# Identification Of Temperature Tolerant Strain Of *Rhizobium* Isolated From *Glycine max* And Study Of Its Metabolites

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***Abstract***

This study focused on isolating a heat-tolerant *Rhizobium* strain from *Glycine max* (soybean) and examining its metabolite profile. Identified through 16S rRNA sequencing, the strain showed tolerance at 35°C to 45°C and produced beneficial compounds such as IAA, proline, trehalose, flavonoids, and phenolics. HPLC analysis highlighted their role in enhancing stress tolerance and plant development. These results indicate the strain's potential for biofertilizer use to boost crop resilience under elevated temperatures.

Index terms – Heat-tolerant *Bradyrhizobium*, Soybean (*Glycine max*) symbiosis, HPLC metabolite profiling, IAA and stress metabolites, Biofertilizer development.

## 1. INTRODUCTION

### 1.1 Leguminous Plants

Plants from the Fabaceae family, commonly known as legumes, play an essential role in agriculture due to their ability to fix atmospheric nitrogen. This natural process boosts soil fertility and minimizes the use of chemical fertilizers. Their symbiotic interactions with nitrogen-fixing microbes make them key components of eco-friendly and sustainable farming methods.

### 1.2 Rhizobium

Rhizobium species establish symbiotic partnerships with leguminous plants, enabling the biological fixation of nitrogen. These beneficial soil bacteria convert nitrogen from the air into forms that plants can absorb and utilize. However, environmental stresses, especially high temperatures, can reduce their effectiveness, highlighting the importance of heat-tolerant strains. Prominent Rhizobium species include *R. leguminosarum*, *R. etli*, *R. phaseoli*, *R. tropici*, *R. galegae*, and *Bradyrhizobium japonicum*. The symbiotic process is initiated by plant-released flavonoids and involves complex molecular signaling mechanisms (Zahran HH. 1999), (Brechenmacher L, Lei Z, Libault M, Findley S, Sugawara M, Sadowsky MJ, et al. 2010 Aug).

### 1.3 *Glycine max L.* (Soybean)

Soybean, scientifically named *Glycine max*, is classified under the Kingdom Plantae.It's It's categorized under the Phylum Magnoliophyta, falls within the Class Magnoliopsida, and is ordered under Fabales. It is a member of the Fabaceae family, Genus Glycine, and Species max. It is part of the Fabaceae family, categorized under the Genus Glycine, with the species identified as max. Soybean (*Glycine max*) is a widely grown legume known for its high nutritional value, containing approximately 38–40% protein and 18–20% oil. It serves various purposes in food, animal feed, and industrial applications. Morphologically, soybeans are annual plants with three-part leaves and seed-bearing pods. Nutritionally, they are packed with proteins, fats, carbohydrates, vitamins, minerals, and beneficial secondary metabolites like saponins and isoflavones(Liu K. 1997), (Medic J, Atkinson C, Hurburgh CR. 2014).

### 1.4 *Bradyrhizobium japonicum*

*Bradyrhizobium japonicum* is a slow-growing, Gram-negative bacterium that forms nitrogen-fixing nodules on soybean roots. It belongs to the group of Plant Growth-Promoting Rhizobacteria (PGPR) and supports plant health by fixing nitrogen, solubilizing phosphorus, producing growth hormones, and offering protection against plant pathogens (Liu Y, Chen Z, Ng CK, Lu Y 2019), (Bhattacharjee RB, Singh A, Mukhopadhyay SN. 2008, *et al.)*. This bacterium also plays a role in maintaining soil quality and shows resilience in adverse conditions. The heat-tolerant Bradyrhizobium japonicum strain demonstrated the ability to suppress harmful pathogens, offering protection to plants and supporting their growth even under elevated temperature conditions.

### 1.5 Temperatures-Tolerant Strains

Soil temperatures ranging from 35 °C to 45 °C can negatively affect the survival and nodulation efficiency of many *Rhizobium* species (Hungria M, Vargas MA, 2005)However, some *Bradyrhizobium* strains exhibit the ability to withstand such high temperatures while still forming successful symbiotic relationships with host plants, (Sadowsky MJ, Bohlool BB, 1986) Identifying and studying these heat-tolerant *Bradyrhizobium* strains is especially important in tropical regions like India, where elevated temperatures are common. Conventional carriers like lignite may underperform under these conditions, whereas gum-based alternatives such as Arabic and Guar gums offer better performance. These gums retain moisture more effectively and

enhance bacterial adhesion to seeds, making them more suitable for delivering inoculants under heat stress, (Basak N, Ghosh A, 2014)

**1.6 Biofertilizers**

Biofertilizers are microbial formulations that promote plant growth by improving the availability of nutrients to plants. Important biofertilizer organisms include *Rhizobium* and *Azotobacter* for nitrogen fixation, and various phosphate-solubilizing bacteria (PSB). These eco-friendly alternatives reduce reliance on chemical fertilizers and support sustainable farming practices (Giller KE, 2001), (Mestrovic T, 2019).

**Benefits of Biofertilizers:**

- Biological nitrogen fixation (e.g., *Rhizobium*, *Azospirillum*)
- Phosphorus solubilization (e.g., PSB, Mycorrhiza)
- Improved soil structure and fertility
- Environmentally sustainable and economically viable
- Boosted plant growth and agricultural productivity

**1.7 Biological Nitrogen Fixation (BNF)**

Biological Nitrogen Fixation is the process by which microbes convert atmospheric nitrogen ($N_2$) into ammonia ($NH_3$), a form plants can utilize. In legumes, *Rhizobium* enters through root hairs and initiates the formation of nodules via infection threads (Miller KJ, Kennedy C, 2013).Within these nodules, the enzyme nitrogenase catalyzes the following reaction-

$$N_2 + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$ (Verma A, Yadav RK, Choudhary M,2016)

The host plant supplies carbon compounds to the bacteria in return for fixed nitrogen (Sprent JI,2007), (Vance CP, Graham PH, Schubert KR,1998).

**1.8 Metabolites and Plant Growth-Promoting Factors-**

- **Primary Metabolites**: Include essential compounds such as amino acids, sugars, and fats, which support growth and metabolic activities (Harper JE, Gibson AH,1987),( Rivas R, Velázquez E, Martínez-Álvarez P, et al.,2009)
- **Secondary Metabolites**: Though not directly involved in basic life functions, these compounds—like flavonoids, alkaloids, and saponins—play crucial roles in plant defense and ecological interactions. In soybeans, isoflavones (e.g., genistein) contribute to stress resistance and offer health benefits (Zhang Y, Li Y, Yang M, et al.,2015),(Zamboni A, La Porta N, Gallo C, et al.,2009),( Janczarek M, Kaczmarek M, Bączek-Kwinta R, et al.,2017).

Plant growth depends on a combination of biological, chemical, and environmental factors. Beneficial microbes like PGPR (*Rhizobium*, *Azospirillum*) and mycorrhizal fungi (*Glomus*) support plant development by improving nitrogen fixation, hormone synthesis, and nutrient uptake (Bashan Y, de-Bashan LE,2005). Chemically, essential macronutrients (N, P, K, Ca, Mg, S) and trace micronutrients (Fe, Zn, Mn, Cu, B, Mo, Cl) are crucial for metabolic and physiological processes (Mengel K, Kirkby EA,2001). Environmental conditions—including light, temperature, water, and soil quality—directly affect photosynthesis, respiration, and nutrient absorption efficiency in plants (Taiz L, Zeiger E, 2010).

## 2. METHODOLOGY

**2.1 Isolation, purification and screening of *Rhizobium* strain-**

YEMA and CRYEMA media were prepared, pH-adjusted, and sterilized via autoclaving. Soybean root nodules were surface-sterilized, crushed aseptically, and plated on YEMA. Resulting colonies were purified using CRYEMA. Isolates were tested for growth at different temperature ranging from 35°C to 45°C for assessing temperature tolerance.

**2.2 Characterization and Identification of Bacterial Isolates**

a) **Colony and Morphology**: *Rhizobium* isolates cultured on YEM agar were examined for morphological characteristics such as shape, size, pigmentation, and surface texture following 2–3 days
b) **Gram Staining**: The Gram staining procedure was employed, to categorize isolates as Gram-positive or Gram-negative.
c) **pH Tolerance Assay**: The isolates were cultured in YEM broth adjusted to pH levels ranging from 4.0 to 9.0. Following incubation, growth was verified by streaking on YEMA plates and incubating at 30°C for 24 to 48 hours.
d) **Salt Tolerance Assay**: Isolates were grown on YEM agar containing various NaCl concentrations (0.5–4%) to evaluate salt tolerance through colony enumeration.
e) **Antibiotic Sensitivity**: The Kirby-Bauer disc diffusion method was applied on YEM agar to assess resistance against Penicillin and Tetracycline (10 µg each), with inhibition zones recorded after 5–7 days.
f) **Heavy Metal Sensitivity**: The impact of Mercury Chloride ($HgCl_2$) at 0.01% and 1% concentrations on bacterial growth was assessed on YEMA plates incubated at 30°C for 48 hours.
g) **BTB Indicator Test**: Bromothymol blue-enriched YEMA medium was used to differentiate between fast and slow-growing strains. Acid production was indicated by a color shift to yellow after 48 hours of incubation.
h) **Starch Hydrolysis Test**: The ability to utilize starch was tested on starch agar; clear halos after iodine application signified starch degradation.
i) **Urease Activity**: Christian's urea agar slants were used to detect urease activity; a pink color change after incubation at 37°C denoted a positive result.

j) **Catalase Assay**: The presence of catalase enzyme was confirmed by immediate bubble formation upon the addition of hydrogen peroxide (30%) to bacterial colonies.
k) **Citrate Utilization**: Growth was observed on Simmon's citrate medium and with a shift in color from green to blue indicated the organism's capacity to use citrate as a sole carbon source.
l) **Indole Production Test**: Isolates were incubated in peptone water at 37°C overnight, followed by addition of Kovac's reagent. A red layer is formed, that indicated positive indole production.

### 2.3 Molecular Characterization

The test isolate was sent to BioKart Bengaluru for genome sequencing to confirm its identity.

### 2.4 Indole-3-Acetic Acid (IAA) Detection via HPLC

IAA was analyzed using a Waters HPLC system equipped with a UV detector and Thermo C18 column. The mobile phase (Methanol:1% Acetic Acid, 60:40) was run at 1 mL/min, with detection at 360 nm and ~5.649 min retention. A 1000 ppm IAA stock was diluted to 10 µg/mL, and 20 µL of centrifuged broth was injected.

### 2.5 Liquid Biofertilizer Development

*Rhizobium* was cultured in YEM broth for 7 days at 28 ± 2°C to reach a cell concentration of approximately $1\times10^9$ cells/mL. The number of viable cells was determined using the plate count technique. Quality assessment involved pH, contamination check, and Gram staining. Cultures were mixed with autoclaved Guar gum.

### 2.6 Seed Trial

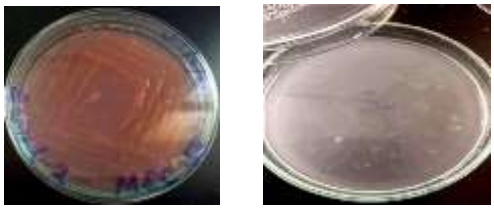
Seeds were surface-sterilized, soaked, and categorized into three treatments: coated with Guar gum, treated with biofertilizer, and untreated controls. Germination was conducted on moist filter paper under controlled conditions, followed by planting in sterile soil to assess growth and nodulation.

### 2.7 Pot Trial

Seeds from each treatment group were sown in pots. Parameters like germination time, viability, growth rate, and plant height were monitored to assess the biofertilizers effectiveness.

## 3. OBSERVATIONS-

**a)** Gummy colonies were obtained on YEMA and CRYEMA plates.



(Fig-1)

**b**) Biochemical tests-

- Citrate Utilization: Blue coloration indicated positive citrate utilization.
- BTB Indicator Test: Yellow coloration indicated moderate-growing, acid-producing strains.
- Urease Activity: Pink coloration on urea slants indicated positive Urease activity.
- Starch Hydrolysis Test: Clear halos around colonies confirmed starch degradation.
- Gram staining showed pink/red-colored, rod shaped cells.
- Indole Production Test: The development of a red-colored layer upon addition of Kovac's reagent indicated the production of indole by the isolates.
- Catalase Assay: Immediate bubbling confirmed Catalase enzyme presence.
- Heavy Metal Sensitivity: Growth inhibited at 1% $HgCl_2$; partial tolerance observed at 0.01%.

**c)** PH Variation Assay: Isolates showed optimal growth at pH 7.0, with reduced growth at extreme pH levels.

**d)** Salt Variation Assay: Isolates tolerated NaCl up to 2%, with diminished growth at 4%

(Fig 2- Citrate test)

(Fig 3- Bromothymol blue test)

(Fig 4- Urease Test)

(Fig 5- Starch hydrolysis)

(Fig 6- Gram -staining)

(Fig 7- IAA test)

(Fig 8 - Catalase Test)

(Fig 9 - Metal test)

(Fig 10 - pH variation)

(Fig 11 - Salt variation)

(Fig 12-Antibiotic resistance)

**e) Seed Trial**

| Treatment | Germination rate | Average Root Length (cm) | Average Shoot Length (cm) |
|---|---|---|---|
| Plate A (Biofertilizer) | 89% | 6.5 ± 0.4 | 5.2 ± 0.3 |
| Plate B (Guar Gum) | 78% | 4.1 ± 0.6 | 3.9 ± 0.4 |
| Control | 70% | 3.5 ± 0.5 | 3.2 ± 0.4 |

i. Mean Germination Rate = 89+78+70/ 3 = 79%

ii. Mean Root Length = 6.5+4.1+3.5 / 3 = ≈ 4.7 cm

iii. Mean Shoot Length = 5.2+3.9+3.2 / 3 = 4.1cm

## 4. RESULTS

The morphological characteristics of the *Rhizobium japonicum* strain indicates that it forms 3.1 mm circular, translucent colonies, is motile, and exhibits a rod-shaped bacterial structure, typical of *Rhizobium* species. The biochemical characteristics revealed, the *Rhizobium* strain tested positive (+) for UV resistance, citrate utilization, bromothymol blue reaction, urease activity, starch hydrolysis, indole production, and catalase activity, indicating strong metabolic adaptability. Gram staining confirmed it as a Gram-negative rod-shaped bacterium, consistent with typical *Rhizobium* morphology.

The Metal test revealed the effect of mercury chloride ($HgCl_2$) on the *Rhizobium* strain. The strain shows no growth at 1%, but tolerates 0.01%, indicating low-level metal resistance. The Effect of pH test indicated that the *Rhizobium* strain grows best at pH 7 and 8, exhibits moderate growth at pH 6 and 9, and fails to grow at pH 4 and 5, suggesting it thrives in neutral to mildly alkaline environments. Salt Variation Assay shows the growth of *Rhizobium* strain in varying concentrations of NaCl. The strain exhibits high growth at 0.5% NaCl, moderate growth at 1% and 2%, and no growth at 3% and 4%, indicating it has a low tolerance to salt. Antibiotic resistance against *Rhizobium* strain displayed, moderate susceptibility to tetracycline with a zone of inhibition of 3.2 cm,

while exhibiting resistance to penicillin with a minimal zone of 0.1 cm, suggesting penicillin has limited effectiveness against the strain.

The partial 16S rRNA genome sequencing matched with *Rhizobium japonicum* (Accession: MK421552.1:1-1382), confirming the molecular identification of the test isolate as *Rhizobium japonicum*. The Phylogram shows that the test isolate clusters closely with various *Rhizobium japonicum* strains, indicating strong genetic similarity.

The HPLC analysis of the *Bradyrhizobium japonicum* strain for Indole-3-Acetic Acid (IAA) reveals an IAA concentration of 41.2 µg/mL in the sample, using a calibration curve with the equation $y = 0.862x + 1.21$ ($R^2 = 0.998$). The IAA retention time in the sample is 7.84 minutes, which closely aligns with the standard IAA's retention time of 7.85 minutes.

The seed trial results showed that plants treated with biofertilizer had the highest growth in terms of plant height, root length, and shoot length compared to Guar gum and control treatments, indicating the superior effectiveness of the biofertilizer in promoting plant development. The pot trial results demonstrated that seeds treated with biofertilizer showed significantly higher germination percentage, plant height, shoot and root length, compared to those treated with Guar gum, highlighting the enhanced growth-promoting effect of the biofertilizer.

**TABLE: 4.1 Morphophological, Characterization of Microorganism**

| S.NO | PARAMETERS | T1 |
|---|---|---|
| 1 | Size | 3.1 mm |
| 2 | Shape | Circular |
| 3 | Opacity | Translucent |
| 4 | Motility | Motile |
| 5 | Bacterium Shape | Rod shaped |

**TABLE: 4.2 Biochemical Test from Isolated Microorganism**

| S.NO | BIOCHEMICAL TESTS | T1 |
|---|---|---|
| 1 | Ultraviolet rays Treatment | + |
| 2 | Citrate Utilization Test | + |
| 3 | Bromothymol Blue Test | + |
| 4 | Urease Test | + |
| 5 | Starch Hydrolysis Test | + |
| 6 | Gram staining | Gram negative rod shaped |
| 7 | Indole Acid production Test | + |
| 8 | Catalase test | + |

**Table 4.3 - Effect of metal Test**

| Metal Salts Strains | HgCl2 | |
|---|---|---|
| *Rhizobium* Strain | 1% | 0.01% |
| | - | + |

| STRAINS | pH | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 |
| T1 | - | - | + | ++ | ++ | + |

**TABLE: 4.4 -Effect of pH**

**Table 4.5 Salt Variation Assay**

| STRAIN | Concentration of NaCl in % | | | | |
|---|---|---|---|---|---|
| | 0.5% | 1% | 2% | 3% | 4% |
| *Rhizobium* | ++ | + | + | - | - |

**(++): High growth; (+): Moderate; (-): No growth**

**Table: 4.6- Antibiotic Resistance test against *Rhizobium***

| S.NO | Antibiotic strains | Zone of Inhibition (diameter in mm) |
|---|---|---|
| 1. | *Tetracycline* | 33.9±35.5 |
| 2. | *Penicillin* | 1.0 ± 0.01 |

Genome sequencing of *Bradyrhizobium japonicum*

>gi|1560959211|gb|MK421552.1| Bradyrhizobium japonicum strain ZA-Bj19 16S ribosomal RNA gene, partial sequence
ACATGCAAGTCGAGCGGGCGTAGCAATACGTCAGCGGCAGACGGGT
GAGTAACGCGTGGGAACGTACCTT
TTGGTTCGGAACAACACAGGGAAACTTGTGCTAATACCGGATAAGC
CCTTACGGGGAAAGATTTATCGCC
GAAAGATCGGCCCGCGTCTGATTAGCTAGTTGGTGAGGTAATGGCT
CACCTAGGCGACGATCAGTAGCTG
GTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAA
CTCCTACGGGAGGCAGCAGTGGGG
AATATTGGAAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGAGT
GATGAAGGCCCTAGGGTTGTAAAG
CTCTTTTGTGCGGGAAGATAATGACGGTACCGCAAGAATAAGCCCC
GGCTAACTTCGTGCCAGCAGCCGC
GGTAATACGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAA
GGGTGCGTAGGCGGGTCTTTAAGT
CAGGGGTGAAATCCTGGAGCTCAACTCCAGAACTGCCTTTGATACT
GAGGATCTTGAGTTCGGGAGAGGT
GAGTGGAACTGCGAGTGTAGAGGCTGAAATTCGTAGATATTCGCAA
GAACACCAGTGGCGAAGGCGGCTC
ACTGGCCCGATACTGACGCTGAGGCACGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCA
CGCCGTAAACGATGAATGCCAGCCGTTAGTGGGTTTACTCACTAGT
GGCGCAGCTAACGCTTTAAGCATT
CCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGACGCAACGCGCAGAACCTTACCAGCCCTTGA
CATGTCCAGGACCGGTCGCAGAGA
TGTGACCTTCTCTTCGGAGCCTGGAGCACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCCCGTCCTTAGTTGCTACC
ATTTAGTTGAGCACTCTAAGGAGA
CTGCCGGTGATAAGCCGCGAGGAAGGTGGGGATGACGTCAAGTCCT
CATGGCCCTTACGGGCTGGGCTAC
ACACGTGCTACAATGGCGGTGACAATGGGATGCTAAGGGGCGACCC
TTCGCAAATCTCAAAAAGCCGTCT
CAGTTCGGATTGGGCTCTGCAACTCGAGCCCATGAAGTTGGAATCG
CTAGTAATCGTGGATCAGCACGCC
ACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
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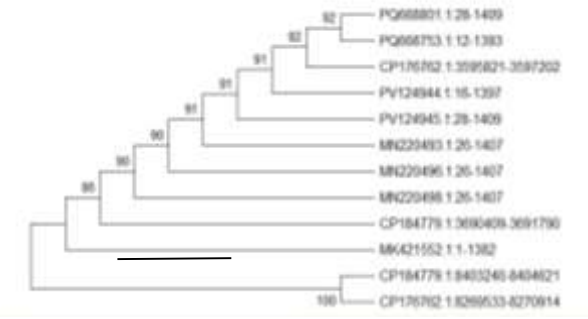
GACTGG



Phylogram of *Bradyrhizobium japonicum*.

**HPLC Report**

Sample: *Bradyrhizobium japonicum* (Temperature-Tolerant Strain)

Analyte: Indole-3-Acetic Acid (IAA)

Instrument: Agilent 1200 Series HPLC

Detector: UV-Vis Detector (λ = 254 nm)

Column: C18 Reverse Phase Column (250 mm × 4.6 mm, 5 µm)

Mobile Phase: Methanol:Water:Acetic acid (60:39:1, v/v/v)

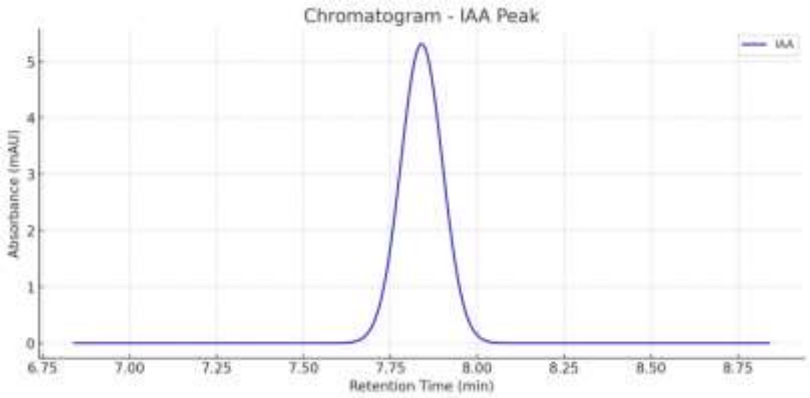
Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Retention Time (Standard IAA): 7.85 min

Chromatogram Summary:

| Peak No | Retention Time (min) | Area (mAU*s) | Height (mAU) | Width (min) | % Area | Compound Name |
|---|---|---|---|---|---|---|
| 1. | 7.84 | 45.27 | 5.32 | 0.14 | 97.3 | IAA |




Quantitative Results:

Standard IAA Concentration: 50 µg/mL

Calibration Curve Equation: $y = 0.862x + 1.21$ ($R^2 = 0.998$)

Sample IAA Concentration: 41.2 µg/Ml

**Table 4.7 – Seed Trial**

| Treatment | Plant Height | | | Average root length (cm) | | | Average shoot length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Seed + Biofertilizer** | 14.2 | 14.1 | 14.3 | 11.1 | 11.2 | 11.0 | 8.3 | 8.4 | 8.2 |
| **Seed + Guar Gum (1%)** | 13.7 | 13.9 | 13.6 | 10.3 | 10.4 | 10.2 | 7.8 | 7.9 | 7.7 |
| **Control (no inoculums)** | 10.5 | 10.6 | 10.4 | 8.2 | 8.3 | 8.1 | 6.0 | 6.1 | 6.0 |

Mean of Each Growth Parameter (All Treatments Combined):

Mean Plant Height = (14.2 + 14.1 + 14.3 + 13.7 + 13.9 + 13.6 + 10.5 + 10.6 + 10.4) / 9 = 12.81 cm

Mean Root Length = (11.1 + 11.2 + 11.0 + 10.3 + 10.4 + 10.2 + 8.2 + 8.3 + 8.1) / 9 = 9.86 cm

Mean Shoot Length = (8.3 + 8.4 + 8.2 + 7.8 + 7.9 + 7.7 + 6.0 + 6.1 + 6.0) / 9 = 7.37 cm

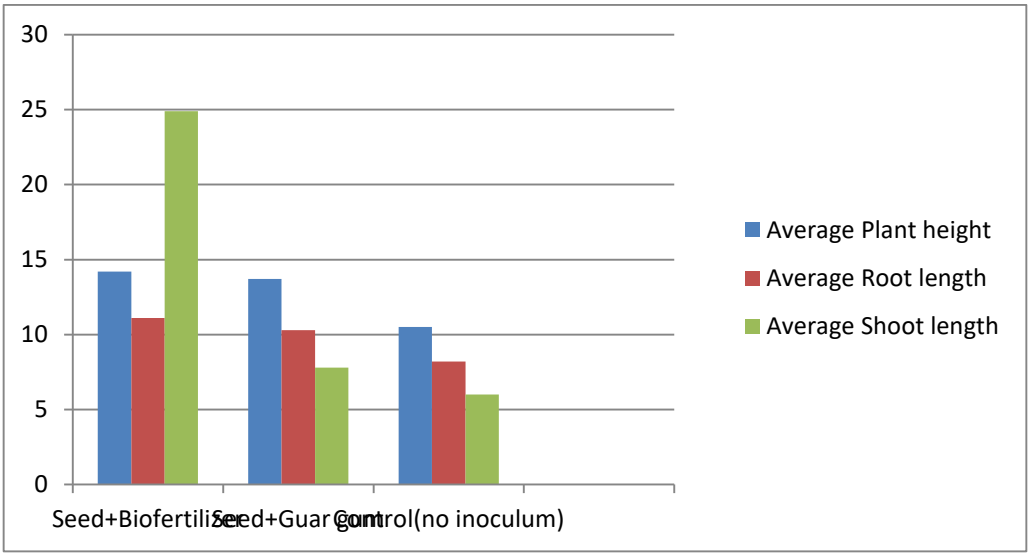
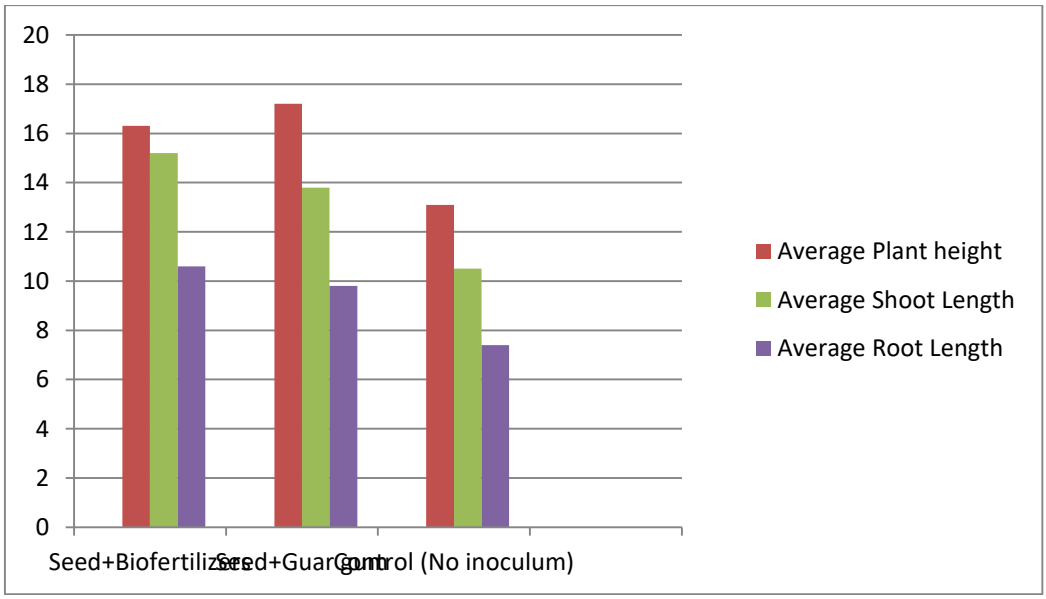
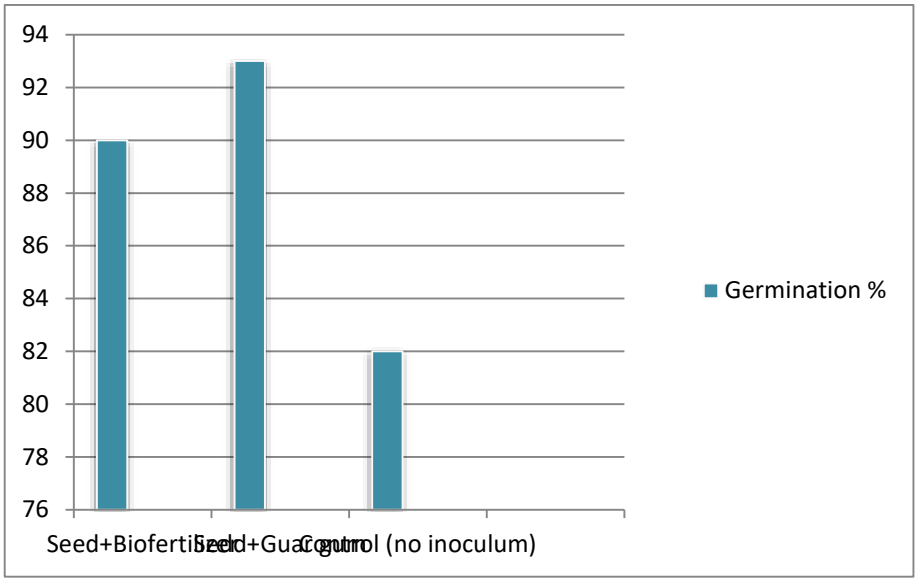



**Table - 4.8- Pot Trial**

| S.No. | Treatment | Germination % | Plant Height (cm) | | | Shoot length (cm) | | | Root length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Seed+Biofertilizer | 90±1.5 | 18.5 | 18.4 | 18.7 | 15.2 | 15.3 | 15.1 | 10.6 | 10.7 | 10.5 |
| 2. | Seed+Guar gum (1%) | 93±2 | 17.2 | 17.4 | 17.1 | 13.8 | 14.0 | 13.7 | 9.8 | 9.7 | 9.9 |
| 3. | Control (no inoculums) | 82±3 | 13.1 | 13.3 | 13.0 | 10.5 | 10.6 | 10.3 | 7.4 | 7.5 | 7.3 |






**CONCLUSION-**

This work aimed to identify a heat-tolerant *Rhizobium* strain from *Glycine max* (soybean) and examine its metabolite profile. The isolate showed strong adaptability to elevated temperatures while sustaining nitrogen fixation and symbiosis. Metabolites like IAA, EPS, organic acids, and osmoprotectants played roles in enhancing plant growth, stress resistance, and soil health. Overall, the findings emphasize the strain's promise for creating temperature-resilient biofertilizers to support sustainable agriculture in heat-prone environments.

**FUTURE PERSPECTIVE-**

Advancing research on temperature-resilient *Rhizobium* strains and their metabolites holds great promise for sustainable farming in the face of global warming. Enhancing the production of key compounds like IAA, organic acids, and osmoprotectants can lead to effective biofertilizers and biostimulants, boosting plant resilience, nitrogen fixation, and productivity under heat stress. Employing omics technologies could further reveal stress-adaptive traits, paving the way for environmentally friendly agriculture and improved food security in a changing climate.

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