Investigating the effect of varying concentrations of Potassium nitrate (KNO_3) solution on the protein concentration in Mung bean, *Vigna radiata* plant, as measured by Lowry protein assay

Word count: 2995

Research question

What is the effect of varying concentrations of potassium nitrate (**KNO_3**) solution (0%, 5%, 10%, 15%, and 20%) **as a nitrogen source** on the protein concentration (**µg/mL**)in mung beans (*Vigna radiata)*, as measured by the Lowry protein assay and identifying the optimal concentration of potassium nitrate for maximizing protein concentration?

1.0 Introduction

Figure 1. *Mung bean plants*

Vigna radiata, commonly known as the Mung bean, is a legume that is cultivated for its raw edible seeds and sprouts, mostly in Asia (Indian subcontinent and East Asia). It is a fast-growing legume that typically grows in warm seasons and is highly tolerant as it does not require large amounts of water, or specific soil types and grows in temperatures above 15 C. [1] Mung beans are known to be a great source of protein, containing about 20.97% to 31.32% protein content[2] and is rich in Zinc, Copper, Iron and Calcium, which makes it an essential legume in vegetarian meals in regions of Southeast Asia due to its health benefits. Consuming mung beans can be beneficial to lowering the level of LDL cholesterol, reducing the risk of high blood pressure, and being a rich source of Iron, it increases the hemoglobin level in the red blood cells.[3]

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[1] Mogotsi, K. K., 2006. *Vigna radiata* (L.) R. Wilczek. In: Brink, M. & Belay, G. (Editors). PROTA 1: Cereals and pulses/Céréales et légumes secs. [CD-Rom]. PROTA, Wageningen, Netherlands.

[2] Anwar, F., Latif, S., Przybylski, R., Sultana, B., & Ashraf, M. (2007). Chemical Composition and Antioxidant Activity of Seeds of Different Cultivars of Mungbean. *Journal of Food Science*, *72*(7), S503–S510. https://doi.org/10.1111/j.1750-3841.2007.00462.x

[3] Nishu, Sood, M., & Bandral, J. (2023). Mungbean -A Legume for Human Health. *Mungbean - a Legume for Human Health*, *10*(03), 52–55. Retrieved from https://indianfarmer.net/uploads/202334.pdf

1.1 Effect of Potassium nitrate on protein concentration in plants

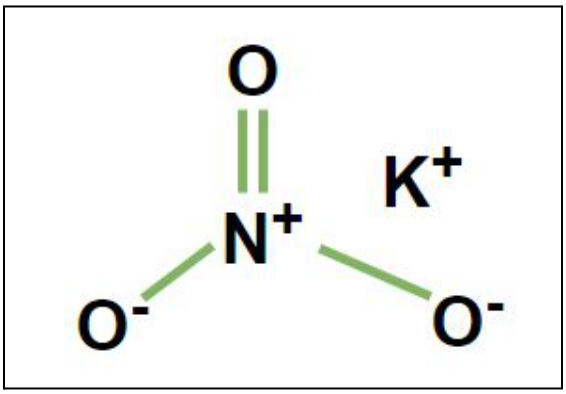


Figure 2. *Structure of Potassium Nitrate*[4]

Potassium nitrate or KNO_3 is an ionic inorganic soluble salt that is the source of two crucial nutrients that are required by plants. Plants require Nitrogen and Potassium for the synthesis of proteins, enzyme regulation, disease resistance, stomatal opening & closing, and facilitating water & nutrient uptake from the soil. Hence, because of potassium nitrate's quality, it is regarded as one of the best fertilizers for plants as it potentially boosts the yield and quality of the plants.[5]

Nitrogen provided by potassium nitrate is an important component of amino acids, which are the basic building blocks of proteins in any living organism. The concentration of potassium nitrate that is applied to plants is crucial as it consists of nitrogen, which is a key component for amino acids during protein synthesis. Hence this study aims to investigate how varying concentrations of potassium nitrate (KNO_3) affect the protein concentration in mung beans (***Vigna radiata***) and to identify the optimal concentration that shall maximize the protein synthesis.

[4] Potassium Nitrate Formula - Structure, Properties, Uses, Sample Questions. (2022, May 9). Retrieved December 10, 2024, from GeeksforGeeks website: https://www.geeksforgeeks.org/potassium-nitrate-formula-structure-properties-uses-sample-questions/

[5] Mosaic. (n.d.). Potassium Nitrate. Retrieved December 10, 2024, from Mosaic Crop Nutrition website: https://www.cropnutrition.com/resource-library/potassium-nitrate/

2.0 Background information

Lowry Protein Assay is a biochemical method developed by Oliver H. Lowry, which is used for quantifying or determining an unknown amount of protein in a sample. The Lowry protein assay consists of the reaction of proteins present in the sample with a mixture of reagents such as copper ions and the Folin-Ciocalteu reagent, which results in the developing of a dark blue color and then is measured at the wavelength (λ) of 660 nm using a colorimeter or a spectrophotometer.

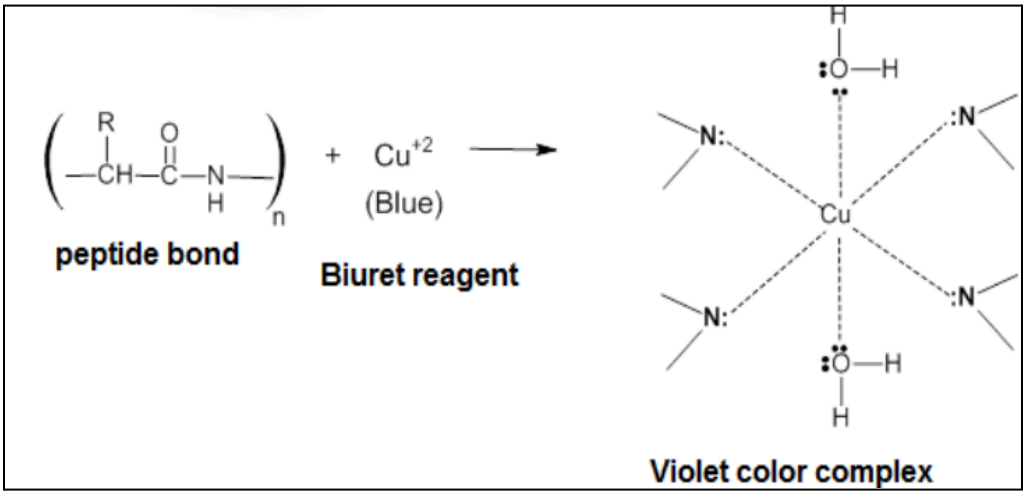


Figure 3. The reaction between the peptide bond and Biuret reagent[6]

The Lowry protein assay's principle is based on two reactions -the Biuret reaction and Lowry's reaction that leads to the formation of a colored compound-complex. The Biuret reaction involves the reaction of copper sulfate (**$CuSO_4$**) with the peptide bonds (present in the proteins) under alkaline conditions, which results in the reduction of copper(I) ions. Moving on, Lowry's reaction involves the reduction of the Folin-Ciocalteu reagent, which will result in the development of a blue-violet color over time, and then the sample can be analyzed in a colorimeter or spectrophotometer at around 660 nm absorbance.[6] The Lowry method was chosen for its high sensitivity and accuracy in measuring protein concentrations under varying KNO_3 levels. Unlike the Bradford assay, which is affected by certain chemicals, Lowry provides more stable and precise readings for complex plant extracts. It is also more accessible and cost-effective than the BCA method.

An initial pilot reading was performed, from which it was deduced that the range of concentrations of f 0%, 5%, 10%, 15%, and 20% KNO_3 represented the effect of both an increase in protein concentration at moderate nitrate levels and a decline at higher nitrate concentrations.

[6] *BZCYL-136 Physiology and Biochemistry: Laboratory 62 EXERCISE 10 ESTIMATION OF PROTEINS BY LOWRY'S METHOD Structure*. (n.d.). Retrieved from https://egyankosh.ac.in/bitstream/123456789/68738/1/Exercise%2010.pdf

3.0 Hypothesis

Alternative Hypothesis (H_1)

The varying concentrations of potassium nitrate (KNO_3) (from 0%, 5%, 10%, 15%, and 20%) will significantly affect the protein concentration (µg/mL) in mung bean (***Vigna radiata***) plants. It is expected that the protein concentration will initially increase with increasing KNO_3 concentration, until reaching a peak at an optimal concentration of KNO_3, where the protein concentration is maximum because of nitrogen's crucial role in amino acid and protein synthesis. However, beyond the optimal concentration, further increases in KNO_3 concentration may result in a decline in protein concentration, potentially because of nitrogen toxicity, osmotic stress, and nutrient imbalance, which hinder the plant's ability to synthesize proteins efficiently.

Null Hypothesis (H_0)

The varying concentrations of potassium nitrate (0%, 5%, 10%, 15%, and 20%) will have no statistically significant effect on the protein concentration (µg/mL) in Mung bean (Vigna radiata) plants.

4.0 Variable(s)

4.1 Independent variable

Independent variable	Why was this changed	How will it be changed
The varying concentrations(0%, 5%, 10%, 15%, 20%) of potassium nitrate solution, (KNO_3).	The varied concentration of potassium nitrate was used to investigate its effect on plant growth, specifically, the protein concentration in the mung bean plant grown in the varied concentration of potassium nitrate.	The varying concentrations of potassium nitrate will be prepared following the methodology mentioned in **Section 6**, Procedure and Methodology.

4.2 Dependent variable

Dependent variable	Why is it measured	How it will be measured
The protein concentration (**µg/mL**) in 500 milligrams of Mung beans (**Vigna radiata**) extract, measured by the Lowry protein assay method.	The protein concentration (**µg/mL**) is measured to determine the effect of potassium nitrate concentration on protein synthesis in mung beans as nitrogen is a key component of amino acids.	The protein concentration will be measured using the Lowry protein assay, where a standard curve will be prepared using BSA as a reference protein and absorbance will be measured at 660 nm.

4.3 Controlled variables

Controlled variable	Purpose of control	Method for control
Plant species	To maintain consistency in the plant's response across different concentrations of potassium nitrate.	Using Vigna radiata across all treatments.
Volume of Potassium Nitrate solution	To maintain equal exposure to potassium nitrate for all the treatments.	Measuring 10 mL of potassium nitrate solution per plant for each treatment.
Growth conditions (light and temperature)	To provide an equal advantage for the Vigna radiata to grow for all treatments.	Placing all plants in the same location with equal light and maintaining temperature at 25°C.
Watering schedule for plants		Adding 10 mL of respective KNO_3 solution on alternate days for one week.
mass of the extracted sample	To ensure that the same sample mass is analyzed for consistent protein measurement.	Using 0.5 grams (or 50 mg) of plant sample for protein analysis.
Amount and type of the soil	To maintain consistent pH and nutrient availability across treatments.	By using the same type and 350 grams of soil in the plant pots for all the treatments.

Table 3. Controlled variables.

5.0 Materials and Apparatus Required

Sr.No	Material - Apparatus	Quantity	Intended use or purpose of use	Uncertainty
1	Digital weighing balance	1 unit	For measuring the accurate mass of soil, extracted samples and chemicals.	±0.01 gram
2	Pestle and mortar	1 unit	For grinding and making the plant tissue homogenous for protein analysis.	-
3	Beaker 500 mL	7	For preparing and storing KNO_3 and mixing protein assay reagents.	-
4	100 mL measuring cylinder	1	For measuring the distilled water used for preparing potassium nitrate solutions	±0.5 mL
5	10 mL measuring cylinder	1	For accurately measuring small volumes of KNO_3 solution and reagents.	±0.05 mL
6	Volumetric flask	3	For preparing and diluting standard protein (BSA) solutions and other reagents.	±0.3 mL
7	Test tubes	10	For storing the reagents for protein analysis and protein extract samples.	-
8	Test tube stand	1 unit	For holding the test tubes.	-
9	Pipette 10 mL	1	For measuring and transferring the samples.	±0.001 mL
10	Centrifuge machine	1 unit	For separating the supernatant and separating any debris.	-
11	Spectrophotometer	1 unit	For measuring sample absorbance and determining protein content.	-
12	plant pots x mL/L	5 pots	For growing the Vigna radiata plants.	-
13	Powder gloves	5 pairs	To protect hands from any potential corrosive or hazardous substances or chemicals	-
14	Protecting goggles	1 pair	To protect hands from any potential corrosive or hazardous substances or chemical	-
15	cuvette	5 units	For holding samples during absorbance reading at 660 nm.	-

Table 2a. List of materials and apparatus required**.**

5.1 Chemicals and biological materials required

Sr.No	Chemicals and biological materials required	Quantity	Intended use or purpose of use	Uncertainty
1	mung beans (**Vigna radiata**) seeds	100 grams	To study the effect of nitrate on protein content in mung beans.	-
2	Potassium Nitrate	70 grams	For preparing the different KNO_3 solutions.	
3	Soil	grams	As a growth medium providing pH and nutrients.	-
4	Distilled water	1 L	To prepare KNO_3 solutions and Lowry assay reagents.	-
5	Copper sulfate crystals	5 grams	For Lowry's reagent in protein concentration measurement.	-
6	Sodium Hydroxide pellets	10 grams	For preparing the alkaline reagent in Lowry's	-
7	Sodium Carbonate	5 grams	To prepare the buffer in Lowry's assay	
8	Sodium Tartrate solution	5 grams	To stabilize reactions in Lowry's	-
9	Folin-Ciocalteau reagent.	5 mL	To develop color indicating protein presence in Lowry's assay.	-
10	Phosphate buffer	150 mL	For leaf homogenization and pH maintenance during assay.	
11	Bovine serum albumin	3 grams	To prepare standard solution for calibrating Lowry's assay.	-

Table 2b. List of chemicals and biological materials required**.**

6.0 Procedure and Methodology

1. Preparation for planting Vigna radiata:

- Start by germinating about 100 grams of Vigna radiata seeds in distilled water for 24 hours.
- Label five identical plant pots as 0%, 5%, 10%, 15%, and 20%, respectively, and then add 350 grams of soil to each pot using an electronic balance.
- Weigh 20 grams of germinated seeds using an electronic balance and then sow them evenly over the soil surface in each pot.
- Water each pot with 50 mL of distilled water immediately after sowing the seeds to initiate growth and ensure that all pots are exposed to the same light and temperature conditions.

2. Preparation for the concentrations of Potassium Nitrate solution:

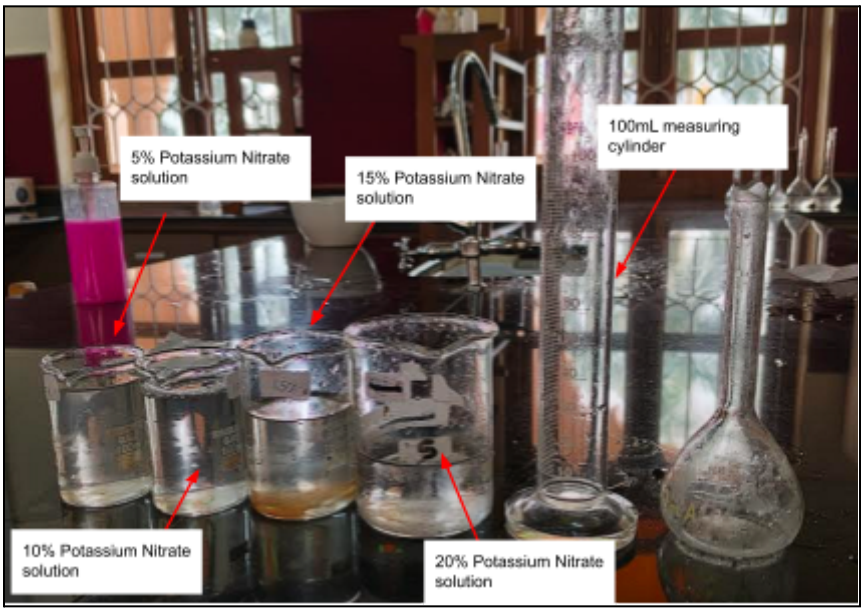


Figure 4. concentrations(%) of Potassium Nitrate solution

Concentration	KNO_3 (grams)	Distilled Water (mL)
5%	5	95
10%	10	90
15%	15	85
20%	20	80

Table 3. Preparation of Potassium Nitrate (KNO_3) Solutions at Different Concentrations

- Start preparing each solution by dissolving the measured KNO_3 powder (in grams) in the appropriate volume of distilled water using a beaker and glass rod, as seen in ***Table 3.***
- Then stir each solution for about 2 minutes to ensure that the KNO_3 is completely dissolved.
- Using a measuring cylinder, 10 mL of the varying concentrations of KNO_3 solutions were added to the plants on alternate days over a 7-day period, to ensure a gradual nitrogen uptake and prevent over-fertilization or stress on roots.

3. Preparation for reagents to be used in the protein analysis:

Preparation for Reagent A

- Start by weighing 2 grams of Sodium Carbonate (**Na_2CO_3**) using the electronic balance and add it to 100 mL of 0.1 N Sodium hydroxide solution.(**NaOH**) and stir for 3 minutes until fully dissolved.

Preparation for Reagent B

- Then, in another beaker, dissolve 0.5 grams of Copper sulfate in 100 mL of 0.1 N Sodium tartrate solution and stir it well for 2 minutes.

Preparation for Reagent C

- In a separate beaker, add 50 mL of **Reagent A** and 1 mL of **Reagent B** and stir them well.

Preparation for Reagent D

- Dilute the Folin-Ciocalteu reagent with an equal 0.1 N Sodium hydroxide (**NaOH**) volume (1:1 ratio) and stir it gently for about 30 seconds.

4. Preparation for standard protein solution:

- In a volumetric flask, dissolve 0.05 gram (50mg) of BSA in 50 mL of distilled water and take 10 mL of this stock standard solution, and dilute it to 50 mL in another volumetric flask for making the working standard solution.

5. Preparing the plant extract (analyte sample):

- Pluck and weigh 0.5 grams of mung bean leaves using an electronic weighing balance for leaves from each mung bean plant grown in successive concentrations of Potassium nitrate.
- Transfer the 0.5 gram of leaves into the mortar and pestle and add 10 mL of phosphate buffer solution.
- Grind the leaves until the mixture is homogeneous. Then, transfer the homogeneous plant mixture extract into a test tube and centrifuge it for about 10 minutes to allow optimal separation of soluble protein from the plant debris.
- After 10 minutes, carefully pipette the supernatant into a new test tube and discard the residue.

6. Measuring the protein content:

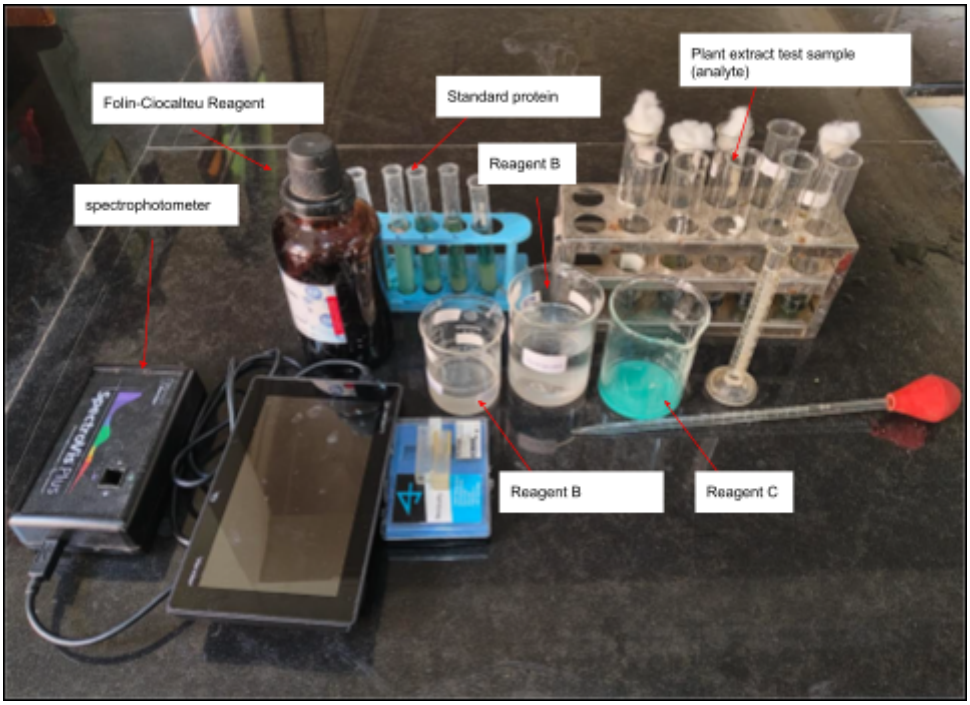


Figure 5. Complete apparatus required to perform the Lowry's protein assay

- Pipette 1 mL of plant extract into a clean test tube
- Then add 0.5 mL of Reagent C to each test tube and mix gently by swirling it.
- Let the solution stand still for about 10 minutes at room temperature.
- Then, add 0.5 mL of Reagent D to each tube and immediately mix it for about 3 minutes.
- Incubate all the tubes at room temperature for 30 minutes to allow the blue-colored complex to develop.
- After the development of the color, transfer 3 mL of the colored analyte into a clean cuvette using a pipette.
- Place the cuvette into a spectrophotometer, and measure the absorbance at 660 nm, and repeat the measurements for all the test samples and also for the BSA standard solution.
- Lastly, use the absorbance values and the BSA standard curve to calculate the protein concentrations.

7.0 Risk assessment

7.1 Safety concerns

Chemicals	Formula	Potential risks	Precautions	First Aid
Sodium Hydroxide pellets	**NaOH**	Corrosive; causes burns to skin and eyes, and respiratory issues.	Wear gloves, goggles, and a mask. Add water carefully; store dry.	Rinse with water for 15 minutes or seek medical help if it is severe.
Folin-Ciocalteu Reagent	-	Irritating to skin and eyes; contains strong acids like phosphomolybdic and phosphotungstic acid	Wear gloves, goggles, and a mask. Avoid contact; rinse immediately if exposed.[7]	Rinse skin or eyes thoroughly or else seek medical attention.
Sodium Tartrate Solution	$C_4H_4Na_2O_6$	May cause mild skin and eye	Wear gloves, goggles, and a mask. Avoid contact; rinse thoroughly if exposed.[8]	Wash the area of contact with soap and water

Table 4. Safety concerns

7.2 Environmental considerations

- Lowry's reagents, containing toxic chemicals like copper sulfate and Folin, must not be poured down drains. Excess potassium nitrate and sodium tartrate should be collected in labeled containers for proper disposal by lab technicians, as they can cause eutrophication and algal blooms.
- The Folin–Ciocalteu reagent in Lowry's assay is harmful to aquatic ecosystems. It must be collected in a labeled hazardous waste container and not poured into sinks or drains. The waste should be handed to lab technicians for proper disposal according to environmental regulations.
- The excess sodium hydroxide is to be neutralized with dilute acid under the fume hood and handed over to the lab technicians for appropriate disposal.

[7] SAFETY DATA SHEET. (2024, February 9). Retrieved December 12, 2024, from https://www.fishersci.fi website: https://shorturl.at/5r1IL

[8] *Safety Data Sheet*. (2022). Retrieved from **https://www.takarabio.com/documents/SDS/SD3003/SD3003-AGHS-EN.pdf**

7.3 Ethical considerations

1. Data integrity is maintained by accurately reporting all the quantitative and qualitative data obtained during this experiment and ensuring the integrity of the experiment's findings.

2 . If the plants are healthy, then they can be transplanted into the soil and allowed to grow instead of being discarded.

3. However if some plants die, then they will be composted or disposed of based on the local organic waste guidelines.

8.0 Raw Data Collection

8.1 Qualitative observations

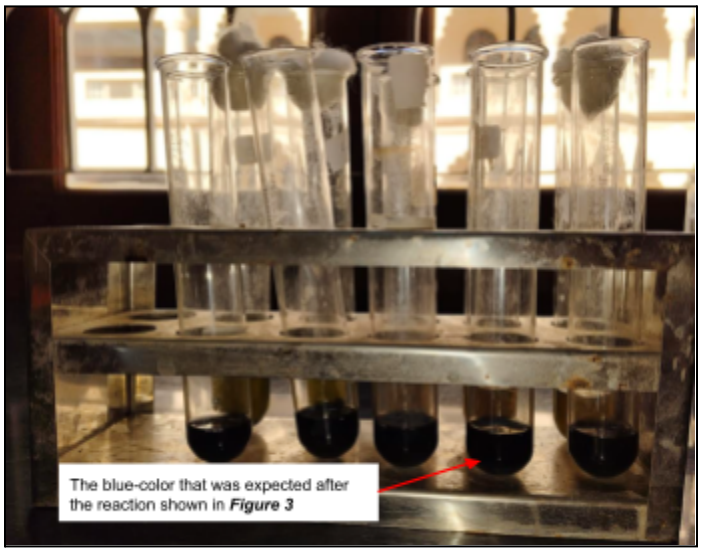


Figure 6. The expected violet color complex for plant samples

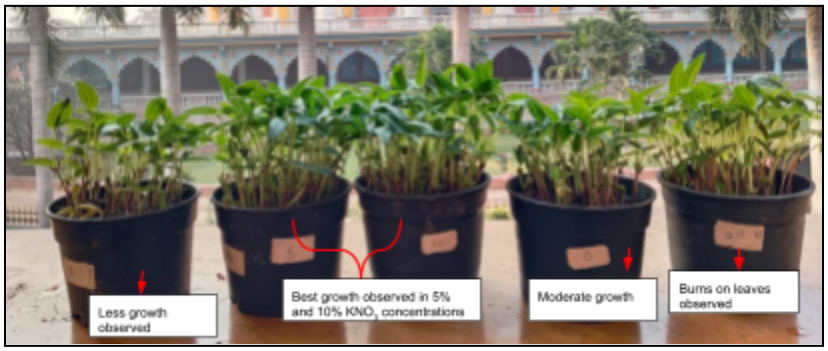


Figure 7. Qualitative observation of the plants after 5 days

8.2 Quantitative observations

Volume of BSA (mL)	Volume of Water (mL)	Absorbance at 660 nm
0	5	0.133
1	4	0.197
2	3	0.222
3	2	0.256
4	1	0.286
5	0	0.369

Table 5. The absorbance of different volumes of BSA

Concentration of KNO_3 (%)	Optical Density (λ600 nm)					Mean Optical Density (λ600 nm)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
0	1.905	1.894	1.745	1.897	1.896	1.867
5	1.936	2.130	2.138	2.132	2.134	2.094
10	2.152	2.154	2.150	1.989	2.155	2.120
15	1.837	1.838	1.832	1.828	1.835	1.834
20	1.833	1.829	1.827	1.820	1.802	1.822

Table 6. The absorbance of plant extracts grown in different concentrations of KNO_3

9.0 Data Processing

Calculation for Standard Protein Curve

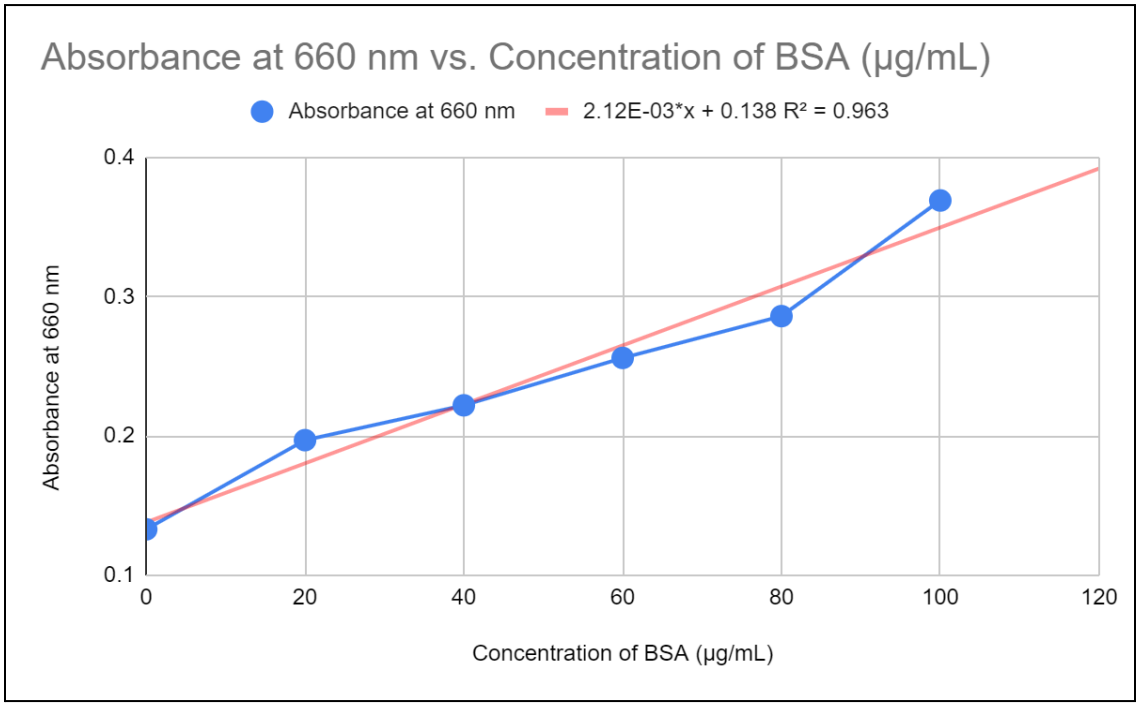
$$Concentration\ of\ BSA\ (\mu g/mL) = \frac{Volume\ of\ BSA(mL) \times Stock\ concentration}{Total\ volume}$$

$$= \frac{3\ mL \times 100 \mu g/mL}{5\ mL}$$

$$= 60\ \mu g/mL$$

Concentration of BSA (µg/mL)	Absorbance at 660 nm
0	0.133
20	0.197
40	0.222
60	0.256
80	0.286
100	0.369

Table 7. The concentration of BSA and corresponding Absorbance at 660 nm



Graph 1. Standard Curve for Protein by Lowry's Method

Equation of line of best fit: $y = 0.00212x + 0.138$

Protein concentration: $(\mu g/mL) = \frac{Mean\ Optical\ Density\ at\ (660\ nm) - 0.138}{0.00212}$

$= \frac{1.867 - 0.138}{0.00212}$

$= 815.57\ \mu g/mL$

Uncertainty propagation

$$Percentage\ uncertainty\ (\%) = (\frac{Uncertainty}{Measurement}) \times 100$$

Uncertainty about weighing KNO_3 for the preparation of solutions

$$uncertainty\ for\ 5\ grams\ KNO3\ \ (\%) = (\frac{0.01}{5}) \times 100 = 0.2\%$$

$$uncertainty\ for\ 95\ mL\ water\ (\%) = (\frac{0.5}{95}) \times 100 = 0.5\%$$

Mass of KNO_3 (gram)	Uncertainty (%)	Volume of water (mL)	Uncertainty (%)
0	0.00	100	0.5
5	0.2	95	0.5
10	0.1	90	0.55
15	0.06	85	0.58
20	0.05	80	0.62

Table 8. Uncertainties for KNO_3 concentrations

KNO_3 concentrations (%)	Total Uncertainty (%)
0	0.5
5	0.7
10	0.65
15	0.64
20	0.67

Table 9. Total uncertainties for KNO_3 concentrations

Uncertainty during the preparation of reagents

Reagent A

$$Uncertainty\ for\ Mass\ (\%) = (\frac{0.01}{2.00}) \times 100 = 0.5\%$$

$$Uncertainty\ for\ volume\ (\%) = (\frac{0.5}{100.0}) \times 100 = 0.5\%$$

$$Combined\ uncertainty(\%) = \sqrt{(0.5)^2 + (0.5)^2}$$

$$= \sqrt{(0.25) + (0.25)} = 0.7\%$$

Reagent B

$$Uncertainty\ for\ Mass\ (\%) = (\frac{0.01}{0.5}) \times 100 = 2.0\%$$

$$Uncertainty\ for\ volume\ (\%) = (\frac{0.5}{100.0}) \times 100 = 0.5\%$$

$$Combined\ uncertainty(\%) = \sqrt{(2.0)^2 + (0.5)^2}$$

$$= \sqrt{(4.0) + (0.25)} = 2.06\%$$

Reagent C

$$Uncertainty\ for\ Volume\ Reagent\ A\ (\%) = (\frac{0.5}{50}) \times 100 = 1.0\%$$

$$Uncertainty\ for\ Volume\ Reagent\ B\ (\%) = (\frac{0.001}{1.0}) \times 100 = 0.1\%$$

$$Combined\ uncertainty(\%) = \sqrt{(1.0)^2 + (0.1)^2} = 1.0\%$$

Reagent D

$$Uncertainty\ for\ Volume\ Folin - Ciocalteu\ (\%) = (\frac{0.5}{50.0}) \times 100 = 1.0\%$$

$$Uncertainty\ for\ Volume\ NaOH(\%) = (\frac{0.5}{50.0}) \times 100 = 1.0\%$$

$$Combined\ uncertainty(\%) = \sqrt{(1.0)^2 + (1.0)^2} = 1.41\%$$

Reagent	Total uncertainty(%)
A	0.7
B	2.06
C	1.0
D	1.41

Table 10. Total uncertainties for Lowry's Reagents

Uncertainty while making the standard protein (BSA) solution

Preparation of the protein stock solution

$Uncertainty\ (\%)\ =\ (\frac{0.3}{50})\ \times\ 100\ =\ 0.6\%$

Transferring 10 mL of the stock solution using a pipette

$Uncertainty\ \ (\%)\ =\ (\frac{0.001}{10})\ \times\ 100\ =\ 0.01\%$

Preparation of the working standard solution

$Uncertainty\ (\%)\ =\ (\frac{0.3}{50})\ \times\ 100\ =\ 0.6\%$

Combined uncertainty

$$Combined\ uncertainty(\%)\ =\sqrt{(0.6)^2\ +\ (0.01)^2\ \ +\ (0.6)^2}$$

$$=\sqrt{(0.36)\ \ +\ (0.0001)\ \ \ +\ (0.36)}\ =\ 0.849\%$$

$$Final\ Combined\ uncertainty\ (\%) for\ 5\% KNO3$$

$$=\sqrt{(0.5)^2\ +\ (0.7)^2\ \ +\ (2.06)^2\ +\ (1.00)^2\ +\ (1.41)^2\ +\ (0.849)^2}$$

$$Final\ combined\ uncertainty(\%)\ for\ 5\%\ KNO3\ \ =\ 2.94$$

$$Sample\ calculation\ for\ standard\ deviation\ for\ 0\%\ KNO_3\ within\ replicates$$

$$\overline{x}=\frac{833.49+828.30+759.91+829.25+828.77}{5}=\ 815.57\mu g/mL$$

$$s\ =\frac{\sqrt{(833.49-815.57)^2+(828.30-815.57)^2+(759.91-815.57)^2+(829.25-815.57)^2+(828.77-815.57)^2}}{5-1}=\ \ 32.34\mu g/mL$$

KNO_3 concentrations (%)	**0%**	**5%**	**10%**	**15%**	**20%**
Standard deviation	31.39	41.68	31.97	1.88	5.43

Table 11. Standard deviation between replicates of each KNO_3 concentrations

One-Way ANOVA Test

A one-way ANOVA will be used to assess whether varying KNO_3 concentrations significantly affect protein levels in *Vigna radiata*. This method compares the means of the five treatment groups (0–20% KNO_3) using absorbance values at 660 nm from the Lowry assay, which correlates with protein content. A p-value below 0.05 indicates a significant difference and supports the alternative hypothesis; a value above 0.05 suggests no significant effect.

Source	Sum of Square ***SS***	Degree of freedom ***df***	Mean Square ***MS***	***F-statistic***
Between treatments	0.4311	4	0.1078	29.866
Within treatments	0.0722	20	0.0036	
Total	0.5033	24	-	

Table 12. ANOVA test summary

Using the ***F-statistic***, the p-value of 3.5149×10^{-8} was obtained as seen in ***Figure 8*** , hence the p-value is less than significance 0.05 (**p < 0.05**), which means the null hypothesis (H_0) is rejected and the alternative hypothesis (H_1) is accepted –indicating that potassium nitrate concentrations have a significant effect on the protein concentration in mung bean plants.

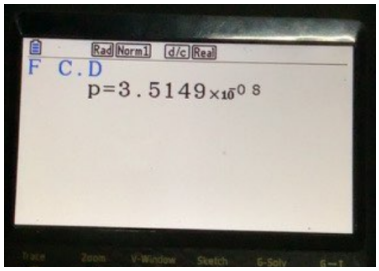


Figure 8. p-value obtained via GDC

The one-way ANOVA revealed a statistically significant difference in absorbance values across treatments ($F = 29.86$, $p < 0.05$). A Tukey's HSD post-hoc test was then conducted to determine which specific KNO_3 concentrations differed significantly in their effect on protein concentration. Both tests were performed using the Social Science Online ANOVA Tool[9], based on raw absorbance data.

[9] Social Science Statistics. (2025). One-Way ANOVA Calculator. Retrieved from Social Science Statistics website: https://www.socscistatistics.com/tests/anova/default2.aspx

Comparison of KNO_3 concentrations(%)	Mean Difference	p-value
0% vs 5%	0.23	0.00007
0% vs 10%	0.25	0.00002
5% vs 15%	0.26	0.00001
5% vs 20%	0.27	0.00001
10% vs 15%	0.29	0.00000
10% vs 20%	0.30	0.00000

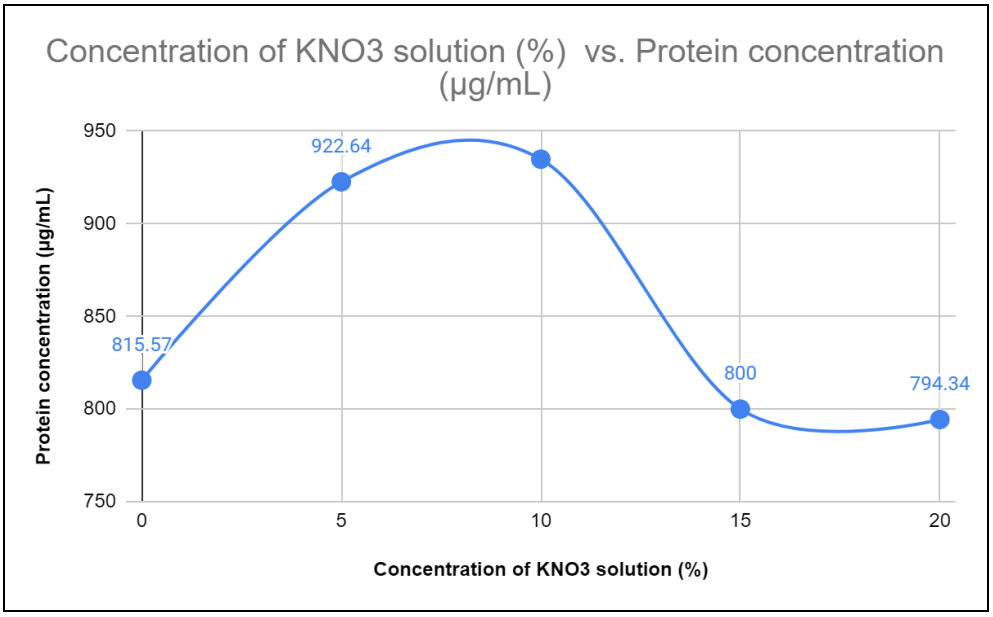
Table 13. Tukey HSD summary

Table 12 shows significantly higher protein levels at 5% and 10% KNO_3 compared to 15% and 20% ($p < 0.05$), indicating an optimal concentration for protein production. No significant difference between 15% and 20% suggests a plateau or decline. Tukey's HSD supports the alternative hypothesis that protein peaks at an optimal level, while higher concentrations may reduce it due to stress or nutrient imbalance.

9.1 Processed data

Concentration of KNO_3 solution (%)	Protein concentration (µg/mL)	Final combined Uncertainty(%)
0	815.57	2.94
5	922.64	2.98
10	934.91	2.97
15	800.00	2.97
20	794.34	2.98

Table 14. Final processed data



Graph 2. Represents the relation between concentration of KNO_3 and protein concentration

The above graph represents the relationship between different concentrations of potassium nitrate or KNO_3 solutions and the protein concentration in the Mung bean (Vigna radiata) plants over ten days.

10.0 Conclusion

This investigation aimed at how varying concentrations of potassium nitrate (KNO_3) from 0%, 5%, 10%, 15%, and 20%, affect the protein concentration in mung beans (***Vigna radiata***), as measured using the Lowry protein assay. After the experiment and analysis of the data, the result represented a significant trend—the protein concentration increased from 815.57 µg/mL at 0% KNO_3 to a peak of 934.91 µg/mL at 10 %, but as the KNO_3 concentrations increased to 15% to 20% the proteins concentration declines to 800 µg/mL and 794.34 µg/mL respectively. Hence, these suggest the presence of an optimal nitrate level that maximizes protein synthesis.

As noted in Section 1.1, nitrogen is essential for amino acid and protein synthesis. Nitrate ions (NO_3^-) from KNO_3 are absorbed by roots and reduced to ammonium (NH_4^+) via nitrate and nitrite reductase. Ammonium is then incorporated into amino acids through the glutamine synthetase–glutamate synthase pathway, leading to protein synthesis. Higher nitrate availability enhances this process, increasing protein production.

However, if the nitrate availability is excessive like 15-20%, then it disrupts the effective protein synthesis because due to excessive nitrate can inhibit root functions, disrupt ion homeostasis, and increase osmotic pressure, leading to reduced photosynthetic efficiency and stress response, as

mentioned in the **Journal of Plant Physiology**[10] and can also inhibit the activity of nitrate reductase and reduce the nitrogen assimilation efficiency.

Additionally, potassium nitrate also influences other physiological processes such as enzyme activation, photosynthesis, and water regulations. In research on **Carbohydrate polymers**[11], it is found that moderate nitrogen significantly corresponds to an increased protein concentration in mungs, while excessive nitrogen reduces the protein concentration. Although the mung bean plants can fix atmospheric nitrogen by root nodules, KNO_3 provides a readily available external nitrogen source, that enhances protein production.

In conclusion, 5% to 10% KNO_3 concentrations could be considered as the optimal range for maximum protein synthesis in Vigna radiata, where 10% KNO_3 results in the highest protein concentration of 934.91 µg/mL. However, beyond 10% KNO_3 concentration, the protein concentration declines because of the nutrient imbalance, stress response, and reduced enzyme activity.

10.1 Evaluation

10.2 Strengths of the experiment

The research question is well-defined and focuses on the relationship between potassium nitrate concentration and protein levels in *Vigna radiata*, aiming to identify the optimal concentration for maximum protein yield. The use of the Lowry Protein Assay provides a sensitive and quantitative method for accurate measurement. Other external variables—such as temperature, light, plant species, KNO_3 volume, watering schedule, and sampled plant parts are controlled to ensure reliability. This investigation also has strong real-world relevance, offering potential applications in agriculture to optimize nitrogen-based fertilizer use for improved growth and protein content.

[10] Zhou, H., Liu, Y., Mu, B., Wang, F., Feng, N., & Zheng, D. (2023). Nitrogen limitation affects carbon and nitrogen metabolism in mung bean (Vigna radiata L.). *Journal of Plant Physiology*, *290*, 154105–154105. https://doi.org/10.1016/j.jplph.2023.154105

[11] Ge, J., Du, Y., Wang, Q., Xu, X., Li, J., Tao, J., … Gao, J. (2024b). Effects of nitrogen fertilizer on the physicochemical, structural, functional, thermal, and rheological properties of mung bean (Vigna radiata) protein. *International Journal of Biological Macromolecules*, *260*, 129616–129616. https://doi.org/10.1016/j.ijbiomac.2024.129616

10.3 Limitations and weaknesses of the experiment

Limitation - weakness	Impact on the results	Potential solutions
Variability in plant growth	Genetic differences may cause inconsistent growth and protein levels, reducing the result's reliability.	Use genetically similar seeds or increase sample size to reduce variability.
Small sample size	Random variation may affect statistical reliability and reduce confidence in conclusions.	Increase number of plants tested per KNO_3 concentration.
Lack of replication across growth stages	Results reflect only one growth stage, possibly missing broader KNO_3 effects over time.	Measure protein concentration at multiple growth stages.
Potential nitrogen toxicity at high KNO_3 concentration	High nitrate levels may stress plants, leading to unpredictable protein synthesis.	Conduct a pilot test to determine a safe KNO_3 concentration range.
Potential measurement errors in Lowry assay	Measurement errors can affect the accuracy of protein quantification, leading to unreliable data.	Use calibrated equipment and replicate readings to minimize human and systematic errors.

Table 14. Represents the limitations/weaknesses, their impact on the results, and potential solutions

10.4 Potential Extensions

1. Investigate the effect of other macronutrients such as phosphorus, sulfur or calcium in ***Vigna radiata*** to determine whether nitrogen alone is the most influential aspect on protein concentration or if any other nutrients also affect it significantly.
2. Comparing other protein assay methods such as Bradford or Biuret assay to compare with the Lowry method and determine the sensitivity and deviation of different protein quantification methods.
3. Investigating the effect of plant growth stages such as seedling, vegetative, and flowering stages on protein concentration to determine how KNO_3 impacts protein synthesis during a plant's growth.
4. Investigating and comparing the effect of other fertilizers such as urea or ammonium sulfate with KNO_3 on the protein concentration and determine which fertilizer source optimizes protein synthesis.
5. Investigating the effect of KNO_3 on the protein concentration in other plant species such as wheat, spinach, lentils, and rice to identify whether KNO_3's effect on protein synthesis is specific to mung bean plants or is it same in other plants.

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