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VALORIZATION OF CHICKEN FEATHER WASTE: KERATIN HYDROLYSIS FOR AMINO ACID-BASED PLANT GROWTH ENHANCEMENT

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ABSTRACT:
This study explores the effective hydrolysis of keratin from chicken feathers and its application as a bioavailable amino acid source to promote plant growth. The Trichloroacetic Acid (TCA) Test confirmed complete keratin hydrolysis, while the Ninhydrin Test validated the presence of primary and secondary amino acids in the hydrolysate. Experimental trials demonstrated that a 2 ml amino acid concentration in 100 ml water optimally enhanced seed germination (83%) and root (6.0 cm) and shoot (8.0 cm) growth. Higher concentrations (>3 ml) negatively impacted growth, likely due to nutrient oversaturation. These findings underscore the potential of feather-derived amino acids as sustainable agricultural supplements, simultaneously addressing waste management challenges. Further studies are recommended to explore long-term applications and species-specific effects.

Keywords:- Keratin hydrolysis, chicken feather waste, amino acids, plant growth enhancement, sustainable agriculture, seed germination, biofertilizers, waste valorization

INTRODUCTION :

Feathers, composed primarily of keratin, hold significant potential as a protein source in both animal feed and agricultural sectors. Keratin makes up approximately 85%–90% of feather content and is rich in essential amino acids that are valuable for animal nutrition (Sharma et al., 2019; Tantamacharik et al., 2022; Banasaz, and Ferraro 2024). However, the structural complexity of keratin makes it indigestible in its native state. This complexity arises from its highly stable molecular structure, reinforced by disulfide bonds that contribute to its rigidity and water-insolubility. To enhance its bioavailability and suitability for various applications, keratin must be hydrolyzed. Hydrolysis disrupts the disulfide and amide bonds, producing simpler and more digestible components such as amino acids, peptides, and proteins. These hydrolyzed products find application in animal feed, fertilizers, and other agricultural uses (Giteru et al., 2023).

Processed feather proteins are increasingly recognized in animal nutrition due to their abundance of essential amino acids. Feather meal, derived from feathers, is particularly high in amino acids such as arginine (ARG), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), threonine (THR), valine (VAL), tryptophan (TRP), and cysteine (CYS) (Adler et al., 2018). These levels often surpass those found in conventional protein sources like fish meal. However, feather meal is deficient in lysine (LYS), methionine (MET), and histidine (HIS), three critical amino acids, which limits its standalone use as a protein source (Machado et al., 2021). Instead, feather meal is typically used as a complementary ingredient in feed formulations to balance nutrient profiles (Campos et al., 2017).

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Traditionally, feather proteins are processed using thermal pressure hydrolysis, a method that employs high temperatures and pressure to break down protein structures. While effective, this method can compromise nutritional value by reducing the bioavailability of some amino acids. In response, alternative methods, including enzymatic hydrolysis and microbial degradation, are being explored to improve the nutritional profile and digestibility of feather proteins (Onifade et al., 1998; Zhou et al., 2020).

Keratin itself is a fibrous structural protein primarily found in vertebrate outer coverings, such as feathers, hair, nails, and skin. It is second only to collagen in biological significance among animal biopolymers. Specialized keratinized structures, like hooves and turtle shells, exhibit mechanical properties such as durability, water resistance, and impact resistance, which enable them to serve specific functions. These materials effectively withstand tension, as in wool, and compression, as in hooves (McKittrick et al., 2012).

Keratin's structural composition integrates crystalline keratin fibers within an amorphous keratin matrix, the latter consisting of high-sulfur and glycine-tyrosine-rich proteins. This composite arrangement imparts versatility and mechanical efficiency, making keratin an essential biological material (Zhao et al., 2019). Its fibrillar structure includes α-helices that assemble into coiled-coil dimers, protofilaments, and intermediate filaments (IFs). These IFs are embedded in the amorphous matrix, providing both structural stability and functional adaptability (Asgarian et al., 2018; Shukla et al., 2021).

Feathers represent an underutilized yet promising protein source for animal feed and agricultural applications. While raw keratin's indigestibility limits its direct use, hydrolysis offers a pathway to unlock its nutritional potential. Further research to refine keratin degradation processes and explore alternative methods can enhance the efficiency and sustainability of feather protein utilization (Reddy et al., 2021).

Keratin-derived amino acids offer significant potential as plant growth promoters (PGPs), serving a vital role in protein synthesis necessary for plant development (Anbesaw, 2022). Plants naturally produce L-amino acids through metabolic pathways that combine carbon and oxygen from the air, hydrogen from soil water, and nitrogen from the soil, a process primarily driven by photosynthesis. When applied as foliar sprays or through soil drenching, these amino acids enhance nutrient uptake and optimize plant metabolism. Moreover, they support the proliferation of beneficial soil microorganisms, which contribute to improved soil fertility and overall plant health. This dual benefit to agriculture and soil health underscores the sustainability of amino acid applications in improving crop yields and quality, particularly during critical growth stages (Li, 2022). The present study highlights the successful hydrolysis of keratin from chicken feathers and its subsequent use as a bioavailable amino acid source to enhance plant growth.

MATERIAL AND METHODS

Experimental Setup

The experimental work utilized analytical-grade chemicals, and all glassware used was of Borosilicate grade, washed, and sterilized to ensure accuracy and safety.

Solution Preparation

Ninhydrin Solution: Prepared by dissolving 0.2 g of ninhydrin in 100 mL ethanol or a mixture of 94 mL water and 6 mL acetone. This solution is used for detecting amino acids, amines, and amino sugars.

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20% TCA Solution: A 20% TCA solution was prepared by mixing 0.2 mL of 100% TCA with 0.8 mL of water. The solution was maintained ice-cold during use.

Sample Collection

Soil samples were collected from the NACSC garden in Wardha, Maharashtra, specifically from the feather dumping site. Samples were stored in sterile plastic bags to avoid contamination.

Preparation of Amino Acid Mixture from Waste Feathers

Cleaning and Drying Feathers: Feathers were washed with 1–2% detergent to remove impurities, followed by sun drying or drying in a hot air oven at a low temperature.

Hydrolysis Process:

- Feathers were loaded into a round-bottom flask, filling approximately 30% of the flask's capacity.
- A 5N HCl solution was prepared by mixing 507 mL demineralized water with 493 mL HCl. The solution concentration was confirmed using a hand refractometer, with readings in the range of 20–23.
- The prepared acid solution (70% of flask volume) was added to the flask containing feathers.

Hydrolysis Reaction:

- The flask was heated using a mantle, with the temperature initially set at 100°C for 1 hour to settle the feathers, followed by 45°C for 6–8 hours.
- Hydrolysis completion was monitored using the TCA test. A 1:1 mixture of the hydrolysate and 20% TCA solution was tested; no precipitation indicated complete hydrolysis.

Post-Hydrolysis Treatment:

- After cooling, the hydrolysate's pH was adjusted using metal oxides or carbonates. The mixture was filtered using a Buchner funnel.
- The filtrate was stabilized with glycerol to prevent crystallization, yielding a 95% recovery of amino acids.

Preparation of Growth Enhancer

Micronutrient Addition: Micronutrients were added to the hydrolysate to enhance its effectiveness. The formulation included:

- Ferrous sulfate (0.7%)
- Magnesium sulfate (0.5%)
- Zinc sulfate (0.25%)
- Copper sulfate (0.2%)
- Manganese sulfate (0.15%)
- Borax (0.12%)
- Ammonium molybdate (0.064%)

pH Adjustment and Filtration:

The pH of the mixture was adjusted to 3.5 using ammonium carbonate before filtration. The final product was a liquid growth enhancer, stabilized for application.

Amino Acid Confirmation:

The presence of amino acids was verified using the ninhydrin test. Amino acids reacted with ninhydrin to produce a blue-purple color (primary amino groups) or yellow (secondary amino groups).

Plant Trials Application Techniques Soil Application:

- The growth enhancer was added to the soil before sowing seeds.
- Two pots were prepared: one treated with the growth enhancer and one control (no treatment).
- Coriander plants were used, and growth was monitored over 1–2 weeks.

Spraying:

- Liquid growth enhancer was sprayed on fenugreek plants after germination.

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- Two pots were used: one treated with the spray and one control.
- Regular watering ensured proper plant growth for comparative analysis.

Growth Assessment Parameters:

- Root and shoot lengths were measured using a scale to assess growth.
- Seed germination rates and vigor were recorded to evaluate the treatment's effectiveness.

Seed Vigor Test:

This test compared treated and control plants, assessing the impact of the growth enhancer on germination and early development.

Interpretation:

Vigorous growth and enhanced germination in treated plants indicated the growth enhancer's positive impact.

Confirmatory Test

The ninhydrin test confirmed amino acid presence. Reactions produced distinct colors: blue-purple for primary amino groups and yellow for secondary groups like proline, validating the presence of free amino groups.

This method provides a sustainable way to utilize waste feathers for agricultural applications, creating an effective plant growth enhancer.

Results

Protein Hydrolysis Confirmation: TCA Test

The Trichloroacetic Acid (TCA) Test was utilized to confirm the complete hydrolysis of chicken feathers. As a potent protein-precipitating agent, TCA effectively precipitates proteins from dilute solutions. In this study, the test demonstrated successful hydrolysis, evidenced by observable precipitation. These findings confirm the efficient breakdown of keratin into simpler proteins or peptides, a crucial step for the derivation of amino acids.

Amino Acid Detection: Ninhydrin Test

The presence of amino acids in the hydrolysate was confirmed using the Ninhydrin Test, a widely employed qualitative analysis for amino acids. The reaction produced characteristic colored products:

- **α-amino acids** generated a blue-purple color.
- **Proline**, a secondary amine, produced a yellow-orange color.

The observed color changes verified the presence of both primary and secondary amino acids in the hydrolysate, demonstrating the successful conversion of keratin into bioavailable amino acids. (Fig 4)

Effect of Amino Acid Concentration on Seed Germination and Growth

Table 1 presents the results of seed germination and early plant growth across varying concentrations of amino acids. The 2 ml concentration yielded the highest germination rate (25 out of 30 seeds) and superior root and shoot growth (4.0 cm and 6.0 cm, respectively). Conversely, concentrations above 3 ml resulted in diminished growth, potentially due to toxicity or oversaturation. Untreated seeds exhibited the lowest growth metrics, highlighting the effectiveness of amino acid supplementation. (Fig 1, 2,5,6)

Plant Growth Trials with Amino Acid-Enriched Medium

Seed Vigor Test: To determine the optimal amino acid concentration for plant growth, seeds were treated with amino acid-enriched liquid media at different concentrations. Germination rates and root and shoot growth were measured on Days 5 and 10.

- On **Day 10**, the 2 ml concentration maintained its superior performance with the highest root (6.0 cm) and shoot (8.0 cm) lengths.
- The 3 ml concentration, while showing moderate results on Day 5, exhibited reduced shoot growth (4.8 cm) by Day 10, likely due to stress from excessive nutrient concentrations.

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- Higher concentrations (4 ml and 8 ml) resulted in poor growth metrics, indicating diminishing returns and potential adverse effects at elevated nutrient levels.
- Untreated plants and those treated with 1 ml exhibited steady but suboptimal growth compared to the 2 ml treatment. (Fig 3,7)

Optimal Concentration for Plant Growth

The study identified 2 ml of amino acid solution per 100 ml water as the optimal concentration for promoting plant growth. This dosage demonstrated significant improvement in germination rates and root and shoot development, establishing it as the most effective concentration for enhancing plant growth through amino acid supplementation.

Discussion

In contemporary agricultural practices, plant growth enhancers have emerged as essential tools for boosting crop productivity and quality, especially under challenging environmental conditions. These biostimulants promote vital physiological processes, improve stress resistance, and foster growth, including root and shoot development. By enhancing seed germination and activating plant biological functions, biostimulants align with the principles of sustainable agriculture. They minimize dependency on synthetic fertilizers and chemical protectants, presenting an environmentally friendly alternative to conventional farming methods (Bulgari et al., 2015).

Amino acid-based biostimulants are particularly noteworthy, as they utilize chicken feathers—a rich and renewable source of amino acids. Research suggests that processing 1 kg of feathers can produce approximately 20 liters of amino acid solution, which can be diluted and applied to crops. This solution contains diverse amino acids, including lysine, which is highly beneficial for the growth of legumes (Sahoo et al., 2023; Raguraj et al., 2024). Feather processing methods include microbial degradation, such as keratin composting with keratin-degrading microorganisms, and hydrolysis. While microbial degradation is time-intensive, hydrolysis offers a quicker alternative. Submerged fermentation, another technique, employs keratinase enzymes to break down keratin in feathers and hair (Lange et al., 2016). The resultant hydrolysate, rich in amino acids, serves as an effective growth enhancer for plants.

This study confirmed the efficacy of hydrolyzed keratin from chicken feathers as a viable amino acid source for plant growth. The findings align with earlier research that underscores the agricultural and environmental advantages of converting feather waste into bioavailable nutrients (Kaur, et al., 2021; Bhari et al., 2021).

Validation of Protein Hydrolysis and Amino Acid Detection

Protein hydrolysis was validated through the Trichloroacetic Acid (TCA) test, which demonstrated complete keratin breakdown into simpler proteins and peptides. TCA's reliability as a protein-precipitating agent for hydrolysis analysis is well-documented (Jin et al., 2011). Additionally, the Ninhydrin Test confirmed the presence of primary and secondary amino acids. The observed color changes corroborate the effective conversion of keratin into bioavailable amino acids, consistent with prior studies on keratin hydrolysis.

Impact on Seed Germination and Plant Development

The research highlighted the critical influence of amino acid concentration on seed germination and early growth. Optimal outcomes were observed with a 2 ml concentration, which yielded the highest germination rate and robust root and shoot development. These findings echo previous studies indicating that amino acids enhance nutrient uptake and physiological activities in plants (Baqir, et al., 2019).

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Conversely, concentrations of 3 ml or higher impeded growth, likely due to nutrient toxicity or osmotic stress.

Challenges with Higher Concentrations

Diminished growth at concentrations of 4 ml and 8 ml underscores the necessity of precise nutrient management. Over-application of amino acids can disrupt metabolic equilibrium, leading to adverse growth effects. This phenomenon aligns with findings in studies examining the effects of nutrient oversaturation. These results emphasize the importance of determining optimal concentrations for maximizing benefits without inducing stress.

Broader Implications for Agriculture and Sustainability

This study presents a sustainable approach to converting feather waste into agricultural inputs. The process addresses environmental challenges associated with feather disposal while enhancing crop productivity. Such practices contribute to global efforts in waste valorization and sustainable farming.

While the study identifies a 2 ml concentration as optimal for plant growth, further research is required to validate these findings across diverse plant species and environmental conditions. Long-term field experiments would provide greater insight into the practical applications of this approach. Additionally, exploring the molecular mechanisms through which amino acids promote growth could enable more targeted agricultural solutions.

CONCLUSION

This study successfully demonstrated the potential of hydrolyzed keratin from chicken feathers as a bioavailable amino acid source for promoting plant growth. The Trichloroacetic Acid (TCA) Test confirmed the effective breakdown of keratin into simpler proteins and peptides, while the Ninhydrin Test validated the presence of both primary and secondary amino acids in the hydrolysate. Experimental trials revealed that a 2 ml amino acid concentration in 100 ml water optimally enhanced seed germination and plant growth, achieving significant increases in root and shoot lengths. In contrast, higher concentrations negatively impacted growth, underscoring the importance of dosage optimization.

The findings highlight the dual benefits of addressing environmental waste management challenges and improving agricultural productivity through feather waste valorization. Utilizing keratin hydrolysates as an eco-friendly biofertilizer presents a sustainable approach for enhancing crop yields while reducing reliance on synthetic fertilizers. Future research should focus on evaluating the long-term effects of amino acid supplementation across diverse plant species and environmental conditions to further establish its practical applications in agriculture.

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Table 1: Seed Germination and Plant Growth Measurements on Day 5

Set	Amino Acid Concentration (ml/100 ml water)	Germination	Root Growth (cm)	Shoot Growth (cm)
Set 1	Untreated	15 out of 30	3 cm	5.5
Set 2	1 ml	20 out of 30	3.5 cm	5.7
Set 3	2 ml	25 out of 30	4 cm	6.0
Set 4	3 ml	19 out of 30	3.5 cm	5.0
Set 5	4 ml	12 out of 30	3.2 cm	4.3
Set 6	8 ml	10 out of 30	-	-

Table 2: Plant Growth Measurements on Day 10

Set	Amino Acid Concentration (ml/100 ml water)	Root Growth (cm)	Shoot Growth (cm)
Set 1	Untreated	5.0	7.0
Set 2	1 ml	5.0	7.0
Set 3	2 ml	6.0	8.0
Set 4	3 ml	6.7	4.8
Set 5	4 ml	4.4	5.6
Set 6	8 ml	4.0	5.0

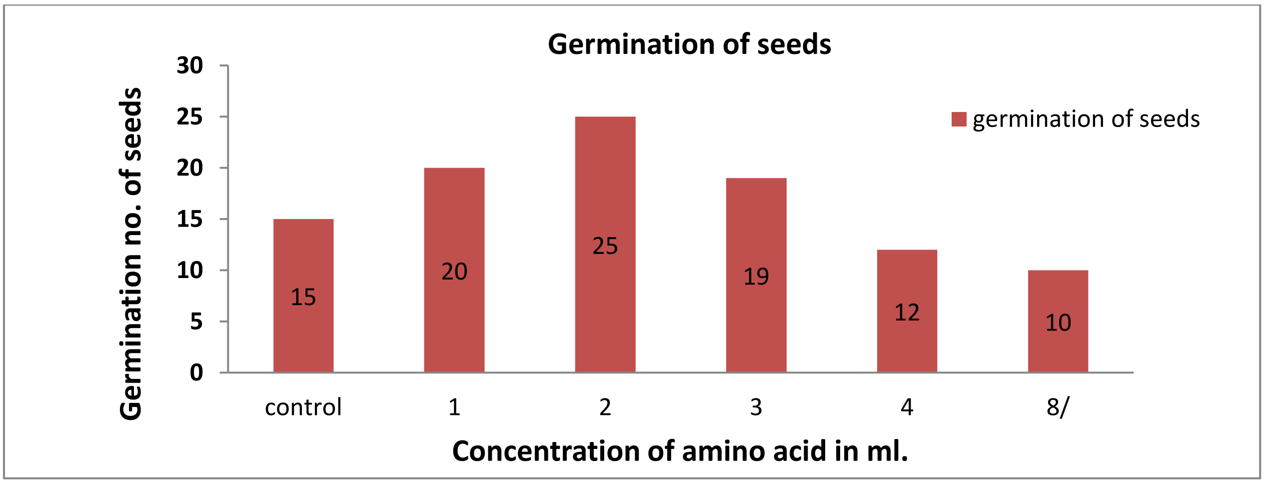

Figure 1: Effect of Amino Acid Concentration on Seed Germination

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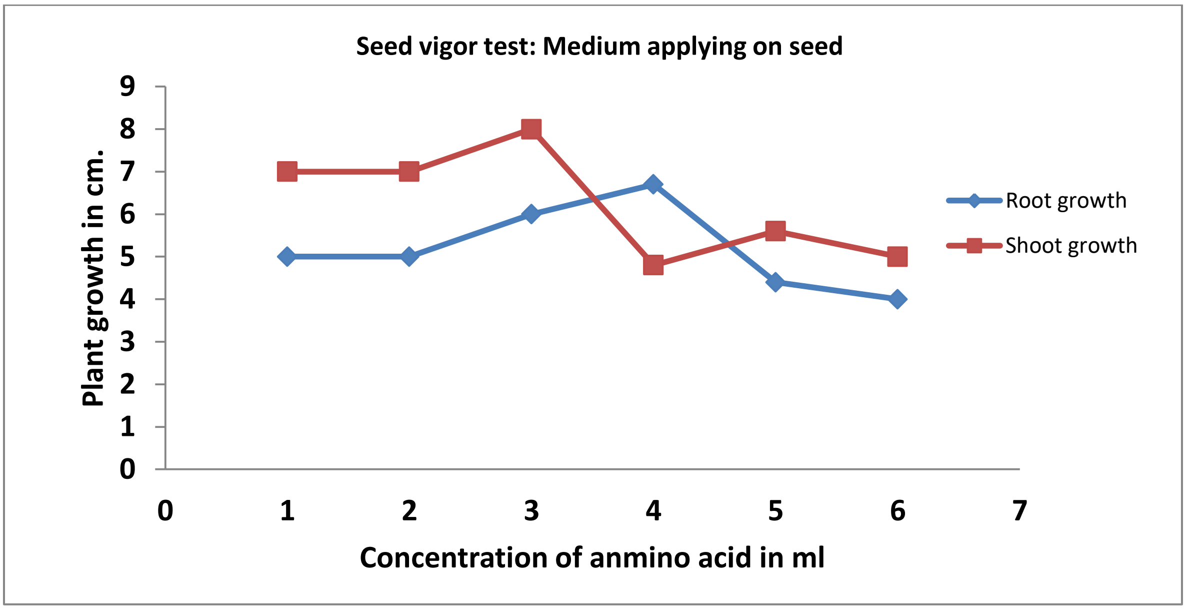

Figure 2: Seed Vigor Test to test the Effect of Amino Acid Concentration on Plant Growth in terms of root and shoot growth (Day 5)

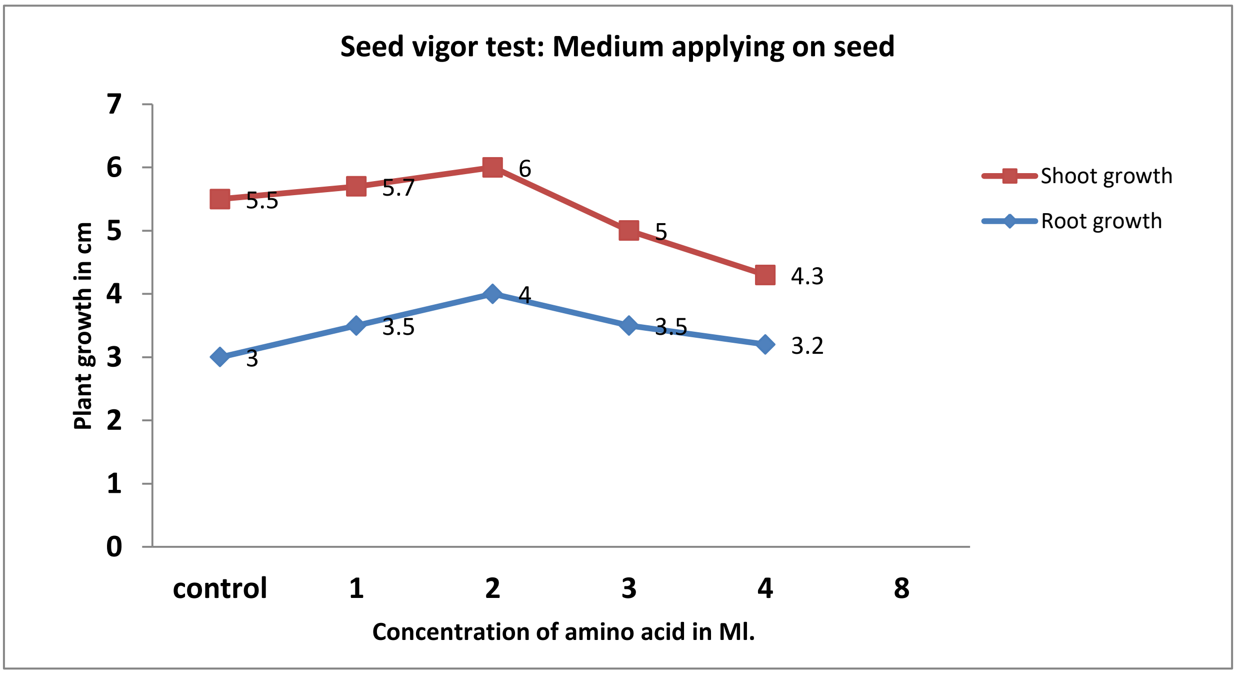

Figure 3: Seed Vigor Test to test the Effect of Amino Acid Concentration on Plant Growth in terms of root and shoot growth (Day 10)

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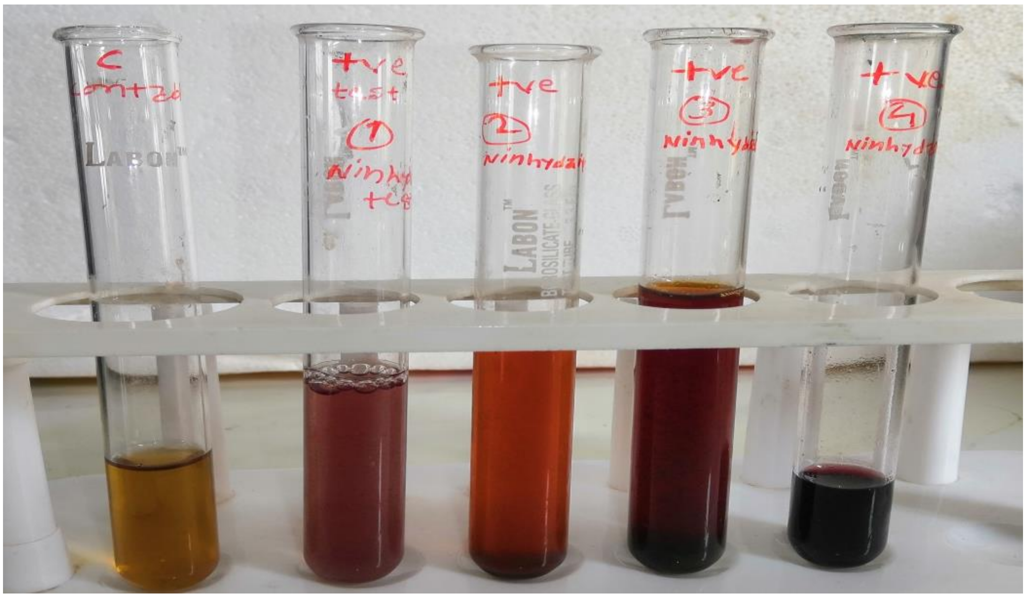

Fig. 4: Amino Acid Detection Test (Ninhydrin reaction)

Fig. 5: Seed Germination across Different Concentrations

Fig. 6: Effect of Liquid Medium on Plant Growth (Day 5)

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Fig. 7: Effect of Liquid Medium on Plant Growth (Day 10)

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