



The Effect of Fish Amino Acid on Germination and Growth of Green Gram (*Vigna radiata*)

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ABSTRACT

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Fermented fish amino acids (FAA) are organic acids known for promoting plant growth due to their essential components. It is widely used in tropical regions, such as India, Thailand, Indonesia, Laos, and other Asian regions, as well as in certain agricultural institutions in the northern Philippines. FAA is applied as a concentrated liquid diluted with water. The present study investigated the effects of FAA on the germination and growth of green gram (*Vigna radiata*), a key leguminous crop vital to human nutrition and global agriculture. Because of its high nutrient content from fish waste, FAA has attracted interest for its potential use as an organic fertilizer in sustainable farming. This study evaluates the effects of different FAA concentrations on germination rates, root and shoot growth, plant biomass, and photosynthetic pigments in green grams (*Vigna radiata*). FAA at 0.6 mg/mL caused a maximum shoot length of about 20 cm on day 6 and the highest root growth (length) at 0.8 mg/mL on day 6 compared to the other concentrations (0.2 – 0.6 and 1 mg/mL). Also, treatment with 1 mg/mL FAA induced the highest amount of plant biomass compared to others. The results show that FAA treatment considerably enhanced their germination rate, as well as shoot and root growth, and other growth parameters in *Vigna radiata*.

Keywords: Fish amino acid (FAA), Green gram, Germination, Sustainable agriculture.

Introduction

Wastes are residual byproducts of human consumption and are significant contributors to environmental pollution. Wastes can vary in type based on their origin. Slaughterhouses, in particular, play a role in providing a favourable environment for flies and pathogenic bacteria to develop and spread diseases. These wastes can be readily degraded by a group of microorganisms called decomposers.^{1,2} Traditionally, fish waste has been used as fertilizer because it contains a large amount of vital nutrients, particularly nitrogen (N) and phosphorus (P), and their breakdown is rapid. After processing, approximately 75% of the fish's overall weight is produced as solid waste, including the intestines, head, skin, bones, fins, and frames. The nitrogen, potassium, phosphorus, and trace minerals found in fish waste can be used as raw materials to produce various nutritious and non-nutritious goods. Using organic waste from soil is an appropriate approach for conserving soil organic matter, improving soil fertility, and supplying vital nutrients for plants.³

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Fertilizers are crucial to modern crop cultivation because they supply vital mineral nutrients that optimize crop growth and yields. High-yielding crop plants may not achieve their maximum potential without receiving a properly balanced number of fertilizers.^{4,5} Organic fertilizers are composed of organic elements and are considered more environmentally sustainable when compared to chemical fertilizers.⁶ Continuous use of chemical fertilizers will decline soil fertility, leading to pH imbalances in the soil, ultimately resulting in low yield. To achieve successful harvesting different types of fertilizers can be used without altering the soil. Organic fertilizer is a type of fertilizer that is produced by decomposing animal and plant waste via biological processes. Moreover, organic fertilizer is environmentally sustainable because it is produced without the use of chemicals. It is commonly considered suitable for promoting plant growth and preserving soil fertility.⁷ Some popular organic fertilizers include bone meal, worm castings, manure, and fish emulsions. To enhance crop growth and development, a variety of organic preparations are used, including vermic, fish amino acid (FAA), egg amino acid (EAA), amirthakaraisal (nectar solution), and panchakavya (a mixture of organic materials for enhance growth). Fish amino acid (FAA) is an organic preparation that is used to enhance crop growth and development of crops.⁸ Additionally, fish excrement is used to make liquid organic manure, which is also known as FAA. Because fish contain various minerals and amino acids, they are extremely valuable to plants and microbes for growth. According to the FAA, commercially accessible fertilizers are an economical method to enhance soil nitrogen (N) levels for plant development and increased agricultural production. However, improper or excessive use of nitrogen fertilizers can result in nitrate contamination in the ground or surface water.^{9,10} Because of this problem, numerous researchers have been conducting studies to develop organic fertilizers that can be used by farmers without negatively impacting the environment and ecosystem. The objective of

this study was to examine the impact of fermented FAA on the growth and development of green gram (*Vigna radiata*).

Materials and Methods

Plant material collection

Green gram (*Vigna radiata*) was used as the planting material to study the effects of FAA. Green gram seeds were purchased from a local store and washed thoroughly with distilled water.

Preparation of fish amino acid

Fish amino acid (FAA) was derived from jaggery (brown sugar) and fish. A clean jar was filled with an equal proportion of solid jaggery and fish slices, including the skin, head, and intestine. This mixture was fermented for up to 21 days and filtered to obtain 200 mL of a liquid portion with a honey-like consistency, as shown in Figure 1.

Germination and growth parameters of green gram after fish amino acids treatment

Fifteen seeds of consistent size, shape, and colour were placed in each Petri dish for treatment. Different concentrations of FAA were sprayed on the seeds to maintain moisture. Distilled water was used for the control. Germination was assessed after 48 hours, and the shoot and root lengths were measured between days 3 and 6 to evaluate growth.

Determination of plant biomass of green gram

The green gram samples for each treatment were placed in envelopes and dried in an oven at 70°C for 24 h. After drying, the samples were weighed using a digital scale.¹¹



Figure 1: Fish amino acid fertilizer

Photosynthetic pigments of green gram

Photosynthetic pigments from green gram seeds were extracted using 80% acetone. Their absorbance at 645, 663, and 480 nm was measured to estimate pigment content.¹² The levels of chlorophyll "a" chlorophyll "b" and carotenoid content were calculated using the following formula, according to the methods described by Hepsibha *et al.*,¹³

Chlorophyll a (mg/g) = $[(12.7 \times A_{663}) - (2.6 \times A_{645})] \times \text{mL acetone}$
 Chlorophyll b (mg/g) = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \text{mL acetone}$
 Total Chlorophyll = Chlorophyll a + Chlorophyll b.
 Carotenoids and Xanthophyll (c, x) = $(1000 \times A_{470} - 1.90 (\text{Chlorophyll a}) - 63.14 (\text{Chlorophyll b})) / 214$
 The pigment level was expressed as milligrams per gram of fresh weight of leaves.

Statistical analysis

In this study, all experimental results were represented as mean values along with their corresponding standard deviations (SD), with each parameter being measured five times (n = 5). Data processing was conducted using GraphPad Prism version 8.0.1 (Graphpad Software Inc., La Jolla, CA). Significant differences among the treatment means were identified using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test ($p < 0.05$).

Results and Discussion

The germination results of the green gram revealed that at a concentration of 0.4 mg/mL, the green gram had a faster germination effect compared with the other concentrations, and after 48 h all of the seeds achieved germination (Figure 2). This finding is supported by previous research on the impact of fish waste fertilizer on the growth of *Amaranthus dubius* (amaranthus) and *Trigonella foenum-graecum* (coriander). Their findings suggest that fish waste fertilizer provides a continuous supply of nutrients to the soil, maintains natural habitats, and enhances plant germination and productivity of the plants.¹⁴ Also, the application of FAA significantly increased the growth of green gram seeds and had a quick germination effect. Shoots and roots developed after 3 days; initial shoot growth was observed at a concentration of 0.4 mg/mL. Interestingly, this initial effect did not last longer. After 6 days, the maximum shoot length was observed at a concentration of 0.6 mg/mL (Figure 3). The study also investigated the effect of FAA among the concentrations. According to the figure, the application of 0.4 mg/mL of FAA around 15.6 cm resulted in the greatest shoot length on day 3 and 0.6 mg/mL around 20 cm on day 6. In a short-term pot experiment, liquid organic fertilizers significantly enhanced root and aboveground development by 10.2-77.8% and 10.7-33.3%, respectively, compared with chemical fertilizers.⁷

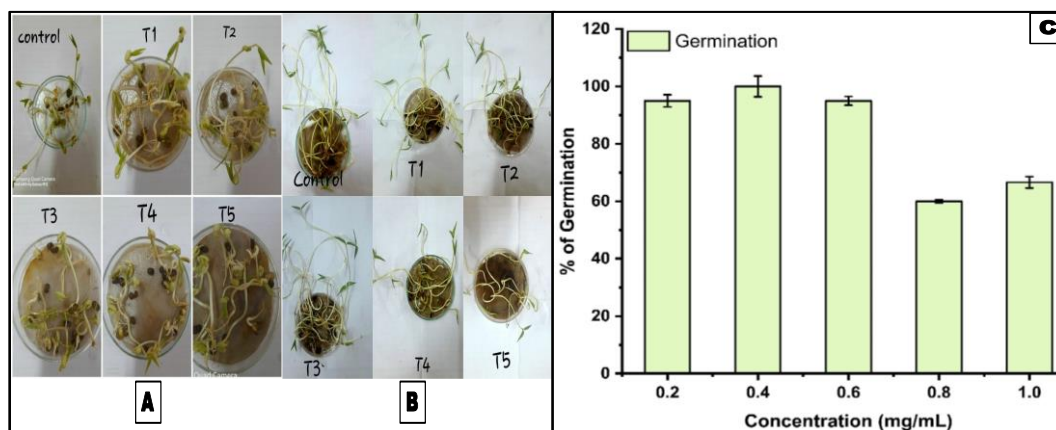


Figure 2: Effect of fish amino acid on the germination of green gram (A) on Day 3, (B) on Day 6, and (C) the percentage of germination

In addition, a prior investigation found that applying 1% of FAA to leaves resulted in a significantly increased plant height, number of leaves per plant, and chlorophyll content by 16.5%, 12.6%, and 8.1%, respectively, compared with the control group.¹⁵ The root growth of green gram determination showed notable variations in root length among the concentrations, as depicted in Figure 4. On day 3, the application of 0.6 mg/mL of FAA resulted in the greatest increase in root length, followed by the application of 0.2, 0.4, 0.8, and 1 mg/mL of FAA. On day 6, the inclusion of FAA affected the root length. The concentration of 0.8 mg/mL of FAA exhibited the highest root length compared with the concentrations of 0.2, 0.4, 0.6, and 1 mg/mL of FAA on day 6. According to Figure 4, the root length increased from day 3 to day 6 when 0.8 mg/mL of FAA was added. Nevertheless, the root length showed a consistent growth from day 3 to day 6 under 0.2, 0.4, 0.6, and 1 mg/mL conditions. This may be due to changes in the plant's nutrient absorption over time or external conditions like light and water availability at the experimental site. These external factors could

interact with the FAA, contributing to the observed differences in root growth. Ji *et al.*,⁷ demonstrated that treating plants with liquid organic fertilizer derived from shrimp extract significantly increased many root characteristics, including dry weight, total length, surface area, volume, and lengths of both tips and thick roots. The results of the determination of plant biomass of green gram are presented in Figure 5. Based on the figure, there were notable variations in plant biomass among the concentrations. Nevertheless, the application of 1 mg/mL of FAA resulted in the highest plant biomass compared with the volumes of 0.2, 0.4, 0.6, and 0.8 mg/mL of FAA. The results demonstrated that FAA enhances the plant biomass of green gram. The nutrients present in FAA stimulate the growth of the green gram. According to Trisilawati *et al.*,¹⁶ the application of organic fertilizer significantly enhanced the root length, fresh and dry weight of the roots, fresh weight of the stolon, fresh and dry weight of the leaves, and fresh and dry weight of the *Centella asiatica* L biomass in the parent plant.

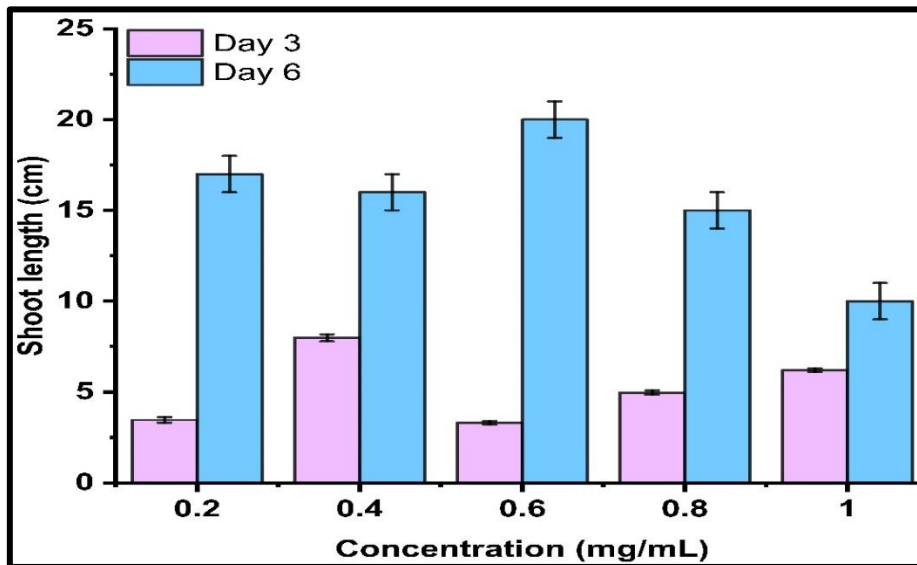


Figure 3: Effects of fish amino acid on the shoot growth of green gram

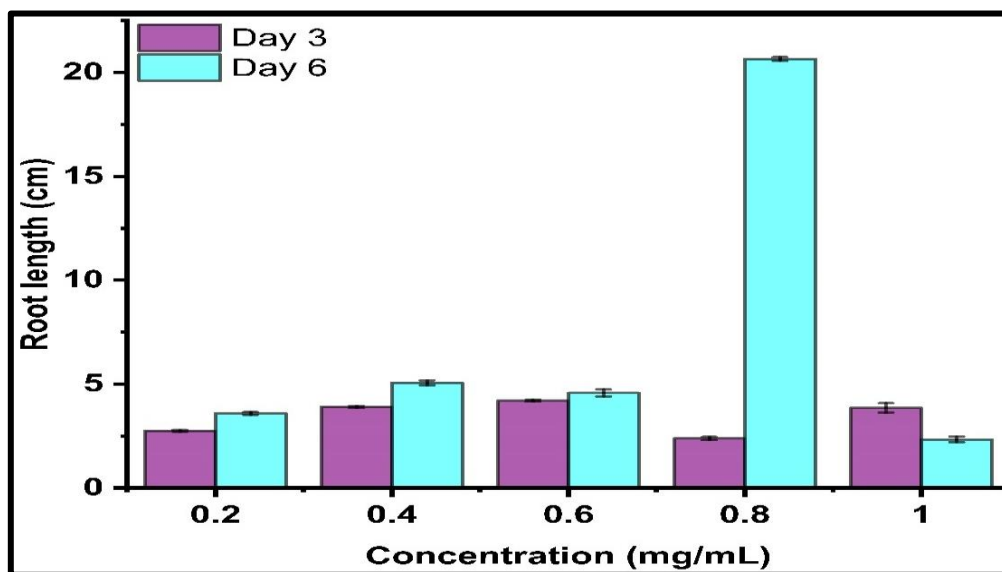


Figure 4: Effects of fish amino acid on the root growth of green gram

Furthermore, investigation of the photosynthetic pigments of green gram showed that the pigment concentrations varied to a great extent depending on the treatment concentration. Findings showed that chlorophyll **a**, chlorophyll **b**, and total carotenoid levels displayed different patterns as the photosynthetic active radiation (PAR) and

nutrient availability levels varied. To be specific, chlorophyll **b** content showed a very noticeable increase in light exposure that was higher than the others, whereas chlorophyll **a** showed a more complex behaviour peaking at moderate light levels.

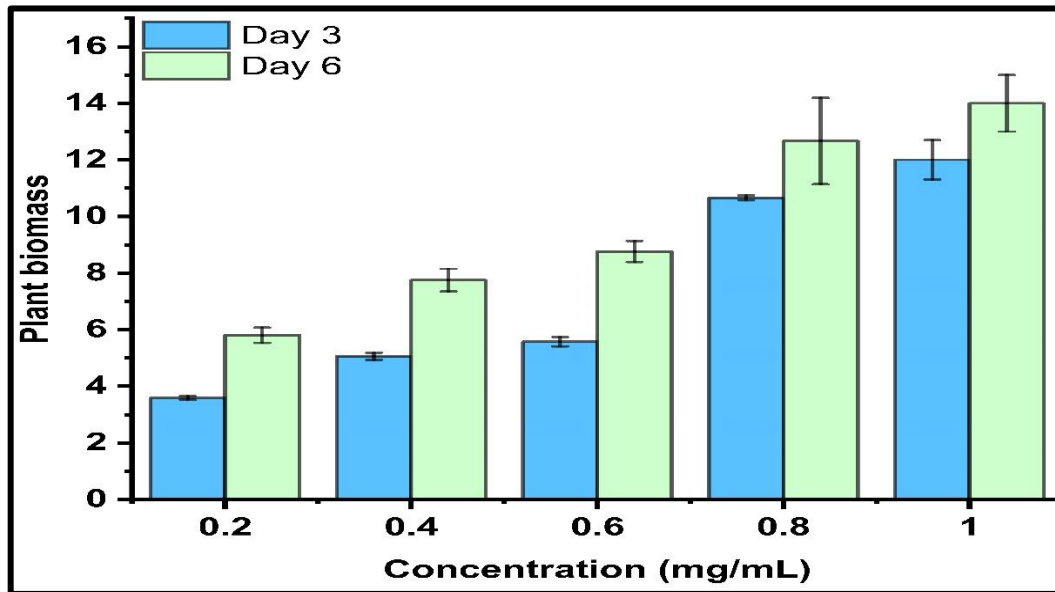


Figure 5: Effect of fish amino acid application on the green gram plant biomass

In addition, total carotenoids were affected by both light level and nutrient supplementation, with maxima at optimum growth conditions. These results affirm the interaction of the environment with photosynthesis in the green gram, providing insights into the control of

photosynthetic processes in this important, leguminous crop (Figure 6). In a previous study by Iqbal *et al.*,¹⁷ a considerable influence was noted on chlorophyll **a**, chlorophyll **b**, total chlorophyll, carotenoid, and protein levels during green gram treatment application.

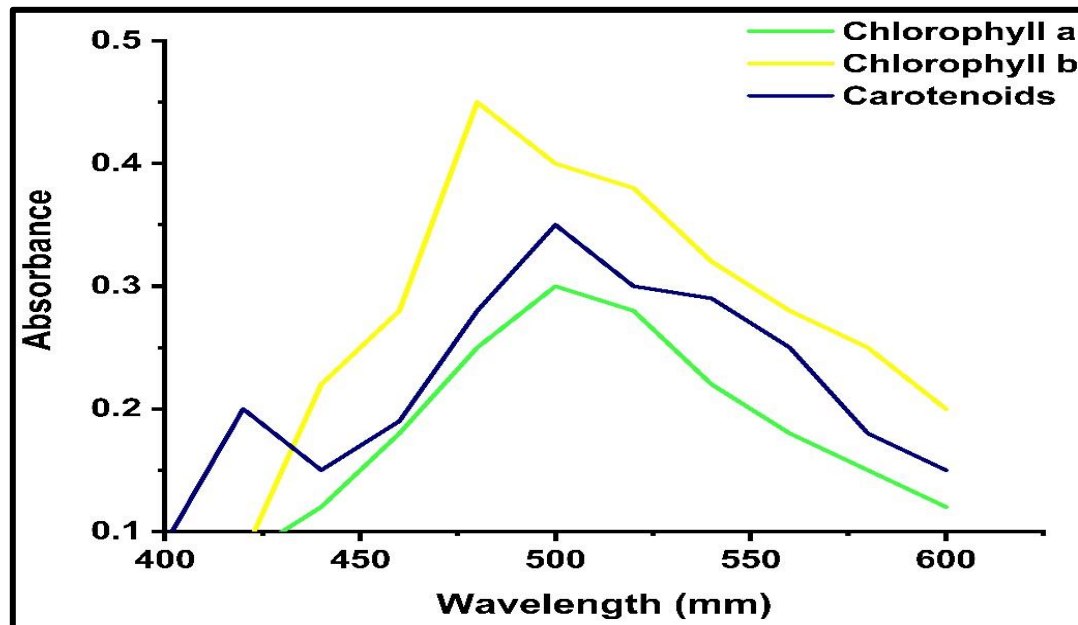


Figure 6: Effect of fish amino acid content on the photosynthetic pigments of green gram

Conclusion

The application of fish amino acid fertilizer had a significant effect on the germination and growth of green gram. Furthermore, as a liquid fertilizer, FAA allows for easy absorption of its nutrients by plants, stimulating rapid development and potentially increasing crop yields. This reduces the need for chemical fertilizers and helps maintain soil fertility. Additional research is necessary to thoroughly understand the

mechanism and determine the most effective methods of applying FAA in agricultural fields.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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