

**POPULATION ESTIMATION OF SOME RHIZOBIA FROM SOIL
SAMPLES COLLECTED FROM HIGH HIMALAYAN
HABITATS OF MANANG, NEPAL**

**A Dissertation Submitted
for the Partial Fulfillment of the Requirements for the
Master's of Science in Botany**

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RECOMMENDATION LETTER

This is to certify that the dissertation work entitled "**Population Estimation of some rhizobia from soil samples collected from high Himalayan Habitats of Manang, Nepal**" has been carried out by Mr. Deepak Mahat under my supervision. The result of this research work has not been submitted for any academic degree to the best of my knowledge. I recommend his thesis for partial fulfillment of his Master's Degree in Botany, Tribhuvan University.

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LETTER OF APPROVAL

This dissertation paper submitted by **Mr. Deepak Mahat** entitled "**Population Estimation of some rhizobia from soil samples collected from high Himalayan Habitats of Manang, Nepal**" has been accepted as a partial fulfillment of Master of Science in Botany.

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ABSTRACT

Rhizobium meliloti, *R. leguminosarum* bv. phaseoli, *R. leguminosarum* bv. trifolii, and *Bradyrhizobium* spp. were isolated from the root nodules of *Argyrolobium roseum*, *Phaseolus vulgaris*, *Trifolium repens* and *Indigofera* spp. respectively from Manang. All the four rhizobial strains were isolated and purified using Yeast extract Mannitol Agar (YEMA) medium and characterized depending upon their growth responses on YEMA-CR medium, YEMA-BTB medium, YEMB medium, Gram staining and rate of multiplication of the bacterial cells. All the isolates were authenticated by using their specific host plant by infection test on sterilized filter paper and plastic pouch using modified Jensen's nitrogen free medium. The bacterial population of *R. meliloti*, *R. leguminosarum* bv. phaseoli, *Bradyrhizobium* spp. and *B. japonicum* was analyzed for each altitude ranging from Khangsar Goth (4190m asl) to Chame (2705m asl) with an altitude difference of about 100m. The effectiveness of *R. meliloti*, *R. leguminosarum* bv phaseoli, *Bradyrhizobium* spp. and *B. japonicum* was determined on the basis of total nitrogen content of the root nodules of *Trigonella corniculata*, *Phaseolus vulgaris*, *Dolichos lablab* and *Glycine max* respectively grown separately in soil of Manang and Kathmandu and showed that *R. leguminosarum* bv. phaseoli was the most effective one among others. Soil in each altitude was analyzed on the basis of pH, moisture, nitrogen, phosphorus, potassium and organic matter content and that overall content was satisfactory for the growth and distribution of bacterial cells. The statistical analysis also revealed the significant relationship between the altitude and no. of bacteria. The bacterial population decreased with an increase in altitudes.

EXECUTIVE SUMMARY

Nitrogen is the most common limiting factor for primary production due to its significant presence in most of the biomolecules. It is the structural component of aminoacids, which are the building blocks of protein. Thus the symbiotic bacteria i.e., rhizobia are responsible for the conversion of atmospheric nitrogen into combined form of organic compound. Successful legume-*Rhizobium* symbiosis definitely increase the incorporation of BNF into soil ecosystems. The legume is the macrosymbiont and the *Rhizobium* is the microsymbiont. The microsymbionts i.e., rhizobia are classified on the basis of the cross inoculation groups of the legumes nodulated by these rhizobia.

The present investigation focuses on the characterization, authentication of rhizobia from Manang as well as their effectiveness and the bacterial population in soils at each altitude ranging from 2705m to 4190m at an altitudinal difference of about 100m. Soil from these altitude were also analyzed for p^H, moisture, nitrogen, phosphorus, potassium and organic matter content.

Rhizobium meliloti (R101), *R. leguminosarum* bv. phaseoli (R103), *R. leguminosarum* bv. trifolii (R109) and *Brdayrhizobium* spp. (B111) were isolated from the root nodules of *Argyrolobium roseum*, *Phaseolus vulgaris*, *Trifolium repens* and *Indigofera* spp. respectively growing in Chame (Manang) area. All the four rhizobial strains were isolated in pure culture on Yeast Extract Mannitol Agar medium. The bacterial colonies grown on YEMA-CR were creamy white to translucent, watery and raised (concave). R101, R103 and R109 showed the acid producing behaviour on YEMA-BTB and YEMB medium where as B111 showed the alkali producing behaviour. All the four isolates showed Gram negative reaction taking up red coloration. The multiplication rate of rhizobial cells increased upto first 72 hours period and later it lowered down showing a hyperbolic curve.

The bacterial isolates R101, R103, R109 and B111 were authenticated by plant infection tests taking up specific hosts *Trigonella corniculata*, *Phaseolus vulgaris*, *Trifolium repens* and *Desmodium* spp. respectively. Pouch test technique was

used. After 18 days of inoculation visible nodules appeared, authenticating the inoculate of bacterial suspension to be rhizobia for all cases.

Soil contained rhizobial population was estimated using standard MPN (Most Probable Number) count method. The population of *R. meliloti*, *R. leguminosarum* bv phaseoli, *Bradyrhizobium* spp. and *Bradyrhizobium japonicum* were estimated for each altitude ranging from Khangsar Goth (4190m) to Chame (2705m) with a difference of about 100m. Throughout the study area, Chame contained the highest population of *R. leguminosarum* bv. phaseoli (70×10^3 cells gm^{-1} soil), *R. meliloti* (48×10^3 cells gm^{-1} soil), *Bradyrhizobium* spp. (112×10^2 cells gm^{-1} soil) and *B. japonicum* (40×10^2 cells gm^{-1} soil). The rhizobial population was estimated on the basis of the nodulation of their specific hosts (*Trigonella corniculata*, *Phaseolus vulgaris*, *Dolichos lablab* and *Glycine max*) and calculated from the Table VIII₂ of Fischer and Yates (1963).

The effectiveness of *R. meliloti*, *R. leguminosarum* bv. phaseoli, *Bradyrhizobium* spp. and *B. japonicum* was determined by analyzing the nitrogen content of the root nodules of *Trigonella corniculata*, *Phaseolus vulgaris*, *Dolichos lablab* and *Glycine max* respectively grown separately in the soils of Manang. They were also grown in soil samples of Kirtipur, Kathmandu. *Phaseolus vulgaris* contained 5.24% total nitrogen in the root noudles and considered *R. leguminosarum* bv. phaseloi as the most effective strain of Manang. *Glycine max* with 3.98% total nitrogen considered *B. japonicum* as the most ineffective belonging to Manang. The soil of each altitude was analyzed for p^H, moisture (%), total nitrogen (%), phosphorus (kg/ha), potassium (kg/ha) and organic matter (%) content and overall content was satisfactory for plant growth. The statistical analysis also revealed the significant relationship between the altitude and no. of bacteria. The bacterial population decreased with the increase in altitudes.

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ACRONYMS

%	Percentage
asl	above sea level
B111	<i>Bradyrhizobium</i> spp. strain
BNF	Biological Nitrogen Fixation
CDB	Central Department of Botany
<i>et al</i>	<i>et albeli</i>
gm ⁻¹	per gram
ha	Hectare
K	Potassium
kg	Kilogram
m	Metre
ml	millilitre
MPN	Most Probable Number
N/N ₂	Nitrogen
no.	number
P	Phosphorus
p ^H	Negative logarithm of hydrogen ion concentration
R101	<i>Rhizobium meliloti</i> strain
R103	<i>Rhizobium leguminosarum</i> bv. phaseoli strain
R109	<i>Rhizobium leguminosarum</i> bv. trifolii strain
ROT	Range of transition
T.U.	Tribhuvan University
YEM	Yeast Extract Mannitol
YEMA	Yeast Extract Mannitol Agar
YEMA-BTB	Yeast Extract Mannitol Agar-Bromo Thymol Blue
YEMA-CR	Yeast Extract Mannitol Agar-Congo Red
YEMB	Yeast Extract Mannitol Broth