

**STUDY ON PRODUCTION AND VIABILITY OF ENCAPSULATED
BRADYRHIZOBIUM JAPONICUM AND THEIR EFFECT ON *VIGNA
UNGUICULATA***

A Dissertation Submitted To The Central Department Of Botany
For The Partial Fulfillment Of Master's Degree In Botany.

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LETTER OF APPROVAL

This Dissertation work entitled '**STUDY ON PRODUCTION AND VIABILITY OF ENCAPSULATED *BRADYRHIZOBIUM JAPONICUM* AND THEIR EFFECT ON *VIGNA UNGUICULATA***' submitted by **Til kumari Chhetri** has been accepted and approved for the partial fulfillment of Master's Degree in Botany.

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RECOMMENDATION

It is hereby recommended that **Ms. Til Kumari Chhetri**, a M.Sc. Botany final year student at Tribhuvan University, Institute of Science and Technology, Kirtipur, Kathmandu has carried out research work entitled “**STUDY ON PRODUCTION AND VIABILITY OF ENCAPSULATED BRADYRHIZOBIUM JAPONICUM AND THEIR EFFECT ON VIGNA UNGUICULATA**” under our supervision. This work has not been submitted for any other academic degree.

This dissertation has been recommended for acceptance as a partial fulfillment of the requirement of Master’s Degree in Botany at Institute of Science and Technology, Tribhuvan University.

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Abstract

Rhizobium a nitrogen fixing bacteria is the essential feature of leguminous plants. Increased cultivation of legume is essential for the restoration of nutrient deficient soil. This study was aimed to isolate, identify, mass culture and immobilize *Rhizobium* in encapsulated form and test their viability effect on the *Vigna unguiculata*. The nitrogen fixing *Rhizobium* was taken from the root nodules of the plant *Glycine max*. Root nodules were collected from the soybean plant planted at the pot at rooftops of house at Putalisadak and they were sterilized, grinded and cultured aseptically in YEMA media containing Congo red. The obtained colony were selected and sub-cultured to get the pure culture of *Rhizobium*. Different biochemical tests as catalase production, pH tolerance test, NaCl tolerance test, Penicillin resistance test and nodulation test were conducted which proved the *Bradyrhizobium japonicum* as the slow growing species. The test showed positive result of catalase production test and nodulation test whereas the pH tolerance test showed more tolerance to the acidic pH and no tolerance of alkaline pH. Similarly result showed that *Bradyrhizobium japonicum* can tolerate 1% and 2% NaCl concentration and it is less resistance to the penicillin disc of 10mg. The mass culture and encapsulation with sodium alginate and adding sucrose as nutrient proved the simplicity for handling. It also resulted that altogether 548 beads can be prepared from the 100ml of the cultured broth and these beads are viable for more than six months. The beads were viable for 190 days and more at 1%, 2% and 3% sucrose concentration but they were viable only for 145 days at 5% and 10% sucrose concentration. There is the significant difference in the shoot length of *Vigna unguiculata* in *Bradyrhizobium japonicum* inoculated samples and the un-inoculated samples.

Table of content

| Title | Page No. |
|---|-----------------|
| Abbreviation | |
| 1. Introduction | 1-14 |
| 1.1. Background | 1 |
| 1.2. Systematic classification | 5 |
| 1.3. Rhizobia | 5 |
| 1.4. History of rhizobial inoculants | 6 |
| 1.5. Need of inoculation | 7 |
| 1.6. Types of inoculation | 8 |
| 1.7. Justification of the study | 11 |
| 1.8. Objectives of the study | 13 |
| 1.9. Limitation of study | 14 |
| 1.10. Hypothesis | 14 |
| 2. Literature review | 15-19 |
| 3. Methodology | 20-28 |
| 3.1 Materials | 20 |
| 3.2 Composition of YEMA media | 20 |
| 3.3 Preparation of media | 20 |
| 3.4 Isolation of the rhizobial strain | 21 |
| 3.4.1 Collection of root nodules | 21 |
| 3.4.2 Surface sterilization of root nodules | 21 |
| 3.4.3 Preparation of inoculants | 21 |
| 3.5 Identification of the <i>Rhizobium</i> | 22 |
| 3.5.1 Catalase production test | 22 |
| 3.5.2 pH tolerance test | 22 |
| 3.5.3 NaCl tolerance test | 22 |
| 3.5.4 Penicillin resistance test | 22 |
| 3.5.5 Nodulation test | 23 |

| | |
|--|--------------|
| 3.5.6 Color change of BTB | 24 |
| 3.6 Mass production of <i>Rhizobium</i> | 24 |
| 3.6.1 Starter culture of <i>Rhizobium</i> | 24 |
| 3.6.2 Mass culture of <i>Rhizobium</i> | 24 |
| 3.7 Immobilization of the <i>Rhizobium</i> | 25 |
| 3.7.1 Encapsulation of <i>Rhizobium</i> with sodium alginate | 25 |
| 3.8 Cross inoculation of the <i>Bradyrhizobium japonicum</i> with cowpea | 26 |
| 3.9 Viability test of encapsulated beads | 27 |
| 4. Results | 29-38 |
| 5. Discussion | 39-42 |
| 6. Conclusion | 43-44 |
| 7. Recommendation | 45 |
| References | 46-56 |

Annex

Lists of tables:

| | |
|---|----|
| Table I: soybean productivity and economic gain due to rhizobial inoculants application | 11 |
| Table1: Enumeration of organism by spread plate technique. | 30 |
| Table2: Biochemical tests on <i>Rhizobium</i> | 31 |
| Table3: Number of beads formed from the 25ml of cultured solution | 32 |
| Table4: viability test of the encapsulated beads of <i>Bradyrhizobium japonicum</i> | 34 |
| Table5: the shoot length of the plants of the cowpea in inoculated and the un-inoculated samples. | 35 |
| Table 6: Result of Mann-Whitney U test performed on shoot length of the cowpea. | 37 |

Lists of figures:

| | |
|---|----|
| Fig 1: shoot length of cowpea inside the greenhouse | 36 |
| Fig 2: shoot length of cowpea in the shaded area | 36 |
| Fig 3: shoot length of cowpea in external environment | 37 |

Abbreviation

| | | |
|-------------------|---|--|
| C.F.U | = | Colony forming unit |
| ml | = | Milliliter |
| Cm | = | Centimeter |
| % | = | Percentage |
| ⁰ C | = | Degree Celsius |
| M | = | Molar concentration |
| Ppm | = | Parts per mole |
| YEMA | = | Yeast Extract Mannitol Agar |
| pH | = | Percentage of hydrogen |
| NaCl | = | Sodium chloride |
| NaOH | = | Sodium hydroxide |
| HCl | = | Hydrochloric acid |
| CaCl ₂ | = | Calcium chloride |
| Rpm | = | Rotation per minute |
| YEM | = | Yeast Extract Mannitol |
| BTB | = | Bromothymol Blue |
| USDA 136 | = | United State Department of Agriculture (<i>Rhizobium</i> collection at Beltsuille originally isolated from soybean) |