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Quantification of Indigenous Rhizobial Populations Associated with Pea Nodulation in Kumaon Region of North-western Himalayas

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study was to explore the diversity of native rhizobial populations in pea-cultivating regions of the Kumaon area in the north-western Himalayas and their role in biological nitrogen fixation (BNF). The research employed completely randomized design (CRD), involving the collection of soil samples from 12 locations to analyze rhizobial populations, soil physicochemical properties, and their effects on pea nodulation and nitrogen accumulation. A positive correlation was observed between soil organic carbon (OC) levels and the most probable number (MPN) of rhizobia, with higher OC supporting greater rhizobial growth and BNF. Sites such as Daranti and Khamaria, with elevated OC levels, exhibited higher rhizobial populations and enhanced nodulation, while regions with lower OC, such as Chafi and Sui, showed reduced activity. Daranti recorded the highest rhizobial count 10,000 g^{-1} of soil, and Timiladiggi demonstrated superior nitrogen fixation efficiency (18.01 mg $plant^{-1}$ BNF). These findings suggest the potential for isolating and using efficient rhizobial strains, such as those from Timiladiggi, as inoculants to improve BNF in less fertile areas. The study also identified climatic influences on soil properties, with cooler, high-altitude regions promoting greater OC accumulation and rhizobial activity. These results emphasize the importance of utilizing efficient rhizobial strains and enhancing soil OC to boost legume productivity and support sustainable agriculture in diverse agroclimatic zones. Future research should focus on metagenomic analyses to characterize the genetic diversity of native rhizobia and uncover their functional traits.

Keywords: Most probable number; rhizobia; nodules; Uttarakhand; biological nitrogen fixation.

1. INTRODUCTION

In North-Western Himalayan region, farming is valued as a significant source of revenue favoured by region's agroclimatic conditions for growing vegetables. People in hilly-agricultural areas practice organic farming which allows the preservation of Himalayan agro-ecosystems. In these agro-ecosystems, biological practices are especially crucial as biological health of soil dictates the various biogeochemical cycles determining the availability of nutrients for the growth of soil microorganisms and plants. Nitrogen (N) is one of the important essential nutrient elements that plays very important role in growth and development of crops (Sulieman, 2011). Approximately, 97% of Indian soils are deficient in available Nitrogen (CSE Report, 2022), depicting the need of nitrogen application in soil. But applying nutrient by mean of inorganic sources has its own negative aspects as it leaks nutrients and GHGs to environment. Increased fertilizer consumption directly contributes to lower N use efficiency (15-30%) (Shukla et al*.,* 2022). Current agricultural policy aims at reducing the utilization of fertilizer by at least 20% and cutting down on fertilizer use while maintaining the soil fertility (European Commission, 2023). Therefore, alternative ways of biological origin to meet the nutrient requirement of crops are highly desired.

Applying nutrients through biological sources *i.e.*, compost, Farmyard manure (FYM) or residue application etc., use of microorganisms is advantageous as these have a direct and positive impact on soil health and crop productivity (Hoque et al*.,* 2022). Biological nitrogen fixation (BNF) is one of the potential natural phenomena to boost crop production while reducing the dependency on chemical sources of nitrogen (Dhillon et al*.,* 2022; Peoples et al*.,* 2009). Legumes play pivotal role in enriching the soil with N, having been included in crop rotations, through biological nitrogen fixation. (Wysokinski et al*.,* 2021, Ntatsi et al*.,* 2019). Legumes may derive up to 90% of the nitrogen from BNF when inoculated with the right strain of bacteria (Ghazi and Karnwal, 2017).

In India, vegetable pea (*Pisum sativum* L.) occupies 10.18 lakh hectare area and mainly is grown in Uttar Pradesh having maximum cultivated area and production followed by Madhya Pradesh, Jharkhand, Assam, Odisha, Manipur, West Bengal, Bihar (GOI Ministry of Agriculture & Farmer Welfare, 2020-21). In Uttarakhand, vegetable pea is cultivated in 13615-hectare area with production of 10,2977 Metric tonnes (State Horticulture Mission Government of Uttarakhand, 2020-21). Vegetable Pea (*Pisum sativum* L.) with its exceptional capacity for symbiotic nitrogen fixation, which has long been recognized as a restorer of soil fertility. Vegetable Pea when grown in the field has the capacity to fix about 165 kg N ha^{-1}, although the usual range is 40–60 kg ha^{-1} (Bourion et al*.,* 2007).

Being short duration crop, vegetable pea (*Pisum Sativum* L.) fits properly between rice – vegetable pea-late sown wheat, rice – vegetable pea- spring maize; and rice - sugarcane crops which have given significant importance to vegetable pea to be included among cropping systems as a restorer of N deficiency which is prominent in Indian soils (Meena et al*.,* 2018).

Multiple numbers of native rhizobial strains are present in nature capable of causing infection in a host plant competing for infection sites with inoculated/ introduced strain. Full benefit of symbiotic nitrogen fixation cannot be achieved, when native strain outcompete the inoculated one that fixes significantly less nitrogen than the inoculated (Yates et al*.,* 2011). So, a thorough knowledge about the native rhizobial population of rhizobia specific to host and their BNF efficacy is essential for harnessing the benefits of nitrogen fixation. The knowledge regarding the native rhizobial population nodulating vegetable pea in Kumaon region of Uttarakhand is lacking. Process of BNF is also susceptible to the biotic and abiotic environment *i.e*., soil moisture conditions, soil aggregation, soil fertility, organic matter, soil microbial diversity etc., affecting growth and nodule initiation process. Thus, when temperate and tropical legumes are exposed to moisture stress, nitrogen fixation suffers. Soils in Uttarakhand have varying soil depths with uneven distribution of nutrients across the region with low soil water holding capacity and limited root zone posing the threat to the growth and productivity of crops.

The Himalayan ecosystems provide a variety of micro-habitats with high biodiversity, within small distances and elevations, showcasing a range of micro-habitats with great biodiversity. In addition, changes in land use patterns, the growth of infrastructure, unsustainable tourism, overexploitation of natural resources, habitat fragmentation, and climate change all pose threats to the Himalayan ecosystems. There are numerous traditional crops cultivated in the Himalayan agro-ecosystems, which have been nurtured by native farming communities for ages. One such crop is Vegetable Pea (*Pisum sativum*L.). Because, these crops have adapted to the local environmental conditions, they possess the inherent qualities to withstand ecological risks and other challenges. Also with its exceptional capacity for symbiotic nitrogen fixation, it has been recognized as a restorer of soil fertility. After dry bean (*Phaseolus vulgaris* L.), vegetable pea is second major legume crop cultivated in 6.18 M ha area with a grain production of 10.48 Million tonnes (Brijbhooshan and Shalini, 2007).

The resident *Rhizobium* populations of each site were enumerated by most probable number plant infection tests (Brockwell 1963) based on the ability of specific rhizobia to produce nodules in a selected species of legume. Depending on the legume type, cropping system, abiotic and biotic environment number of nodulating rhizobia below 50 g^{-1} soil could be a limiting factor for the symbiotic growth of legumes (Slattery et al*.,* 2004).

Depending on the physicochemical characteristics of the agroecosystem, rhizobial diversity and abundance differ. Given that oxygen has a major role in controlling microbial activity, the majority of rice fallow soils either had low concentrations of native rhizobia—roughly 100 rhizobia per gramme of dry soil—or none at all. To ascertain whether artificial rhizobia inoculation is necessary, it is vital to count the rhizobial populations in the soil. Since there are no reliable molecular markers to distinguish rhizobia from other soil bacteria, the most probable number (MPN) method is used to count the population of rhizobia by observing the presence or absence of root nodules. The enumeration of rhizobial MPNs is predicated on the idea that nodules will form in nitrogen-free media when a single, viable rhizobial cell is present in the root region of a young legume host of the proper type (Senthilkumar et al*.,* 2021).

Some rhizobia can potentially form nodules even in presence of other strains exhibiting rhizobial competitiveness (Yates et al., 2011; Onishchuk et al., 2017). Soil physicochemical properties like pH, temperature, moisture, availability of nutrients and their efficient use by microbes and soil type affects, rhizobial competitiveness and ultimately nodulation (Rathi et al., 2021; Kasper et al., 2019).

In addition to BNF, several rhizobia strains have lately shown a variety of additional PGP activities. This makes the isolation and creation of effective multi-trait rhizobia isolates for French beans necessary to promote sustainable production, environmental safety, and economising nitrogenous fertiliser (Yadav and Raverkar, 2021). With higher rates of soil N application at planting, there are fewer nodules in the plant. The degree of strain competition will be highly influenced by how well the rhizobial strains adapt to the particular soil conditions (Poole et al., 2018).

Similarly, Nath et al. (2015), enumerated resident pea and lentil rhizobia in acidic soils of Assam and reported the population ranging from 9 to 14700 g^{-1} soil. In Vertisols of Madhya Pradesh, a survey of soybean rhizobial populations in the rhizosphere of post-summer rainy season revealed a low number ranging from 0.5–3.3 × 10^3 cells g^{-1} soil but improved in the rhizosphere of cool season as 3.6–9.6 × 10^3 cells g^{-1} (Ansari and Rao, 2014). In an Ethiopia, indigenous *Rhizobium leguminosarum* var.*viciae* in soils ranged from 30 to 5.8 × 10^3 cells g^{-1} dry soil (Argaw, 2013).

These microbial species are spatially distributed based on the niche selection and agroecological zones which vary considerably. There is a need for inoculation in legumes where rhizobial population at the time of sowing is below threshold.

2. MATERIALS AND METHODS

2.1 Sample Collection

The sample collection of this study was conducted in October 2022across 6 districts Almora, Nainital, Pithoragarh, Bageshwar, Champawat and Udhamsingh Nagar in Kumaon region of Uttarakhand. The sampling was conducted before the sowing of vegetable pea from the fields where pea is common crop in crop rotation. Soil samples (1 kilogram from each location) from the fields with varying altitude ranging from approximately 200 m to 2200m above MSL and climatic conditions with the temperature ranging from 25-29 ℃ of N-WHimalayan region werecollected. (Figs. 1 & 2, Table 1) and stored in cool place. The samples were divided in two parts out of which one part was stored in refrigerator for determination of MPN studies. The other portion of soil sample was stored in cool place and processed for the determination of various chemical and physico-chemical properties.

2.2 Enumeration of Native Rhizobia Calculation

The population of rhizobia specific to vegetable pea in the soil was determined using the plant infection and Most Probable Number (MPN) technique, as described by Vincent (1970). A 10-fold dilution series with four replications was prepared, starting with an initial dilution of 1:10 by mixing one gram of soil (equivalent to dry weight) with 9 ml of sterile water in a test tube and vigorously shaking with vortex shaker. This suspension was further diluted up to 10^8 dilutions under aseptic conditions, and the number of rhizobia was calculated using an MPN table.

Table 1. Sampling location from NWH Kumaon region of Uttarakhand

Altitude, Latitude and Longitude				
Place	**Altitude**	**N-E**	**Block**	**District**
Podhar	1894.2	29.565-79.6912	Lamgara	Almora
Timiladiggi	1630.8	29.6208-79.3856	Tarikhet	Almora
Chafi	1302.3	29.3676-79.5872	Bhimtal	Nainital
Dhari	1306.9	29.3115-79.6946	Bhimtal	Nainital
Baram	1200	29.5118-80.2145	Dharchula	Pithoragarh
Daranti	1588	30.09307-80.25216	Munsiari	Pithoragarh
Leti	2175	30.00327-80.03060	Kapkot	Bageshwar
Sama	1986	29.97463-80.04544	Kapkot	Bageshwar
Sui	1672	29.4141-80.07215	Lohaghat	Champawat
Raikot Kunwar	1631	29.41256-80.09393	Lohaghat	Champawat
Khamaria	242.5	29.1979-79.1173	Bajpur	US Nagar
Nh 309	206.2	29.0178-79.2816	Gadarpur	US Nagar

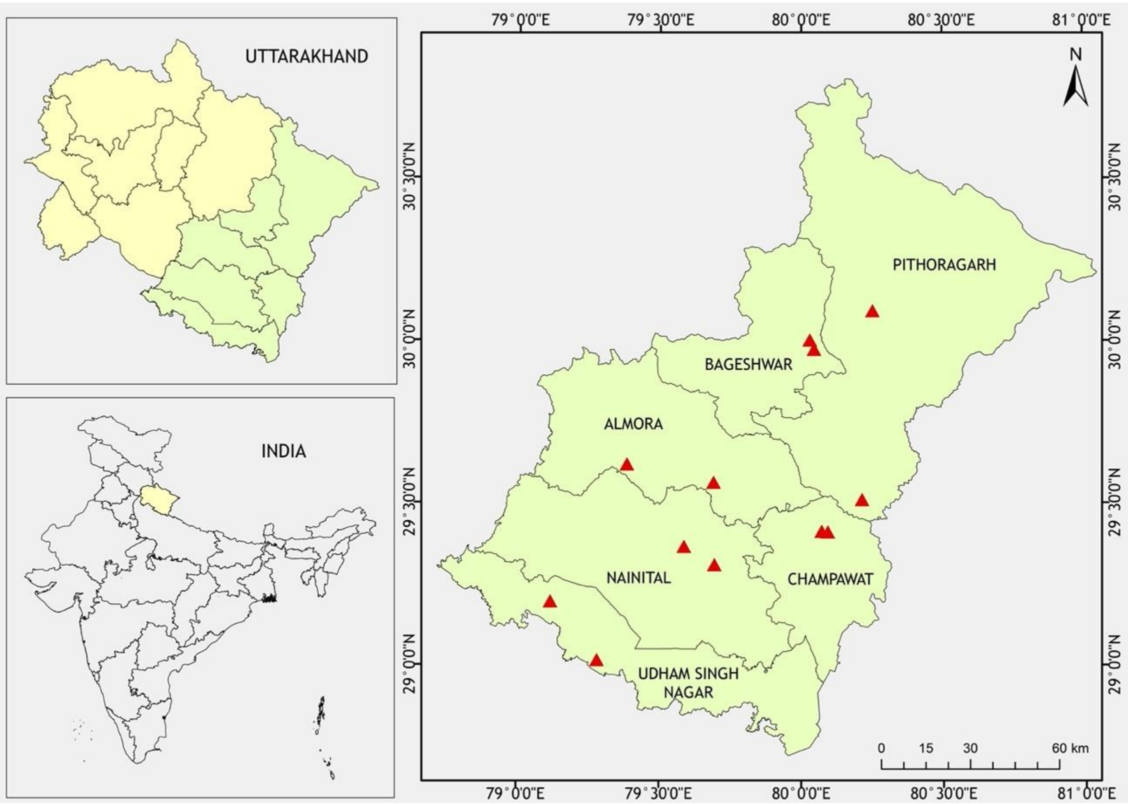

Fig. 1. Sampling location from NWH Kumaon region of Uttarakhand

Fig. 2. Sampling locations

The uniform and undamaged vegetable pea seeds were surface sterilized immersing in a 1% $HgCl_2$ solution for 3 minutes. After draining off the excess bleach, the seeds were rinsed five times with sterile distilled water and then soaked overnight. The surface-sterilized vegetable pea seeds were sown at a depth of 2-3 cm in a sterilized plastic pot containing acid washed sterilized sand. After seed germination (around 3-4 days), a 1 ml aliquot of the respective dilution of the soil suspension was inoculated at the base of each seedling. The plants were grown in a growth chamber under controlled conditions, with a light intensity of 2400 Lux and a temperature ranging from a minimum of 13°C to a maximum of 24-25°C **(**plate 1)**.** The plants were watered every day, alternating with sterile nitrogen-free solution (Hoagland Solution). Uninoculated seeds were also included as check, maintained at a rate of 10% of the total pots. After 45 days of germination or at flowering, the root systems under individual dilution were examined and the presence or absence of nodules was recorded. Further the

MPN of native rhizobia nodulating Vegetable pea in a individual soil sample was obtained with the aid MPN table employing the formula:

$$\text{MPN} = \frac{m \times d}{v \times g}$$

Where,

m= likely number at dilution 1 in the series used for the entry (correspond to the particular number of + tube)
d= dilution represented by tube 1
v=volume of aliquot
g= weight of sample

2.3 Observation

In addition to nodulation, several plant growth parameters were recorded to determine the location-specific variations influencing the plant development.

- Dry shoot mass (g plant^{-1})
- Dry root mass (g plant^{-1})
- Total dry biomass (g plant^{-1})
- Amount of N2 fixed (Plant^{-1})

2.4 Soil Analysis

A composite soil sample particularly from plot was collected from different locations at 0-15cm depth. The soil samples were collected from five random spots in each plot and mixed into one composite sample. For chemical analysis soil samples were processed by shade drying and passed through 2mm sieve. In current experiment soil parameters such as pH, Electrical conductivity (EC), organic carbon (OC), available nitrogen (alkaline $KMnO_4$ method) was determined in year 2022. Soil pH and electrical conductivity (EC) was determined by (1:2.5 soil water suspension) using Beckman glass electrode pH meter and EC meter (Jackson, 1973). The air-dried soil samples were passed through 0.2mm sieve to analyze soil organic carbon (SOC). SOC was determined by modified Walkley and Black method (Jackson, 1973) using 1N $K_2Cr_2O_7$, conc. H_2SO_4, sodium fluoride, orthophosphoric acid and ferrous ammonium sulphate solution. Available (mineralizable) nitrogen (N) in soil was determined by using alkaline permagnate ($KMnO_4$-N) method (Subbiah and Asija, 1956).

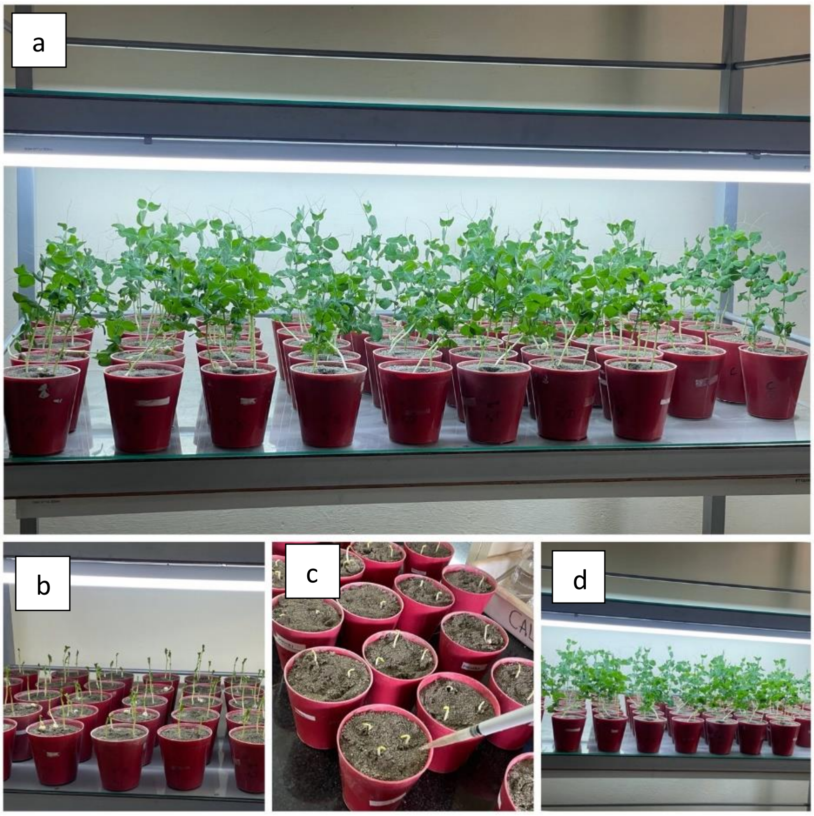

Plate 1. (a & d) Set up for enumeration of native rhizobia in soils of N-W Himalayas of Uttarakhand nodulating French bean under controlled conditions, (b & c) Germinating seeds in growth chamber

2.5 Statistical Analyses

A triplicate sample were exposed to assess the standard deviation among replications. Additionally, the correlations between native rhizobial population nodulating Vegetable pea and soil characteristics along with amount of biological nitrogen fixed was also determined using R software.

3. RESULTS AND DISCUSSION

The purpose of this experiment was to determine the optimal native rhizobial population required for efficient nodulation of *Pisum sativum*, leading to maximum dry matter production and nitrogen accumulation in legume crops. Most probable number (MPN) of vegetable pea rhizobia and BNF. By analysing the impact of soil suspension dilution (Table 4 & Fig. 2) on vegetable pea plant growth and nodulation were observed the outcomes explained below:

The physicochemical properties of soils, including pH, electrical conductivity (EC), nitrogen (N) content, and organic carbon (OC) percentage, varied across the studied villages in the Kumaon region. The pH of soils ranged from 5.42 (Sama) to 7.77 (Timiladiggi), indicating slightly acidic to near-neutral conditions. The soil from different areas exhibited different pH, highest pH value (7.77± 0.047) reported at Timiladiggi and lowest at Sama, the acidic soil (pH 5.42 ± 0.056) however soils from Sui, Leti, Baram, Khamaria, Chafi, Podhar, Raikot Kunwar, Daranti were found to be slightly acidic in nature. Generally, neutral to slightly acidic soils are considered conducive to Rhizobium proliferation, as observed by Vincent (1970) and later studies by Jaiswal et al. (2016), who suggested optimal nodulation and nitrogen fixation in slightly acidic soils. Electrical Conductivity (EC) The EC of soils ranged from 0.33 dS m^{-1} (Sui) to 0.87dS m^{-1} (Leti). A higher EC generally indicates higher salt content, which can impact microbial activity. The soils from Sui exhibited the lowest EC (0.33 ± 0.017 dS m^{-1}), which might be beneficial for Rhizobium survival. Studies by Somasegaran and Hoben (1994) emphasized that Rhizobium thrives best in low-salinity environments. Saline-alkaline soil poses a significant challenge in agriculture by disrupting soil microbial community structure and impairing plant growth (Zhang et al., 2024). Conversely, the highest EC recorded in Leti (0.87 ± 0.005dS m^{-1}) could inhibit bacterial colonization, as suggested by Zahran (1999). However, the soil sample from Leti were taken from pea rhizosphere therefore the resident rhizobium population were reportedly high. The effect of EC on nodulation needs further investigation, as the correlation between high EC and reduced nodulation was not clearly evident in this study. Nitrogen (N) Content in soil, from Leti(272.49 ± 12.27 kg ha^{-1})recorded the highest which falls under medium range, followed by Khamaria (230.24±6.73), Baram (223.80±9.94), Daranti (214.38±13.07) all under low range of Nitrogen, Chafi with lowest nitrogen levels (76.09 ± 10.22 kg ha^{-1})of all . The availability of nitrogen is crucial for plant growth and nodulation efficiency, as observed by Peoples et al. (1995), who linked higher nitrogen content to increased symbiotic nitrogen fixation. Organic carbon (OC) content also varied, with Khamaria exhibiting the highest OC (230.24±6.73) followed by Dhari (0.95±0.245), Daranti (0.93 ± 0.30%), Leti (0.923±0.12) and Timiladiggi (0.917±0.279).OC is a critical factor influencing soil microbial biomass and rhizobial populations. Higher OC content can enhance soil structure and nutrient availability, thus promoting microbial activity and pea nodulation. These results align with the findings of Sharma et al. (2019), who reported increased nodule formation and nitrogen fixation in soils with high organic matter. Overall Comparison of Villages Based on the combined analysis of physicochemical parameters, Khamaria, Daranti, Baram, and Timiladiggi showed the most favorable conditions for rhizobial proliferation and pea nodulation. The high nitrogen and organic carbon content in these regions indicate enhanced microbial activity and better pea nodulation, correlating with findings from similar studies in leguminous crops (Singh et al., 2021). In contrast, villages like Chafi and Sui, with lower nitrogen content and organic carbon, demonstrated less favourable conditions for nodulation, suggesting that improvements in soil organic matter might boost rhizobial populations and nitrogen fixation.

The Fig. 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 OC %, nitrogen intake varied amongst the various locations. The range of most probable number (MPN) of native vegetable pea rhizobia in soil was 300 to 10000 g^{-1} (Fig. 2) which was less than 0.01% percent of the culturable fraction of soil microbes. The correlation matrix and the correlation map reveals the relationship between the physicochemical and biological properties of the soils supporting vegetable pea nodulation in Kumaon region. Electrical conductivity (EC) shows a positive correlation with nitrogen content (r= 0.618) and organic carbon (OC) (r= 0.353), suggesting that higher EC levels are associated with increased nutrient availability. This aligns with the findings by Singh et al. (2021), who highlighted the role of electrical conductivity in influencing nutrient solubility in soil of hilly region. pH shows a significant positive correlation with Biological Nitrogen Fixation (BNF) (r= 0.534), indicating that neutral to slightly alkaline soils favour nodulation and subsequent nitrogen fixation. The observation is supported by a study (Sharma et al., 2019) reported optimal pea nodulation at pH values between 6.5 and 7.5. Organic Carbon (OC) demonstrated positive correlation with both shoot dry weight (SDW) and root dry weight (RDW) (r=0.35), suggesting that increased organic matter in soil enhances plant biomass, which is consistent with previous research by Verma et al. (2020), showing organic matter plays a vital role in soil fertility and plant growth. Most probable number (MPN), which reflects the rhizobial population, shows a positive correlation with SDW (r=0.543) and RDW (r= 0.522), indicating that higher rhizobial activity positively influences plant biomass production. This observation supports the notion that rhizobia contributes directly to plant growth by enhancing nutrient uptake efficiency (Kumar et al., 2021). Nitrogen (N) content correlates strongly with EC (r= 0.618), suggesting a link between nitrogen availability and soil conductivity. However, nitrogen content shows a weak correlation with Biological Nitrogen Fixation (BNF) (r= -0.16). Total Nitrogen (TN) exhibited a significant positive correlation with Biological nitrogen fixation (BNF) (r= 0.907), reinforcing the essential role of BNF in contributing to nitrogen content in soils where peas are cultivated.

Notably, Daranti and Khamaria stand out with elevated Rhizobia counts of 10,000 and 5800 respectively. On contrary, Champhi and Podhar characterized by lower Rhizobia counts of 170to 310, underlining the impact of Rhizobia abundance on plant biomass. Although the Rhizobia count of Champhi was least among all 12 sites, which is close to the threshold level (Nazih and Weaver, 1994). Sama, Daranti, Dhari, Sui, Liti, Timiladiggi and Raikot Kunwar demonstrate varying patterns, emphasizing the complex relationship between Rhizobia populations and plant biomass accumulation. For example, regardless of Daranti's high Rhizobia count of 10,000, the BNF is reportedly low as compared to other locations this suggests that whilst the rhizobial count was higher in Daranti (Munsiari, Pithoragarh), actual nitrogen fixation potential was not as high compared to the Timiladiggi (Tarikhet, Almora) thus need the identification and inoculation of efficient rhizobia to reach up to the maximum potential of nitrogen fixation.

Among all locations, the sample of Raikot Kunwar were collected from the rhizospheric soil of Vegetable pea. In Raikot Kunwar, (Block Champawat, Lohaghat), the rhizobial count was around 3100 $g^{-1}$of soil, and it contributed to a reasonable amount of biological nitrogen fixation of 8.41 mg $plant^{-1}$.

In Timiladiggi (Tarikhet, Almora) the native population was 1699 g^{-1} of soil, with maximum 18.01 mg $plant^{-1}$ BNF among all the locations. This may be attributed to the presence of efficient rhizobium isolates in the soils of this region that can perform efficiently.

In Leti, (Kapkot block, Bageshwar), the average rhizobial count was 3100 g^{-1}, the biological nitrogen fixation was 8.53 mg $plant^{-1}$. Whereas, Sama of the same block have the native rhizobial population of 580 g^{-1} with nitrogen fixation value was 4.26 mg $plant^{-1}$ which was still much greater than Baram and Sui despite the low rhizobial count. This implies that the strain of rhizobia found in Leti was more efficient in fixing nitrogen than than Baram and Sui but there is a possibility to increase the BNF through identification and inoculation of efficient rhizobia.

The native rhizobial population of Baram (Block Pithoragarh), NH 309 (Block Gadarpur) and Sui (Block Lohaghat) of Champawat district was 1000, 1700 and 1700 g^{-1} of soil, respectively displayed intermediate nitrogen fixation values of with 2.32, 3.75 and 3.15 mg $plant^{-1}$, respectively, thus require the inoculation of specific efficient rhizobia.

High native rhizobial count and BNF in Timiladiggi, Daranti, Raikot Kunwar, and Khamaria emphasize a positive correlation between rhizobial numbers and efficacy of biological nitrogen fixation, highlight the potential of host legume *i.e.* Vegetable pea or homologues legume through the stimulatory effect resulting higher number of rhizobia and BNF in plant. (Janatiet al., 2021; Dinnage et al., 2019) 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^{-1}) 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. High rhizobial populations can contribute to more effective nitrogen fixation *i.e.* Daranti, Raikot Kunwar, and Khamaria, since higher rhizobial counts typically correlate with better nitrogen fixation values (Dinnage et al., 2019; Raverkar, 2017; Naziah and Weaver, 1994). Also, the soil sample are found to be low in available nitrogen content thus help to stimulate nitrogen fixation and Nodulation (Hellsten and Kerstin 2000).

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 utilized 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. 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. Furthermore, biological nitrogen fixation (BNF) exhibits a significant positive correlation with nitrogen uptake in roots 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 nitrogen fixation and plant growth, which is essential for photosynthesis.

Table 2. Physiochemical and biological properties of growing areas in Kumaon region of Uttarakhand

Village	pH	EC (dSm^{-1})	N (Kg ha^{-1})	OC %
Podhar	6.65±0.167	0.46±0.066	138.26±7.84	0.63±0.214
Timiladiggi	7.77±0.047	0.61±0.033	152.84±9.13	0.917±0.279
Chafi	6.55±0.136	0.35±0.021	76.09±10.22	0.863±0.245
Dhari	7.03±0.125	0.44±0.012	124.48±11.45	0.95±0.245
Raikot Kunwar	5.81±0.365	0.40±0.005	203.08±7.59	0.717±0.182
Leti	6.09±0.082	0.87±0.005	272.49±12.27	0.923±0.12
Sama	5.42±0.022	0.72±0.016	187.05±9.42	0.77±0.222
Daranti	6.21±0.066	0.49±0.005	214.38±13.07	0.933±0.304
Baram	5.71±0.008	0.64±0.005	223.80±9.94	0.62±0.283
Sui	6.03±0.121	0.33±0.017	196.61±7.11	0.64±0.371
Khamaria	6.55±0.255	0.66±0.050	230.24±6.73	0.973±0.349
Nh 309	6.47±0.170	0.55±0.045	226.75±12.95	0.783±0.146

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

Table 3. Correlations between various physicochemical and biological properties of vegetable pea growing soils in Kumaon region of Uttarakhand

	EC	pH	OC	N	MPN	SDW	RDW	BNF	TN
EC	1.000	-0.146	0.353	0.618	0.096	0.248	-0.09	-0.05	-0.21
pH	-0.146	1.000	0.408	-0.460	-0.061	0.317	0.275	0.534	0.545
OC	0.353	0.408	1.000	0.026	0.438	0.388	0.035	0.327	0.237
N	0.618	-0.460	0.026	1.000	0.469	0.117	0.088	-0.16	-0.28
MPN	0.096	-0.061	0.438	0.469	1.00	0.543	0.522	0.087	-0.14
SDW	0.248	-0.317	0.388	0.117	0.5435	1.000	0.566	0.501	0.141
RDW	-0.097	0.275	0.035	0.088	0.522	0.566	1.00	0.198	-0.04
BNF	-0.0502	0.534	0.327	-0.16	0.087	0.501	0.198	1.00	0.907
TN	-0.210	0.545	0.237	-0.28	-0.14	0.141	-0.04	0.907	1.00

*** Represent significance at 0.01% LOS and * Represent the significance at 5 % LOS*

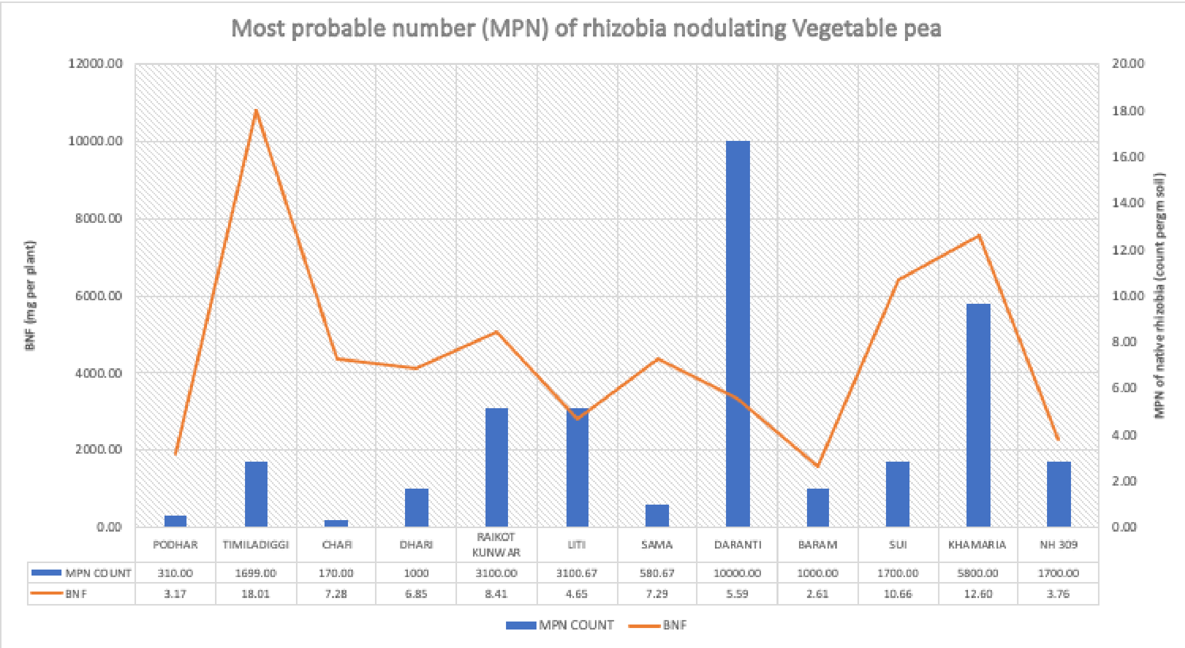

Fig. 3. Most probable number (MPN) of rhizobia nodulating Vegetable pea in soils of hilly region of Uttarakhand and Biological Nitrogen Fixation (BNF)

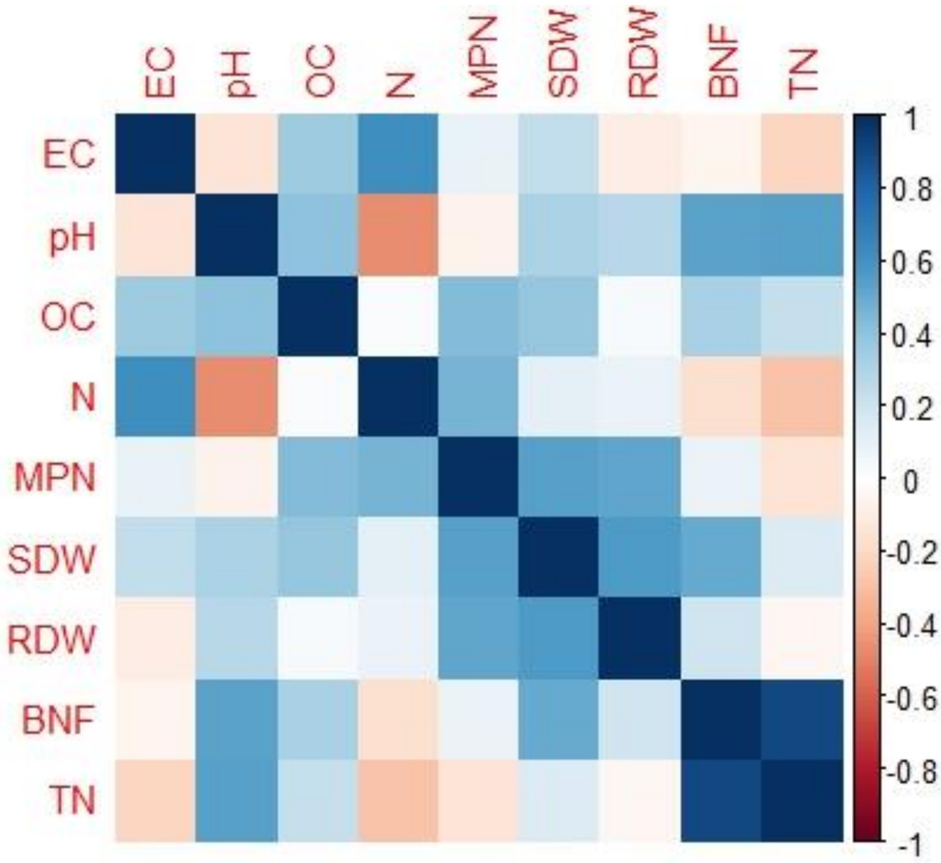

Fig. 4. Correlation map showing correlations between various physicochemical and biological properties of vegetable pea growing soils in Kumaon region of Uttarakhand

Table 4. Effect of soil suspension on Vegetable pea growth under MPN setup

Location	Most Probable Number of Rhizobia	Biological nitrogen fixation	Shoot dry weight	Root dry weight	Total dry weight	Shoot N	Root N
	(Count g^{-1})	(mg per plant)	(g per plant)	(g per plant)	(g per plant)	%	%
Podhar	310.00	3.17	0.590	0.505	1.095	1.404	1.317
Timiladiggi	1699.00	18.01	0.705	0.413	1.118	2.853	1.793
Chafi	170.00	7.28	0.543	0.300	0.843	2.380	1.280
Dhari	1000	6.85	0.476	0.336	0.812	2.464	1.370
Raikot Kunwar	3100.00	8.41	0.597	0.461	1.058	2.214	1.429
Leti	3100.67	4.65	0.603	0.330	0.933	1.680	1.420
Sama	580.67	7.29	0.580	0.281	0.861	1.737	1.457
Daranti	10000.00	5.59	0.678	0.461	1.138	1.625	1.230
Baram	1000.00	2.61	0.477	0.331	0.808	1.568	1.485
Sui	1700.00	10.66	0.512	0.311	0.823	2.548	1.547
Khamaria	5800.00	12.60	0.607	0.506	1.113	2.269	1.653
Nh 309	1700.00	3.76	0.520	0.325	0.845	1.625	1.480

4. CONCLUSION

This study emphasizes the interconnectedness of soil organic carbon, rhizobial populations, biological nitrogen fixation (BNF), and available nitrogen in pea-cultivated soils across the Kumaon region of Uttarakhand. The findings reveal that soils richer in organic carbon tend to harbor larger rhizobial populations, which subsequently enhance nitrogen fixation. A moderately positive correlation was identified between organic carbon and the most probable number (MPN) of rhizobia, along with a positive link between MPN and BNF, underscoring the significance of improving soil organic matter to promote nitrogen fixation in legumes like peas.

Interestingly, a weak negative correlation was observed between available nitrogen and BNF, highlighting the role of nitrogen feedback inhibition in soils with elevated nitrogen levels. This suggests that managing nitrogen inputs carefully is essential to prevent adverse effects on symbiotic nitrogen fixation. Considering the diverse climatic conditions across Kumaon, from the colder, high-altitude areas such as Bageshwar and Pithoragarh to the milder, low-altitude regions like Nainital and Almora, the results offer key insights. Cooler and wetter climates at higher elevations appear more conducive to organic carbon build up, fostering favourable conditions for rhizobial activity and nitrogen fixation. On the other hand, warmer and drier areas face challenges such as reduced soil moisture and organic matter, which may limit rhizobial proliferation and nodulation.

In summary, the study highlights the need for region-specific soil management strategies, particularly those aimed at enhancing organic matter, to boost legume productivity and nitrogen fixation in Kumaon. Customized approaches, taking into account the region's climatic variability, could improve nitrogen fixation efficiency, reduce reliance on chemical fertilizers, and support sustainable agricultural practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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