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Keywords: Chemo-priming, Simulated microgravity, Two-dimensional clinostat, Phaseolus vulgaris, Indole-3-Acetic Acid (IAA), Thiourea, Sodium Nitroprusside (SNP), Bean seeds, Seed germination, Plant adaptation, Space agriculture, Controlled Environment Agriculture.

THE EFFECTS OF IAA, SNP AND THIOUREA GROWTH STIMULATORS ON CHEMOPRIMED BEAN SEEDS PRESENT IN MICROGRAVITY USING A 2D CLINOSTAT

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ABSTRACT

Research into the effects of microgravity on plants has increased significantly because of its importance to long-duration space missions and the possibility of cultivating food crops in extraterrestrial environments. This study examined the effects of Indole-3-Acetic Acid (IAA), Thiourea (T. urea), and Sodium Nitroprusside (SNP) on chemoprimed bean seeds exposed to simulated microgravity using a two-dimensional (2D) clinostat. The seeds were divided into a control group treated with water and experimental groups treated with IAA, Thiourea, or SNP at concentrations of 500 ppm and 1000 ppm. Following treatment, the seeds were maintained under either simulated microgravity using the 2D clinostat or normal gravity conditions. Germination percentage, root and shoot lengths, root number, fresh weight, dry weight, and other observable growth characteristics were evaluated. The results

showed that all treatment groups recorded high final germination percentages, although their responses varied according to the growth stimulant, concentration, and gravity condition. SNP at 500 ppm gave the most favorable results, producing the quickest germination, highest germination index, and strongest seedling vigour under both gravity conditions. IAA at 500 ppm promoted early germination and improved seedling establishment, whereas the 1000 ppm treatment generally reduced growth, indicating that higher IAA concentrations may have inhibitory effects. Thiourea enhanced germination uniformity and root development, while the 1000 ppm treatment supported vigorous seedling growth under simulated microgravity. Overall, the study demonstrates that IAA, Thiourea, and SNP may enhance crop establishment under controlled conditions and could be useful in the development of agricultural systems for future space exploration. Among the treatments tested, SNP at 500 ppm exhibited the most consistent and effective overall performance.

1. INTRODUCTION

Phaseolus vulgaris L., or common bean, is a widely cultivated leguminous crop with considerable nutritional and economic importance. It provides the human diet with important nutrients such as plant protein, dietary fibre, carbohydrates, vitamins, and essential minerals. This makes it particularly valuable in developing countries, where animal protein may be less affordable or readily accessible. In Nigeria, beans are commonly consumed due to their relatively low cost and availability, while also contributing to improved food security and nutritional status. The crop further supports sustainable agriculture through its ability to fix atmospheric nitrogen biologically, which enhances soil fertility.

Gravity is an important environmental stimulus that influences the pattern and direction of plant growth and development. Plants have developed specialized systems that enable them to perceive gravitational forces and modify their growth accordingly, a response known as gravitropism. Roots generally respond positively to gravity by growing downward, whereas shoots respond negatively by growing upward and away from the gravitational pull. Gravity sensing involves the settling of statoliths in specialized plant cells, which subsequently promotes the redistribution of hormones, particularly auxin. The resulting changes in auxin concentration affect cellular elongation and growth, thereby controlling the directional development of roots and shoots (Blancaflor & Masson, 2003).

Microgravity can cause considerable changes in the physiological and biochemical processes of plants, influencing activities such as seed germination, cell division, cell elongation, gene expression, and plant morphology (Wolverton & Kiss, 2009). These changes may disturb normal plant development, decrease crop productivity, and pose challenges to successful crop production in space. Therefore, researchers have continued to explore strategies that could help plants adapt to or overcome the effects of altered gravitational conditions.

Clinostats are specialized laboratory devices used to mimic microgravity by rotating biological samples continuously around a horizontal axis. The rotation changes the direction of the gravitational stimulus acting on the plant, thereby reducing its ability to perceive a constant gravitational direction (Hoson et al., 1997; Okonkwo & Ibrahim, 2023). This makes clinostats valuable tools for investigating plant growth and developmental responses under simulated microgravity and other altered-gravity conditions.

Plant growth regulators (PGRs), including Indole-3-Acetic Acid (IAA) and Thiourea (T. urea), are important in the regulation of plant growth and development. They influence several processes, including cell division, cell elongation, seed development, and reproductive growth. Bello and Okonkwo (2024) investigated the effects of growth stimulators on common bean seed development and identified IAA as a potential agent for improving seed quality and growth performance. Understanding the response of common bean seeds to IAA application may contribute to the improvement of desirable agronomic characteristics. Their study also suggested that auxin treatment can affect seed quality, growth performance, and chemical composition, which may have implications for increasing common bean production and nutritional value.

Sodium Nitroprusside (SNP) is another compound that has potential as a plant growth stimulator because it acts as a source of nitric oxide (NO). Nitric oxide is an important signaling molecule that participates in several plant processes, including seed germination, root growth, responses to environmental stress, antioxidant defense, and cellular metabolism (Jiao et al., 2022). SNP application has been reported to promote germination and improve plant tolerance to unfavorable environmental conditions through signaling pathways involving nitric oxide (Zhang et al., 2008).

The application of growth stimulators during seed priming has been associated with improved germination and seedling establishment, especially under unfavorable environmental conditions (Xiong et al., 2021). Studies involving simulated microgravity have also reported positive effects of growth stimulators on germination and physiological responses in crop plants. Ikhajiagbe et al. (2023) found that treatment with selected growth stimulators improved the germination

characteristics of *Zea mays* under clinostat-simulated microgravity. Similarly, Orukpe et al. (2025) reported enhanced germination indices and physiological responses in chemoprimed maize seedlings exposed to simulated microgravity. These findings indicate that growth stimulators could be useful in promoting crop establishment and maintaining plant development under altered-gravity conditions.



Figure 1. Brown Beans (*Phaseolus vulgaris*)

Despite these advances, information regarding the effects of IAA, Thiourea and SNP on the germination and early growth of *Phaseolus vulgaris* under simulated microgravity remains limited. Therefore, this study investigates the effects of Indole-3-Acetic Acid (IAA) and T.urea and Sodium Nitroprusside (SNP) on the germination and early seedling growth of *Phaseolus vulgaris* under clinostat-simulated microgravity conditions.

Botanical Classification

- **Kingdom:** Plantae
- **Phylum (Division):** Magnoliophyta (Angiosperms)
- **Class:** Magnoliopsida (Dicotyledons)
- **Order:** Fabales
- **Family:** Fabaceae (Leguminosae)
- **Genus:** *Phaseolus*
- **Species:** *Phaseolus vulgaris* L.

Common bean (*Phaseolus vulgaris*) is an economically and nutritionally important legume cultivated extensively in many parts of the world. It provides essential nutrients such as protein, carbohydrates, vitamins, minerals, and unsaturated fatty acids, particularly linoleic and oleic acids. For many communities with limited access to animal protein, beans serve as an affordable alternative source of nutrients. Breeding programmes have mainly concentrated on improving bean yield, resulting in the development of numerous improved varieties. However, less

emphasis has been placed on nutritional quality and flavor. Comparative studies of traditional landraces and modern varieties have assessed their protein, fat, fatty acid, and mineral composition, including selenium (Se), zinc (Zn), and iron (Fe). Findings revealed that the LR05 landrace had greater protein and mineral contents, especially Se and Zn, than the modern varieties.

Auxins are naturally produced plant hormones that regulate a wide range of physiological and developmental processes. They are involved in cell division, elongation, and differentiation and can influence the germination of seeds and tubers. Auxins also contribute to the formation of roots and xylem and stimulate the production of lateral and adventitious roots. In addition, they are involved in plant responses to environmental stimuli such as light and gravity. Other processes influenced by auxins include flowering, fruit development, photosynthesis, pigment production, metabolite synthesis, and adaptation to stressful conditions (Tsakelova et al., 2006).

Indole-3-acetic acid (IAA) is one of the principal naturally occurring auxins and has been widely investigated because of its important functions in plants. Although IAA is commonly referred to as the major auxin, a large proportion of it occurs in conjugated forms within plant tissues. These conjugated forms serve important functions in IAA storage and transport and help protect the hormone from degradation. They therefore contribute to maintaining appropriate levels of biologically active IAA in different plant tissues (Seidel et al., 2006).

Sodium Nitroprusside (SNP) is an important source of nitric oxide (NO), which functions as a signaling molecule in plants. NO is involved in regulating several physiological activities, including seed germination, root development, photosynthesis, antioxidant defense, and responses to environmental stress (Jiao et al., 2022). Studies have demonstrated that SNP treatment can improve germination and promote stronger seedling development. For example, Zhang et al. (2008) reported that SNP enhanced wheat seed germination and reduced oxidative damage under stressful conditions. Similar findings in other crops have shown that SNP application can increase chlorophyll levels, improve photosynthetic performance, and support greater biomass production.

The growth-promoting effects of SNP are partly associated with its ability to improve the antioxidant defense system of plants. SNP can stimulate enzymes such as superoxide dismutase, catalase, and peroxidase, which help neutralize reactive oxygen species and protect plant cells from oxidative damage. Nitric oxide can also interact with other plant hormones, influencing cellular signaling pathways that regulate growth and developmental processes (Jiao et al., 2022).

Thiourea, also known as thiocarbamide, is a sulfur- and nitrogen-containing compound with amino, imino, and thiol functional groups that contribute to its biological activity. It has been applied to enhance plant growth and productivity under both favorable and stressful environmental conditions. Depending on the desired effect, thiourea may be administered through seed treatment, foliar spraying, or incorporation into the growth medium. Applying thiourea to seeds can improve germination, while foliar treatment may enhance gas exchange and photosynthetic activity. Its addition to the growth medium can also encourage root formation and development. Generally, the effects of thiourea are more evident in tissues that are directly exposed to the treatment.

2. MATERIALS/METHODS

Study Area

This work was carried out in the National Space Research Laboratory in an incubation room with controlled humidity and temperature, adequate ventilation and aeration, sunlight and water that were added to the petri dish containing the beans to be grown.

Equipment

Weighing balance, Clinostat.

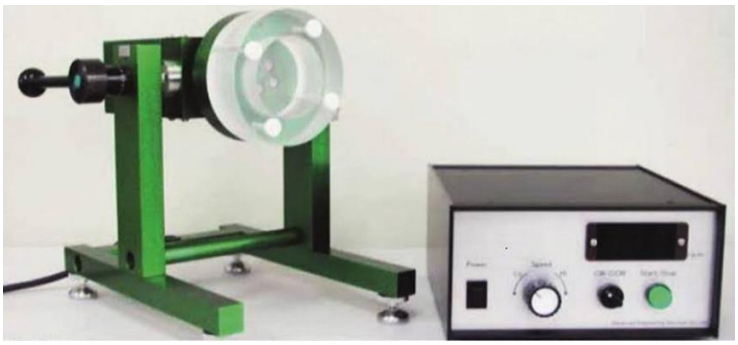


Figure 2.1. A Clinostat



Figure 2.2. Petri dish mounted on a clinostat

Apparatus

Petri dishes, Cotton wool, Filter paper, beaker, Measuring cylinder, Masking tape.

Reagents

Indole-3-acetic acid (IAA), Sodium Nitroprusside (SNP), Thiourea, Distilled water.

METHODS

Sample Collection

The sample, Beans (*Phaseolus vulgaris*), was purchased on the 18th of January, 2026 from a store at UNIBEN maingate, Benin City, Edo state. The Laboratory provided the reagents used.

Sample Preparation

The bean samples were thoroughly examined and checked for holes and bruises to make sure they are viable for the experiment

Experimental Groups

1. Control Group: This group is baseline for the experiment and contains only water.

2. IAA Treatment Groups: Different concentrations of IAA at 500ppm and 1000ppm respectively where used to treat this group.

3. SNP Treatment Groups: Different concentrations of SNP at 500ppm and 1000ppm respectively where used to treat this group.

4. Thiourea Treatment Groups: Different concentrations of Thiourea at 500ppm and 1000ppm respectively where used to treat this group

Experimental Design

Different concentrations of growth stimulators (500 ppm, and 1000 ppm) and distilled water as control are used to treat the seeds.

Number of seeds used: 210 (30 seeds per treatment). The beans seeds was carefully selected to make sure they are viable.

Number of petri dishes used: Fourteen (14) petri dishes were used with 15 primed seeds placed in each petri dish.

Priming method: 20ml of the respective prepared concentration was used to prime each batch of seeds for 1 hour before sowing.

Planting: The base of sterilized petri dishes was filled with cotton wool and primed seeds were planted on them.

Replicates: Each treatment was replicated in 2 petri dishes.

Gravitational conditions: Each petri dish was used to represent either microgravity or gravity, with the microgravity petri dish mounted on a two-dimensional clinostat.

Preparation of Reagents

A total of Four (4) plastic bottles were thoroughly cleaned and labelled with each filled with 250 ml of distilled water.

Growth regulator solutions of 500 ppm and 1000 ppm were prepared as follows:

1. 500 ppm solution: 0.125 g of the solute was weighed and dissolved in 250 ml of distilled water.
2. 1000 ppm solution: 0.25 g of the solute was weighed and dissolved in 250ml of distilled water.

Calculations:

To Prepare 500ppm Solution:

500mg (0.5g) of solute is dissolved in 1000ml of Distilled Water

For 250ml:

0.5g = 1000ml

X = 250ml

Cross multiply the equation

X=125/1000 this would equal 0.125g

To Prepare 1000ppm Solution:

1000mg (1g) of solute is dissolved in 1000ml of Distilled Water

For 250ml:

1g = 1000ml

X = 250ml

Cross multiply the equation

X=250/1000 this would equal 0.25g

Seed Priming

1. A total of seven (7) beakers where prepared and labelled (H_2O, IAA 500ppm and 1000ppm, SNP 500ppm and 1000ppm, and Thiourea 500ppm and 1000ppm).

2. A total of 210 viable seeds where picked and set apart and 30 seeds were placed in each beaker.

3. 20ml of each concentration (500 and 1000ppm) of the Reagents (IAA, SNP, Thiourea) where poured into the beaker containing the seeds, and the seeds were left to prime for 60 minutes.

4. While the seeds where priming Fourteen (14) petri dishes where prepared and labelled with each growth regulator and concentration respectively. Afterwards an appropriate amount of cotton wool was spread in each Petri dish to act as the base for the plant and a source of Carbon.
5. At the 60mins mark, the seeds were removed from the beaker and then implanted into the Petri dishes containing the cotton wool and the 15 seeds were separated in the Petri dishes each.
6. One of the two petri dishes representing each growth stimulator was mounted on the clinostat to represent Microgravity and left to spin at 0.5 rpm and the other was left at normal gravity.
7. Afterwards the growth was observed for a total of 120 hours.

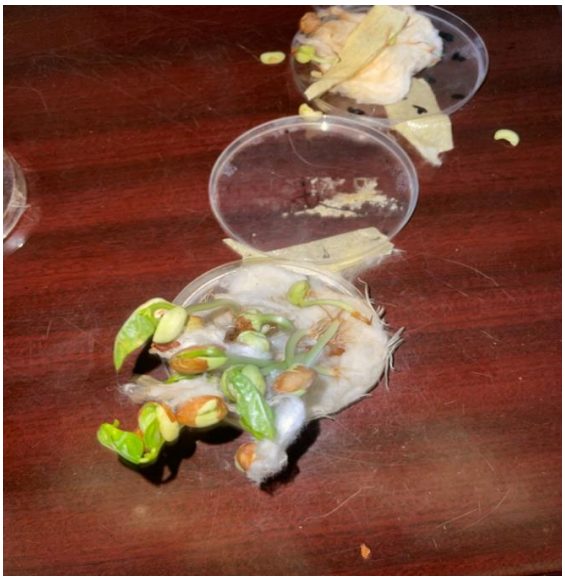


Figure 3.1. SNP 500ppm microgravity at 120 hours.



Figure 3.2. SNP 500ppm gravity at 120 hours

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3. RESULTS

Table 1. Showing the germination rate at different intervals (24hrs-120hrs)

Treatment	24 HRS	48 HRS	72 HRS	96 HRS	120 HRS
Ctrl (H20) G	_	3	6	15	15
Ctrl (H20) 0.5rpm	_	4	8	15	15
IAA 500ppm µg	4	15	15	15	15
IAA 500ppm(g)	2	15	15	15	15
IAA 1000ppm µg	_	2	7	12	15
IAA 1000ppm(g)	_	1	5	9	15
T.UREA 500ppm µg	_	5	12	13	13
T.UREA 500ppm(g)	_	8	11	15	15
T.UREA 1000ppm µg	3	10	15	15	15
T.UREA 1000ppm(g)	5	10	15	15	15
SNP 500ppm µg	2	13	15	15	15
SNP 500ppm(g)	5	15	15	15	15
SNP 1000ppm µg	_	4	7	12	15
SNP 1000ppm(g)	2	5	10	15	15

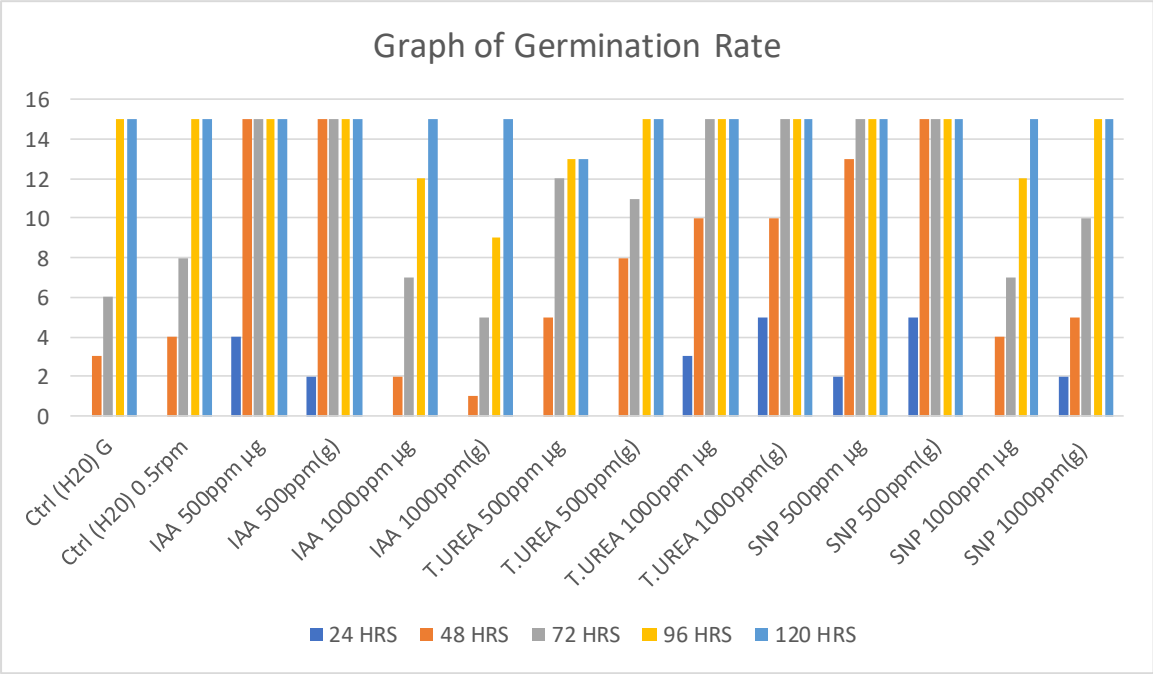


Figure 4.1 Germination Rate Graph

From the graph, during the first 24 hours, little or no germination was observed across all treatments because the seeds were still undergoing water imbibition and activation of metabolic processes necessary for germination.

Between 24 and 48 hours, germination increased rapidly, with seeds treated with SNP at 500 ppm exhibiting the highest germination rate, indicating earlier radicle emergence and faster seedling establishment than the other treatments.

Seeds primed with IAA at 500 ppm also showed a significant increase in germination rate during this period, although the increase was slower than that observed with SNP, while IAA at 1000 ppm displayed a delayed germination response

Thiourea-treated seeds exhibited a more gradual but continuous increase in germination rate between 48 and 96 hours, suggesting that Thiourea enhanced stress tolerance and maintained metabolic activity rather than promoting extremely rapid germination.

By 96 to 120 hours, the germination curves for all treatments reached a plateau, indicating that nearly all viable seeds had completed germination.

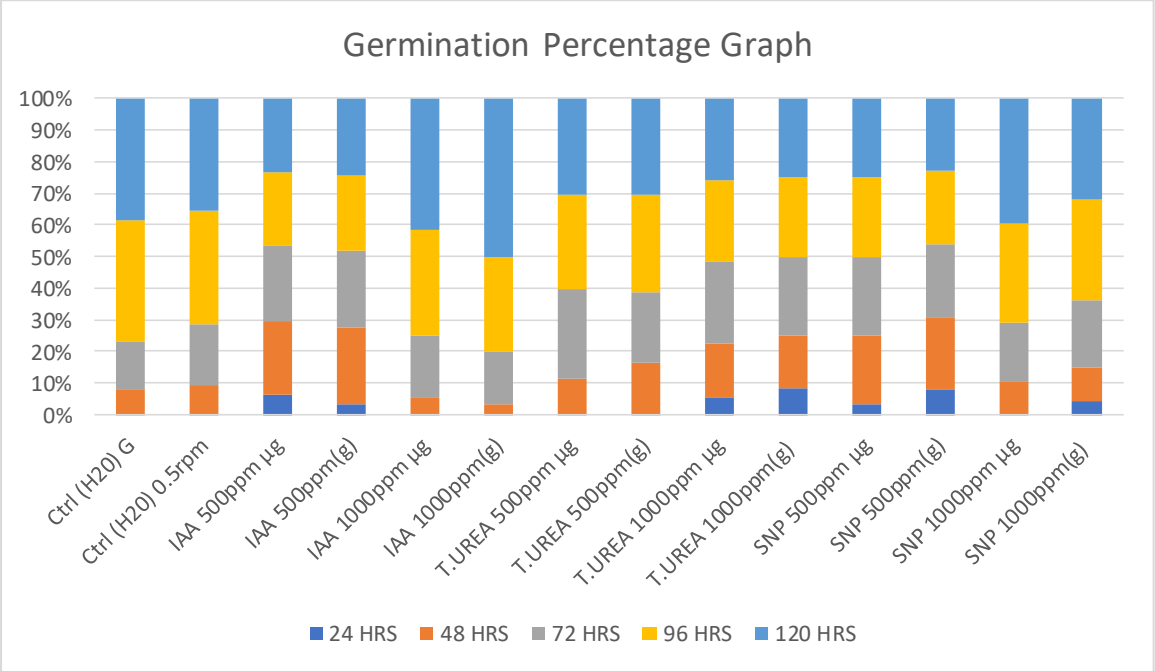


Figure 4.2 Germination Percentage Graph

The germination percentage graph shows the proportion of *Phaseolus vulgaris* seeds that successfully germinated under each treatment throughout the experimental period. At the beginning of the experiment, germination percentages were low for all treatments because only a few seeds had completed radicle emergence during the early hours of incubation. As germination progressed, the percentage of germinated seeds increased steadily, with SNP at 500 ppm consistently recording the highest germination percentages at each observation period, indicating greater germination uniformity and improved seed vigour. Seeds treated with IAA at 500 ppm also achieved high germination percentages, demonstrating that moderate auxin concentration

effectively promoted embryo development and successful seedling establishment. In contrast, IAA at 1000 ppm produced comparatively lower germination percentages, suggesting that excessive auxin concentration reduced germination efficiency and delayed seedling emergence. Thiourea treatments exhibited a gradual increase in germination percentage throughout the experiment, with Thiourea at 1000 ppm achieving high final germination percentages, reflecting its ability to maintain seed viability and enhance tolerance to simulated microgravity stress. By the end of the experiment (120 hours), all chemically primed treatments recorded higher final germination percentages than the untreated control, confirming the beneficial effects of seed priming.th.

PARAMETERS

1. Germination Percentage at 72 Hours:

Percentage of seeds that germinated within the first 72 hours.

$$GP72h = \frac{\text{Number of seeds germinated at 72h}}{Total\ seeds\ sown}) \times 100$$

2. Germination Time (h):

The time taken for a seed to germinate after sowing.

$$GT = t$$

where *t* the germination time of an individual seed.

3. Mean Germination Time (MGT):

The average time required for seeds to germinate.

$$MGT = \frac{\sum nt}{\sum n}$$

where:

- n = Number of seeds germinated at time t
- t = Time of germination

4. Median Germination Time (T_{50}) (h):

Time required for 50% of the final germinated seeds to germinate.

$$T_{50} = t_i + \left(\frac{(N/2) - n_i}{n_j - n_i}\right)(t_j - t_i)$$

where:

- N = Total number of seeds
- ni = Cummulative germination time before 50%
- nj = Cummulative germination time after 50%

- ti and tj = Their corresponding times

5. First Germination Percent (%):

Percentage of seeds germinated at first count

$$FGP = \frac{\text{Seeds germinated at first count}}{Total\ seeds\ sown} \times 100$$

6. Final Germination Percentage (FGP):

The percentage of seeds that germinated by the end of the experiment.

$$FGP = \frac{Total\ germinated\ seeds}{Total\ seeds\ sown} \times 100$$

7. Mean Daily Germination (MDG):

Average percentage germinated per day

$$MDG = \frac{FGP}{Days\ to\ final\ germination}$$

8. Germination Index (GI):

A measure of speed and completeness of germination.

$$GI = \sum \frac{n_i}{t_i}$$

where:

- ni = Number of seeds germinated at time t
- ti = Time corresponding to germination count

9. Time for the First Germination (T_0):

Time when the first seed germinated.

$$T_0 = t_{first\ germination}$$

10. Time to Last Germination (Tg):

The time from sowing until the last seed germinates.

$$T_g = t_{last\ germination}$$

11. Time Spread of Germination ($T_g - T_0$):

Duration between first and last germination.

$$TSG = T_g - T_0$$

12. Peak Period of Germination:

The time at which the highest number of seeds germinate.

$$PP = t_{maximum\ germination}$$

13. Germination Rate Index (GRI):

Measures germination speed based on cumulative germination.

$$GRI = \sum \frac{GP_i}{t_i}$$

GRI = (GP1 ÷ t1) + (GP2 ÷ t2) + ... + (GPn ÷ tn)

Where GP1 is germination percentage at 1st day, GP2 is germ percent at 2 days, GPn is germination percent at n days.

14. Corrected Germination Rate Index (GRIcorrected):

$$GRI_{corrected} = \frac{GRI}{FGP}$$

15. Rate of Germination (hrs/seed):

Equivalent to MGT when expressed as time per seed.

$$RG = \sum \frac{nt}{n}$$

16. Daily Germination Speed:

$$DGS = \frac{1}{MGT}$$

17. Seedling Vigour Index I (SVI-I):

Combines germination percentage and shoot length.

$$SVI - I = FGP \times Shoot\ length$$

18. Seedling Vigour Index II (SVI-II):

Combines germination percentage and seedling length.

$$SVI - II = FGP \times (Root\ length + Shoot\ length)$$

19. Seedling Vigour Index III (SVI-III):

Combines germination percentage and seedling dry weight.

$$SVI - III = FGP \times Dry\ weight$$

20. Coefficient of Velocity of Germination (CVG):

A measure of germination speed.

$$CVG = \frac{\sum n_i}{\sum n_i t_i} \times 100$$

21. Germination Capacity (GC):

$$GC = \frac{FGP}{n}$$

where,

- n = Number of seeds used in the experiment

22. Speed of Accumulated Germination (SAG):

A measure of cumulative germination speed.

$$SAG = \sum \frac{n_i}{t_i}$$

where,

- ni = Cumulative germinated seeds at time i
- ti = Time corresponding to cumulative germination

23. Modified Timson's index, germination energy index (GEI):

A measure of germination performance over time.

$$GEI = \frac{\sum G}{t}$$

where,

- G = Cumulative germination percentages

t = Total germination period

Table 2.1. Germination indices of the germinant.

PARAMETERS	CTRL(H20) g	CTRL(H2O) µg	IAA 500ppm µg	IAA 500ppm(g)	IAA 1000ppm µg	IAA 1000ppm(g)
Germination Percent (%) at 72hrs	40	53.33	100	100	46.67	33.33
Germination Time(hrs)	96	96	48	48	120	120
Median Germination Time(T50)(hrs)	76	69	31.64	34.15	74.4	87
Final Germination Percent(%)	100	100	100	100	100	100
Mean Daily Germination	20	20	20	20	20	20
Germination Index	4.75	5.08	9.5	7.5	4.52	4.03
Time for the First Germination(T0)	48	32	24	16	40	24
Time for the Last Germination(Tg)	96	96	48	48	120	120
Time Spread of Germination(Tg-To)	48	48	24	24	72	72
Peak Period of Germination	96	96	48	48	72	120
Germination Rate Index or Speed (GRI)	31.67	33.89	77.04	89.33	30.11	26.89

Corrected germination rate index (GRIcorrected)	0.32	0.34	0.77	0.89	0.3	0.27
Rate of Germination (hrs/seed)	4.75	5.08	9.5	7.5	4.52	4.03
Daily Germination Speed	0.29	0.31	0.58	0.63	0.28	0.25
Length of Roots(cm)	2.9	1.3	1.2	4.55	4	2.85
Length of Shoot(cm)	12.35	4.9	7.1	6.6	1.1	2.45
Number of Roots	10	6	9	10	7	9
Wet Seedling Weight(g)	1.24	0.55	0.98	0.95	0.79	0.78
Dry Seedling Weight(g)	0.15	0.1	0.26	0.25	0.3	0.34
Original Seed Dry Weight(g)	0.35	0.35	0.28	0.34	0.35	0.28
First Germination Percent(%)	20	26.67	26.67	13.33	13.33	6.67
Seedling vigour I	1,235	490	710	660	110	245
Seedling vigour II	1,525	620	830	1,115	510	530
Seedling vigour III	15	10	26	25	30	34
Mean germination time, MGT	3.4	3.2	1.73	1.6	3.6	4
Coefficient of velocity of germination	29.41	31.25	57.69	53.57	27.78	25.42
Germination capacity	6.67	6.67	6.67	6.67	6.67	6.67
Speed of accumulated germination	7.25	8.42	11.5	9.5	9.33	7.42
Modified Timson's index,Germinaation energy index GEI	39	42	64	62	38	30

Table 2.2. Germination indices of the germinant.

Parameters	**Ctrl (H20) g**	**Ctrl (H20) µg**	**SNP 500ppm g**	**SNP 500ppm µg**	**SNP 1000ppm g**	**SNP 1000ppm µg**
Germination Percent (%) at 72hrs	40	53.33	100	100	46.67	66.67
Germination Time (hrs)	96	96	72	48	120	96
Mean Germination Time	3.4	3.2	2	1.67	3.47	2.87
Median Germination Time (T50) (hrs)	76	69	36	30	74.4	60

First Germination Percent (%)	20	26.67	13.33	33.33	26.67	13.33
Final Germination Percent (%)	100	100	100	100	100	100
Mean Daily Germination	20	20	20	20	20	20
Germination Index	4.75	5.08	8.17	10	4.85	6.42
Time for the First Germination (T0)	41	39	18	16	38	20
Time for the Last Germination (Tg)	96	96	72	48	120	96
Time Spread of Germination (Tg-To)	55	57	54	32	82	76
Peak Period of Germination	96	96	48	48	96	72
Germination Rate Index or Speed (GRI)	31.67	35.26	54.44	66.66	32.33	42.77
Corrected germination rate index (GRIcorrected)	0.32	0.35	0.54	0.67	0.32	0.43
Rate of Germination (hrs/seed)	4.75	5.08	8.17	10	4.85	6.42
Daily Germination Speed	0.29	0.31	0.5	0.6	0.29	0.34
Length of Roots (cm)	2.9	1.3	2.2	2.95	1.35	4.25
Length of Shoots (cm)	12.35	4.9	4.8	9	3.7	3.5
No. of Roots	10	6	12	15	10	9
Wet Seedling Weight (g)	1.24	0.55	0.71	1.28	0.59	0.74
Dry Seedling Weight (g)	0.15	0.1	0.4	0.24	0.36	0.33
Original Seed Dry Weight (g)	0.35	0.35	0.35	0.35	0.35	0.35
Seedling vigour I	1235	490	480	900	370	350
Seedling vigour II	1525	620	700	1195	505	775
Seedling viguor III	15	10	40	24	36	33
Coefficient of velocity of germination	29.41	31.25	50	60	28.84	34.88
Germination capacity	6.67	6.67	6.67	6.67	6.67	6.67
Speed of accumulated germination	260	280	400	433	253	313
Modified Timson's index, germination energy index (GEI)	39	42	60	65	38	47

Table 2.3. Germination indices of the germinant.

PARAMETERS	CTRL(H2O)g	CTRL(H2O) µg	T.UREA 500ppm µg	T.UREA 500ppm(g)	T.UREA 1000ppm µg	T.UREA 1000ppm(g)
Germination Percent (%) at	40	53.33	80	73.33	100	100

72hrs						
Germination Time(hrs)	96	96	96	96	72	72
Median Germination Time(T50)(hrs)	78	69	56.57	46.5	39.43	36
Final Germination Percent(%)	100	100	86.67	100	100	100
Mean Daily Germination	20	20	17.3	20	20	20
Germination Index	4.75	5.08	5.08	6	8.17	9.17
Time for the First Germination(T0)	48	48	48	48	24	24
Time for the Last Germination(Tg)	96	96	96	96	72	72
Time Spread of Germination(Tg-To)	48	48	48	48	48	48
Peak Period of Germination	96	96	72	48	48	72
Germination Rate Index or Speed (GRI)	31.67	33.89	33.9	40.01	54.44	61.11
corrected germination rate index(GRIcorrected)	0.32	0.34	0.34	0.4	0.54	0.61
Rate of Germination (hrs/seed)	4.75	5.08	5.08	6	8.17	9.17
Daily Germination Speed	0.29	0.31	0.44	0.37	0.47	0.5
Length of Roots(cm)	2.9	1.3	1.5	1.9	3.33	3.77
Length of Shoot(cm)	12.35	4.9	0.73	2.7	4.7	2.27
Number of Roots	10	6	3	9	18	6
Wet Seedling Weight(g)	1.24	0.55	0.63	0.99	0.81	0.77
Dry Seedling Weight(g)	0.15	0.1	0.32	0.37	0.21	0.3
Original Seed Dry Weight(g)	0.35	0.35	0.34	0.27	0.28	0.35
First Germination Percent(%)	20	26.67	33.33	53.33	20	33.33
Seedling vigour I	1,235	490	73	270	470	227
Seedling vigour II	1,525	620	223	400	803	604
Seedling vigour III	15	10	32	37	21	30
Mean germination time, MGT	3.4	3.2	2.3	2.73	2.13	2
Coefficient of	29.41	31.25	43.33	36.59	46.88	50

velocity of germination						
Germination capacity	6.67	6.67	6.67	6.67	6.67	6.67
Speed of accumulated germination	7.25	8.42	9.75	11.35	13	15
Modified Timson's index,Germinaation energy index GEI	39	42	43	49	58	60

Table 3.1: Biochemical stress marker responses of Phaseolus vulgaris to SNP chemo-priming (500ppm and 1000 ppm) under simulated microgravity (MG) and normal gravity (G) conditions.

Treatment / Tissue	Prot (g/dL)	SOD (U/g Prot)	CAT (U/g Prot)	GPx (U/g Prot)	MDA (mmol/g Prot)	Sugar (mg/mL)	Proline (µmol/g fw)	Vit C (µg/mL)
SNP 500ppm (MG) Root	1.144	1.288	0.834	1.937	0.316	1.19	2.028	62.044
SNP 500ppm (MG) Shoot	1.055	1.491	0.898	2.104	0.272	0.663	2.972	41.606
SNP 500ppm (MG) Seed	1.43	1.21	0.405	1.547	0.272	0.874	4.775	44.161
SNP 500ppm (G) Root	1.788	0.825	0.534	1.246	0.364	0.737	1.828	37.591
SNP 500ppm (G) Shoot	1.09	1.185	0.869	2.028	0.554	1.004	1.244	39.051
SNP 500ppm (G) Seed	2.431	0.1	0.164	0.892	0.203	3.225	3.759	38.321
SNP 1000ppm (MG) Root	1.591	1.015	0.598	1.378	0.396	0.813	5.384	44.526
SNP 1000ppm (MG) Shoot	1.448	1.014	0.646	1.525	0.39	1.079	4.372	37.226
SNP 1000ppm (MG) Seed	1.269	1.315	0.757	1.746	0.573	0.662	4.956	44.161
SNP 1000ppm (G) Root	1.287	1.144	0.741	1.715	0.504	0.765	2.313	42.336
SNP 1000ppm	1.895	0.483	0.508	1.177	0.422	0.669	3.288	39.781

(G) Shoot								
SNP 1000ppm (G) Seed	1.144	1.342	0.528	1.916	0.56	1.496	4.172	41.606

Table 3.2: Biochemical stress marker responses of Phaseolus vulgaris to IAA chemo-priming (500ppm and 1000 ppm) under simulated microgravity (MG) and normal gravity (G) conditions.

Treatment / Tissue	Prot (g/dL)	SOD (U/g Prot)	CAT (U/g Prot)	GPx (U/g Prot)	MDA (mmol/g Prot)	Sugar (mg/mL)	Proline (µmol/g fw)	Vit C (µg/mL)
IAA 500ppm (MG) Root	1.234	1.316	0.611	1.787	0.376	0.614	2.931	37.226
IAA 500ppm (MG) Shoot	0.554	2.787	0.567	3.403	0.916	0.876	2.694	41.241
IAA 500ppm (MG) Seed	0.822	1.892	1.158	2.685	0.507	0.555	3.338	38.686
IAA 500ppm (G) Root	0.983	1.358	0.967	2.242	0.207	0.499	1.013	40.876
IAA 500ppm (G) Shoot	0.947	0.993	0.978	2.314	0.26	1.082	2.494	38.686
IAA 500ppm (G) Seed	1.234	0.915	0.583	1.802	0.195	2.751	1.9	47.445
IAA 1000ppm (MG) Root	1.251	1.013	0.759	1.761	0.404	0.445	2.288	39.781
IAA 1000ppm (MG) Shoot	0.429	2.08	1.22	4.691	0.578	2.232	2.203	39.416
IAA 1000ppm (MG) Seed	0.411	2.56	2.292	5.361	0.458	1.166	4.7	39.416
IAA 1000ppm (G) Root	1.037	0.827	0.92	2.148	0.421	0.587	3.216	40.876
IAA 1000ppm (G) Shoot	1.09	0.299	0.446	2.004	0.346	0.506	4.456	37.591
IAA 1000ppm (G) Seed	1.055	0.835	0.663	2.112	0.542	1.624	3.491	42.336

Table 3.3: Biochemical stress marker responses of Phaseolus vulgaris to Thiourea chemo-priming (500ppm and 1000 ppm) under simulated microgravity (MG) and normal gravity (G) conditions.

Treatment / Tissue	Prot (g/dL)	SOD (U/g Prot)	CAT (U/g Prot)	GPx (U/g Prot)	MDA (mmol/g Prot)	Sugar (mg/mL)	Proline (µmol/g fw)	Vit C (µg/mL)
Thiourea 500ppm (MG) Root	0.769	1.251	1.238	2.887	0.605	0.422	5.291	46.35
Thiourea 500ppm (MG) Shoot	1.216	1.217	0.528	1.823	0.405	1.496	4.85	44.526
Thiourea 500ppm (MG) Seed	2.181	0.617	0.393	1.014	0.143	1.729	4.4	40.876
Thiourea 500ppm (G) Root	1.108	1.103	0.846	2.02	0.415	0.461	3.522	37.956
Thiourea 500ppm (G) Shoot	2.145	0.565	0.249	1.04	0.303	0.746	3.394	38.321
Thiourea 500ppm (G) Seed	1.323	0.874	0.724	1.672	0.157	2.584	2.506	43.431
Thiourea 1000ppm (MG) Root	1.323	0.805	0.717	1.666	0.276	0.53	4.509	41.971
Thiourea 1000ppm (MG) Shoot	2.628	0.507	0.357	0.848	0.07	0.542	4.041	47.445
Thiourea 1000ppm (MG) Seed	1.108	1.301	0.839	1.999	0.264	2.374	2.525	44.161
Thiourea 1000ppm (G) Root	0.965	0.159	0.844	2.315	0.825	0.536	3.025	41.241
Thiourea 1000ppm (G) Shoot	1.019	0.521	0.703	2.174	0.4	2.079	3.966	37.226
Thiourea 1000ppm (G) Seed	1.269	0.758	0.415	1.743	0.426	2.187	4.747	40.146

Note: Prot = total protein; SOD = superoxide dismurase; CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; MG = simulated microgravity; G= normal gravity. Values represent single determinations per treatment-tissue combination.

4. DISCUSSION

The present study demonstrated that seed priming with Indole-3-Acetic Acid (IAA), Sodium Nitroprusside (SNP), and Thiourea improved the germination and early growth of *Phaseolus vulgaris* under both normal gravity and simulated microgravity conditions. Although all treatments enhanced germination compared with the untreated control, their effectiveness varied depending on the type of growth stimulator and concentration used.

Germination was minimal during the first 24 hours but increased rapidly between 24 and 72 hours as the seeds became metabolically active. Seeds primed with Sodium Nitroprusside (SNP) at 500 ppm exhibited the fastest germination rate, indicating earlier radicle emergence and quicker seedling establishment than the other treatments. Indole-3-Acetic Acid (IAA) at 500 ppm also enhanced germination rate, whereas IAA at 1000 ppm delayed germination, suggesting that higher auxin concentrations may inhibit early seedling development**.** Thiourea, particularly at 1000 ppm**,** showed a gradual but sustained increase in germination rate throughout the experimental period. By 120 hours, all treatments had reached a plateau, with SNP demonstrating the greatest ability to accelerate germination under simulated microgravity.

Simulated microgravity altered normal germination dynamics by reducing the rate of seedling establishment and affecting root orientation. Nevertheless, chemopriming substantially alleviated these adverse effects. Improved germination characteristics in treated seeds suggest that all three growth stimulators enhanced stress tolerance by maintaining cellular homeostasis, improving antioxidant activity, and promoting efficient utilization of stored food reserves during early development.

Among the treatments evaluated, SNP at 500 ppm consistently produced the greatest improvement in both germination percentage and germination rate, followed by IAA at 500 ppm and Thiourea at 1000 ppm. These findings demonstrate that appropriate chemical priming can serve as an effective strategy for improving crop establishment under simulated microgravity and may contribute significantly to future controlled-environment agriculture and space-based food production systems.

5. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

1. Sodium Nitroprusside (SNP) at 500 ppm should be considered the preferred growth stimulator for enhancing the germination and early seedling development of Phaseolus

vulgaris under simulated microgravity conditions because it consistently produced the best overall performance.

2. Indole-3-Acetic Acid (IAA) should be applied at moderate concentrations, particularly 500 ppm, since higher concentrations may reduce germination performance and early seedling growth.
3. Thiourea should be considered an effective alternative growth stimulator for improving germination uniformity, root development, and seedling establishment under reduced-gravity conditions.
4. Future studies should investigate combined applications of IAA, SNP, and Thiourea to determine whether synergistic interactions among auxin signaling, nitric oxide, and sulfur metabolism further enhance plant adaptation to simulated microgravity.
5. Additional concentration ranges and priming durations should be evaluated to determine the optimum treatment conditions for maximizing germination and seedling vigour.
6. Similar investigations should be conducted on other economically important food crops, including maize, rice, soybean, cowpea, and wheat, to determine species-specific responses to these growth stimulators.
7. Long-term studies extending beyond the seedling stage should evaluate the effects of IAA, SNP, and Thiourea on vegetative growth, flowering, pod formation, yield, and seed quality under simulated and actual microgravity environments.
8. More advanced microgravity simulation systems, such as three-dimensional clinostats and random positioning machines, should be incorporated into future research to improve the accuracy of ground-based microgravity studies.
9. Increased investment in space biology and controlled-environment agriculture research is recommended to support the development of sustainable crop production technologies for future lunar, Martian, and orbital missions.

CONCLUSION

This study demonstrated that growth stimulators can improve the germination and early development of Phaseolus vulgaris under both normal gravity and simulated microgravity conditions. Among all treatments, SNP at 500 ppm gave the best results by promoting faster germination, better seedling growth, and improved overall performance. Indole-3-Acetic Acid at 500 ppm also enhanced germination and early growth, whereas excessive concentration reduced performance, demonstrating the importance of maintaining optimal auxin levels. Thiourea

effectively improved germination uniformity, root development, and seedling establishment, particularly under simulated microgravity, highlighting its value as a stress-mitigating growth stimulant.. Overall, the findings indicate that chemical seed priming represents an effective strategy for reducing the adverse effects of simulated microgravity on common bean germination and early development. Among the treatments evaluated, SNP at 500 ppm exhibited the greatest overall potential for supporting crop establishment in controlled-environment agriculture and future space-based food production systems.

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