, 1956**). For estimation of available P, soil was extracted with Bray’s P-I (**Bray and Kurtz 1945**) reagent. Dehydrogenase activity was assessed with Lenhard's method (**Lenhard, 1956**), while microbial biomass carbon was analysed using the chloroform fumigation extraction method (**Voroney *et al.,* 1993)**, and the microbial population was enumerated through serial dilution and plating technique **(Rolf and Bakken, 1987; Chhonkar *et al.,* 2007)** (Table 2).

### Statistical Analysis

In this study, a triplicate sample were analysed to assess the standard deviation among replications. Additionally, the correlations of native rhizobial population and soil characteristics along with amount of biological nitrogen fixation was also examined using SPSS version 16.0 software package (SPSS Inc., Chicago, USA).

Table 1: Physiochemical and biological properties of Rajmash growing areas in hilly region of Uttarakhand

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Village | | Block | | District | | pH | | EC | | N | | P | | OC | | B | | F | | A | | T | | MBC - | | DHA | |
|  | | (dSm−1) | | (Kg ha−1) | | (Kg ha−1) | | (%) | | Log10 | | | | | | | | µg g −1soil | | µg TPF /gsoil/hr | |
| 1 | | Jhalla | | Bhatwari | | Uttarkashi | | 6.15±0.06 | | 0.34±0.06 | | 233±24.3 | | 12.8±1.92 | | 0.80±0.12 | | 8.67±0.02 | | 5.57±0.06 | | 6.61±0.08 | | 8.67±0.02 | | 322.0±26.1 | | 12.5±3.55 |
| 2 | | Dharali | | 5.61±0.02 | | 0.36±0.06 | | 325±60.8 | | 10.4±1.69 | | 0.82±0.42 | | 8.70±0.01 | | 5.14±0.35 | | 6.59±0.15 | | 8.70±0.01 | | 333.0±26.9 | | 19.6±0.44 |
| 3 | | Jogura | | Munsiari | | Pithoragarh | | 5.15±0.07 | | 0.34±0.06 | | 180±20.5 | | 7.77±1.14 | | 0.66±0.41 | | 8.26±0.08 | | 4.66±0.07 | | 5.95±0.11 | | 8.31±0.08 | | 255.3±20.8 | | 6.51±0.40 |
| 4 | | **Daranti** | | 6.21±0.07 | | 0.48±0.08 | | 292±25.4 | | 12.2±2.61 | | 0.83±0.37 | | 7.91±0.00 | | 4.98±0.37 | | 6.05±0.07 | | 7.94±0.00 | | 342.0±27.8 | | 17.7±1.45 |
| 5 | | Sama dhura | | 5.92±0.03 | | 0.83±0.13 | | 239±22.7 | | 12.7±2.29 | | 0.76±0.23 | | 8.80±0.04 | | 4.73±0.31 | | 6.33±0.09 | | 8.81±0.04 | | 320.3±25.7 | | 10.2±0.32 |
| 6 | | **Baram** | | Dharchula | | 5.71±0.01 | | 0.63±0.11 | | 279±56.7 | | 13.9±2.10 | | 0.79±0.37 | | 8.4±0.11 | | 4.94±0.36 | | 6.32±0.03 | | 8.41±0.11 | | 266.7±21.6 | | 14.8±0.83 |
| 7 | | **Liti** | | Kapkot | | Bageshwar | | 6.08±0.10 | | 0.87±0.14 | | 260±25.3 | | 12.5±1.86 | | 0.60±0.00 | | 8.64±0.00 | | 4.17±0.00 | | 5.67±0.03 | | 8.65±0.00 | | 339.7±27.4 | | 12.0±1.02 |
| 8 | | Selisama | | 5.42±0.02 | | 0.74±0.12 | | 159±22.8 | | 11.5±1.00 | | 0.81±0.40 | | 8.51±0.01 | | 5.09±0.44 | | 6.44±0.05 | | 8.53±0.01 | | 249.0±20.4 | | 10.0±1.62 |
| 9 | | **Gwaldam** | | Tharali | | Chamoli | | 6.33±0.04 | | 0.84±0.14 | | 373±30.7 | | 13.4±2.56 | | 0.69±0.38 | | 8.58±0.00 | | 4.56±0.14 | | 6.02±0.28 | | 8.58±0.01 | | 259.0±21.2 | | 14.8±0.83 |
| 10 | | Mudiani | | Champawat | | Champawat | | 6.30±0.09 | | 0.52±0.09 | | 168±20.4 | | 7.6±1.39 | | 0.58±0.36 | | 7.89±0.05 | | 3.80±0.04 | | 5.2±0.36 | | 7.91±0.05 | | 259.3±20.8 | | 6.5±0.40 |
| 11 | | Sui | | Lohaghat | | 6.03±0.14 | | 0.33±0.06 | | 195±21.4 | | 16.8±2.06 | | 0.62±0.13 | | 8.076±0.28 | | 4.00±0.08 | | 5.68±0.19 | | 8.17±0.28 | | 222.0±18.0 | | 3.9±0.16 |
| 12 | | Raikotkunwar | | 6.14±0.14 | | 0.40±0.07 | | 288±27.1 | | 9.50±1.59 | | 0.48±0.00 | | 7.92±0.01 | | 3.88±0.03 | | 5.21±0.61 | | 7.93±0.00 | | 142.0±11.4 | | 9.6±0.73 |

Values are means of the replications. Value following ± is standard deviation. Places written in bold font depict the one where sample drawn from French bean rhizosphere itself.

Figure2: Most probable number of rhizobia nodulating Rajmash in soils of hilly region of Uttarakhand, chlorophyll content and biological nitrogen fixation

Table 2: Correlations between various physicochemical and biological properties of Rajmash growing soils in hilly region of Uttarakhand

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | EC | pH | N | P | OC | MBC | DHA | T mic | Bac | Fun | Actin | BNF | Chla | Chlb | Tchl | DW | N con | MPN |
| EC | 1 | 0.267 | -0.029 | 0.049 | 0.057 | 0.252 | 0.191 | .403\* | .403\* | -0.025 | -0.008 | -0.146 | 0.219 | 0.319 | 0.179 | -0.288 | 0.28 | .399\* |
| pH |  | 1 | 0.064 | 0.31 | -0.074 | 0.317 | 0.178 | -0.024 | -0.026 | -0.145 | -0.063 | -0.081 | -0.034 | -0.075 | -0.068 | 0.03 | 0.001 | .493\*\* |
| N |  |  | 1 | 0.123 | 0.265 | .373\* | .752\*\* | 0.199 | 0.194 | 0.267 | 0.281 | .455\*\* | .593\*\* | 0.326 | .622\*\* | 0.048 | 0.271 | 0.178 |
| P |  |  |  | 1 | 0.162 | 0.113 | 0.14 | 0.175 | 0.174 | 0.173 | 0.254 | 0.174 | -0.087 | -0.082 | -0.007 | -.542\*\* | -0.194 | 0.225 |
| OC |  |  |  |  | 1 | .611\*\* | .580\*\* | .492\*\* | .483\*\* | .919\*\* | .788\*\* | .488\*\* | .513\*\* | 0.172 | .527\*\* | -0.187 | 0.233 | 0.248 |
| MBC |  |  |  |  |  | 1 | .516\*\* | .474\*\* | .468\*\* | .485\*\* | .500\*\* | .346\* | .484\*\* | .440\*\* | .608\*\* | 0.096 | .556\*\* | .354\* |
| DHA |  |  |  |  |  |  | 1 | .359\* | .353\* | .521\*\* | .429\*\* | .507\*\* | .618\*\* | .356\* | .685\*\* | 0.038 | .347\* | .383\* |
| T mic |  |  |  |  |  |  |  | 1 | 1.000\*\* | .608\*\* | .513\*\* | .502\*\* | 0.228 | .785\*\* | .529\*\* | -0.293 | 0.301 | .432\*\* |
| Bac |  |  |  |  |  |  |  |  | 1 | .601\*\* | .504\*\* | .501\*\* | 0.222 | .788\*\* | .525\*\* | -0.292 | 0.3 | .429\*\* |
| Fun |  |  |  |  |  |  |  |  |  | 1 | .802\*\* | .591\*\* | .526\*\* | 0.288 | .562\*\* | -0.193 | 0.18 | 0.268 |
| Actin |  |  |  |  |  |  |  |  |  |  | 1 | .429\*\* | .386\* | 0.166 | .406\* | -.350\* | 0.097 | 0.168 |
| BNF |  |  |  |  |  |  |  |  |  |  |  | 1 | .422\* | .521\*\* | .736\*\* | 0.203 | 0.315 | 0.029 |
| Chla |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 0.329 | .799\*\* | 0.205 | .362\* | 0.118 |
| Chlb |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | .707\*\* | 0.217 | .522\*\* | .407\* |
| Tchl |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 0.273 | .535\*\* | 0.274 |
| DW |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | .467\*\* | -0.106 |
| N con |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 0.127 |
| MPN |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |

**\*\*** Represent significance at 0.01% LoS and **\*** Represent the significance at 5 % LoS.

## Result and Discussions

### Most probable number (MPN) of French bean rhizobia and BNF

The soils of all the site were mostly acidic with pH below **6.5**. The organic C content was also low and varies in between 0.40 to 0.83%. Similarly, all the sites were found to be low in available N. The **figure 2** suggests that the number of rhizobia in the soil vary across various locations. The crop grown, season *etc.* in each location may have influenced the rhizobia population. Other metrics, including nitrogen intake, chlorophyll content, and total chlorophyll content, varied amongst the various locations.

The range of most probable number (MPN) of native French bean rhizobia in soil was 100 to 31000/g **(Figure 2).** which represents less than 0.01% percent of the culturable fraction of soil microbes. Among all locations, the sample of **Baram, Darati, Liti, and Gwaldam** were collected from the rhizospheric soil of Rajmash. The data in Figure 2 indicates variations in nitrogen fixation across the places where samples were drawn from the field after a significant time of harvesting of French bean or from its rhizosphere.

In **Baram**, (Block Dharchula, **Pithoragarh),** the rhizobial count was around 17,000 /gof soil, and it contributed to a maximum biological nitrogen fixation of 11.14 mg/plant . Where as in **Daranti (Munsiari,** Pithoragarh) and **Gwaldam, (Tharali, Chamoli)** instead of having highest number of native rhizobia (31000 /g) the biological nitrogen fixation, was 4.92 and 4.88 mg/plant . In **Jogura and Sama dhura (Munsiari Block, Pithoragarh)** the native population was 1700 and 3100/g of soil, respectively with 4.54 and 5.61 mg/plant BNF**.** This suggests that whilst the rhizobial count was higher, actual nitrogen fixation potential was not as high compared to the **Baram**, thus need the identification and inoculation of efficient rhizobia to reach up to the maximum potential of nitrogen fication and dry matter accumulation.

Conversely, in **Liti**, of (**Kapkot** block, **Bageshwar)** where the average rhizobial count was 5,800 /g, the biological nitrogen fixation tracked was 5.62 mg/plant . Whereas **Selisama** of the same block have the native rhizobial population of 100/g with nitrogen fixation value was 5.28 mg/plant which wasstill much greater than **Daranti and Gwaldam** despite the decreased rhizobial count. Although the Rhizobial count of Selisama was least among all 12 sites, which is close to or below the threshold level (**Naziah and Weaver, 1994)**. This implies that the strain of rhizobia found in **Liti** is more efficient in fixing nitrogen than **Daranti** and **Gwaldam but there is a possibility to increase the BNF** through identification and inoculation of efficient rhizobia**.**

**Jhalla** and **Dharali (Bhatwari** block, **Uttarkashi)** exhibits a rhizobial count of 17,000/g and 5800/g of soil, respectively and a comparatively high nitrogen fixation value of 9.92 mg/plant and 8.99 mg/plant, respectively.

The native rhizobial population of **Mudiani (Block Champawat)**, **Sui** and **Raikot Kunwar (Block Lohaghat)** of **Champawat** district was **1700, 1000** and **3100** /g,respectivelyexhibit intermediate nitrogen fixation values of with **4.06, 3.61** and **4.36** mg/plant, respectively, also require the inoculation of specific efficient rhizobia.

**High native rhizobial count in Baram, Darati, Liti, and Gwaldam** emphasize the relationship between **rhizobial numbers** and efficacy of **biological nitrogen fixation**, highlighting the potential of the French bean rhizosphere to support nitrogen-fixing bacteria and enhance nitrogen availability for plant growth. Increase in the number of rhizobia in the rhizosphere of host legume or homologues legume through the stimulatory effect has been reported **(Janati *et al*., 2021; Dinnage *et al*., 2019; Thies *et al*., 1995).** Similarly, In the Bhopal region of Madhya Pradesh and Durg of Chhattisgarh, **Raverkar *et al.* (2005)** noticed a low soyabean native rhizobial population that remained below threshold (1000 /g) especially in the summer. These rhizobial populations rose in Bhopal, where soyabean was continually grown, by 10 to 25 folds during the monsoon season, but only by 3 to 8 folds in Durg, where heterologous crops were grown alternately. Also, high rhizobial populations can contribute to more effective nitrogen fixation *i.e., Baram, Jhalla, Dharali,* since higher rhizobial counts typically correlate with better nitrogen fixation values **(Dinnage *et al*., 2019; Raverkar, 2017; Naziah and Weaver, 1994).** Correspondingly, all the soil sample are found to be low in available nitrogen content thus help to stimulate nitrogen fixation and Nodulation **(Hellsten and Kerstin 2000).**

The log 10 scores range from 7.94 to 8.70, indicating varying microbial abundance. As **Dharali** has the highest score (8.70), suggesting a larger population, while **Daranti** has the lowest (7.94). Although, Serial dilution and plate count method only provide the information about only a fraction of the soil microbe; the culturable fraction only, however DHA provides corelative information on the catabolic potential and is widely used for the generalised index of soil biological activity, which indirectly can relate to the population of nitrogen fixer in soil and the microbial biomass carbon (Figure 2 & Table 2).

Higher MBC relates to the high amount of organic carbon and increased microbial activity as depicted by High DHA. Villages like Sui (MPN of 1,000 /g) and Raikot kunwar (MPN of 3,100 /g) have lower MPN values compared to other villages. Interestingly, these villages also have relatively lower OC values (0.62 and 0.48, respectively) and MBC (222 and 142 µg /g, respectively). suggesting lower presence of rhizobia in the soil is associated with relatively lower organic carbon content (Table 2). Villages with a higher MPN of native rhizobia tend to have higher MBC which can be associated with more active and efficient microbial communities capable of utilizing available organic carbon. And this demonstrates that a larger concentration of rhizobia in the soil may help to enhance the amount of carbon in the microbial biomass. For example, villages such as Jhalla, Daranti, Sama dhura, and Gwaldam, which have higher MPN values (17,000 and 31,000 /g, respectively), also have correspondingly higher MBC values (ranging from 259.0 to 342.0 µg/g of soil). Similarly, Villages with lower total microbe counts, such as Jogura (log 10 score of 8.31) and Mudiani (log 10 score of 7.91), also tend to have lower OC values (0.66 and 0.58%, respectively) and lower MBC (255.3 and 259.3 µg/g of soil, respectively) and lower DHA (6.51 and 6.5 µg TPF /gh−1, respectively) shows a relation between decreased overall microbial population and reduced microbial activity in the soil (table 2).

It is also important to consider that various factors, such as environmental conditions, soil characteristics, and the presence of other microorganisms *i.e.,* PGPR, asymbiotic Nitrogen fixer *etc.*, can also influence nitrogen fixation rates in each location **(Zheng *et al*., 2019). The existence of asymbiotic nitrogen-fixing microorganisms in the soil solution utilised for inoculation might have altered the overall nitrogen fixation measurements obtained.** This suggests that the total soil ecosystem, including the presence and interactions of various microbial populations, is essential for understanding the dynamics of nitrogen fixation and how it affects plant growth. The relationships between soil properties *i.e.,* pH, EC, available nutrient content, organic carbon (OC), microbial biomass carbon (MBC), dehydrogenase activity (DHA), and rhizobial counts (Rhizobia /g soil), and other microorganism *i.e.,* culturable bacteria, fungi and actinomycetes *etc.*, can provide insights into the microbial dynamics and their influence on nitrogen fixation, considering both symbiotic and asymbiotic microbes (figure 2 and table 2).

### **Correlation deduction**

The data indicates that higher levels of organic carbon in the soil are positively associated with increased available nitrogen (table 2). Furthermore, organic carbon content demonstrates a significant positive correlation with dehydrogenase activity (DHA) and microbial biomass carbon (MBC), total microbial number. This implies that higher levels of organic carbon in the soil are linked to greater microbial activity and biomass. Microorganisms rely on organic carbon as an energy source, which leads to enhanced microbial processes. Additionally, increased dehydrogenase activity, an indicator of microbial metabolic activity, is connected to higher microbial biomass in the soil. Moreover, positive correlation between the total count of microorganisms in the soil biological nitrogen fixation (BNF) and plant nitrogen concentration suggests that the overall microbial community contributes to nitrogen-fixing processes, either through symbiotic or asymbiotic means. The positive strong correlation between MPN and BNF also prove it. Furthermore, biological nitrogen fixation (BNF) exhibits a significant positive correlation with chlorophyll a, total chlorophyll and nitrogen uptake indicate a direct relationship between nitrogen content in plants, dry matter accumulation, and higher biological nitrogen fixation. It implies that nitrogen-fixing processes actively contribute to plant growth and the synthesis of chlorophyll, which is essential for photosynthesis.

## Conclusions

Higher soil organic carbon levels are linked to better nitrogen availability, boosting microbial activity and biomass. This enhances nitrogen fixation, crucial for plant growth and chlorophyll production, which supports photosynthesis. The study focused on counting French bean rhizobia in Uttarakhand's NWH region. Baram, Jhalla, and Dharali had high rhizobial counts and efficient nitrogen fixation. However, Daranti and Gwaldam, despite high counts, showed lower fixation. Sama Dhura, Liti, Selisama, and Mudiani had lower but sufficient counts, indicating efficient fixation. Areas like Daranti, Gwaldam, and others with poor fixation, and those with low counts like Sui and Selisama, need inoculation with efficient rhizobia. Future research could explore factors affecting rhizobial counts and better strain interactions

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