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Studies on effect of foliar application of amino acids and vitamins on total chlorophyll content of turmeric (*Curcuma longa* L.) leaves

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Abstract

Application of biologically active amino acids and vitamins would be environmentally friendly approach in achieving sustainable agriculture. Considering increasing global demand and environmental challenges, this study explores novel approaches to enhance its sustainable production and improve quality. Amino acids are an organic form of N and play a role in plant protein structure. Vitamins can be used as convenient and affordable organic fertilizer to increase the efficiency of the plant and preserve its nutrients. This research was conducted to evaluate the physiological changes with respect to chlorophyll content of turmeric leaves induced by foliar applied amino acids (glutamine, lysine, glycine, aspartic acid and phenyl alanine at four concentrations of 50, 100, 150 and 200 ppm each) and vitamins (ascorbic acid, thiamine, folate and niacin at four concentrations of 25, 50, 75 and 100 ppm each) including control. The results revealed that chlorophyll content of turmeric leaves was significantly increased content by the application of amino acids and vitamins and it was found high with enhanced concentration of amino acids and vitamins. Among the different amino acids, glutamine 150 ppm (4.49 mg g⁻¹) recorded the highest total chlorophyll content and for the vitamins, ascorbic acid 100 ppm (4.38 mg g⁻¹) associated with highest as compared to other treatments and control.

Keywords: Amino acids, vitamins, turmeric leaves and total chlorophyll content

Introduction

Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae and is originated from tropical Southeast Asia (Ferreira *et al.*, 2013) [12] and is often called as “Indian saffron” and “Golden spice” (Ravindran *et al.*, 2007) [33]. India is largest producer and consumer of turmeric in the world by contributing 80% of the total world production. Turmeric is a monocotyledonous herbaceous plant usually reaches a height of 1.0 -1.5 m (approx.) characterised by dark green, oblong leaves and produces underground rhizomes. Turmeric has long been revered in Indian traditional Ayurvedic medicine for its healing properties. The rhizomes are widely utilized as spice, natural dye, cosmetics and pharmaceutical industries (Reshma and Vishwanath, 2020) [34]. Beyond its culinary uses, turmeric is known for its antifungal and antioxidant properties, which helps to guard against free radical damage. Turmeric comprises main curcuminoid, curcumin which imparts yellow colour and is widely utilized in the medicinal industry for its various beneficial properties, including antioxidant, anti-arthritis, antimutagenic and antimicrobial effects also shown potential in combating Alzheimer's disease (Mane *et al.*, 2018) [25].

Amino acids are organic compounds and the fundamental building blocks of proteins, which have structural, metabolic and transport functions in plants like respiration and photosynthesis to increase plant development and yield (Calvo *et al.*, 2014) [9]. Commercial amino acids can increase crop yields by improving nutrient and water uptake, enhance fertilizer assimilation, increase the photosynthetic rate and boost up dry matter production (Liu *et al.*, 2008) [23]. Amino acids play a crucial role in various physiological functions, including osmotic control and the functioning of plant growth regulators like auxin and gibberellin, they also enhance plant tolerance to various environmental stresses (Ibrahim *et al.*, 2010) [19]. Due to diverse roles of amino acids in plant metabolism, applying them externally can offer significant benefits, stimulate plant growth and improve quality. Nikiforova *et al.* (2006) [30] cited that spraying of

amino acids triggered the growth of aerial plant parts above the soil surface and promoted the establishment of the root system.

Glutamine is the first amino acid synthesized in nitrogen assimilation in plants. It is a building block for protein synthesis and N-donor for the biosynthesis of amino acids, nucleic acids, amino sugars and vitamin B coenzymes (Amin *et al.*, 2011) [5]. In agriculture, lysine can be utilized as a unique fertilizer synergist to improve drought resistance and chlorophyll synthesis and is a precursor of glutamate, a crucial signalling amino acid that controls plant development and environmental reactions (Ashouri *et al.*, 2023) [7]. Glycine promotes the production of chlorophyll, initiates vegetative growth and helps plant to improve cell membrane stability and protection against peroxidation (Abd-Elkader *et al.*, 2020) [2]. Phenylalanine is crucial for the interaction between primary and secondary metabolism and serves as a precursor for several plant substances that are essential for plant growth, development, reproduction and stress resistance (Ali and Mohammed, 2020) [3]. Aspartic acid is essential for plant growth and stress tolerance because it is utilized in the synthesis of hormones, organic acids in the tricarboxylic acid cycle, nucleotides, sugars in glycolysis and other amino acids (Han *et al.*, 2021) [16].

Vitamins are organic compounds that occur in various forms and are thought to be necessary for the proper growth of plants because they participate in physicochemical processes that sustain the plant functions. By acting as coenzymes, altering variables that regulate many physiological processes, including enzyme synthesis and stimulating growth even at low concentrations, these bio-regulator chemicals have a major impact on plant growth (Aziz *et al.*, 2009) [8]. The application of vitamins promotes alpha-ketoglutaric acid production, which forms proteins and amino acids when it reacts with ammonia. This process helps regulate the occurrence of disorders and promotes the manufacture of natural hormones such as IAA, cytokinin and gibberellins, as well as promoting cell division, plant pigments, enzymes, organic compounds and overall plant metabolism (Samiullah *et al.*, 1988) [38]. Ascorbic acid serves as a significant redox buffer and a cofactor for enzymes that control hormone production, photosynthesis and the regeneration of other antioxidants (Rafique *et al.*, 2011) [32]. Folic acid plays a crucial role as a cofactor in different plant metabolic processes. Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺) are the active forms of niacin (Vitamin B₃), which is a substitute derivative of pyridine (Abdelhamid *et al.*, 2013) [1]. Thiamine, is an essential element of the vitamin-B group and helps to mitigate drought stress (Sadak *et al.*, 2022) [37].

Glutamine at 200 ppm give rise to enhanced leaf chlorophyll content, yield and bulb quality of garlic reported by Hegazi *et al.* (2016) [18] and Noroozlo *et al.* (2019) [31] in lettuce. Hassan *et al.* (2020) [17] found that cassava plants sprayed with glutamine (200 mg l⁻¹), exhibited substantial improvements in vegetative growth parameters (plant height, leaf count, lateral branches, leaf area and chlorophyll content). Abd-Elkader *et al.* (2020) [2] reported that the dahlia plants sprayed with a mixture of amino acids (100 ppm) recorded the highest growth, chlorophyll, total carbohydrates and amounts of N, P and K content in the leaves. Lei *et al.* (2022) [22] reported that the perennial ryegrass treated with 2 mM aspartic acid exhibited considerably higher levels of chlorophyll and antioxidant activity. In Gerbera, foliar spraying 150 mg l⁻¹ phenylalanine was superior in plant relative chlorophyll content, percentage of NPK and total carbohydrates

in the leaves (Sabreen and Saeed, 2019) [35].

Ascorbic acid (vitamin c) is known as a growth regulating factor which influences many biological processes. Elbohy *et al.* (2018) [10] found that Zinnia plants were treated topically with a combination of 300 ppm ascorbic acid and 300 ppm salicylic acid had the highest values for plant height, total fresh and dry weights of aerial parts, chlorophyll a and b content in leaves and Gahory *et al.* (2022) [14] in coriander. Foliar spraying of faba bean (*Vicia faba*) plants with 30 mg l⁻¹ folic acid resulted in significant improvements in chlorophyll and nutritional content in the leaves as well as higher green pod and fresh seed yield per plant (Al-Maliky *et al.*, 2019) [4]. Dahlia plants treated with 100 ppm thiamine showed the highest flower yield, flower characteristics and chlorophyll content, outperforming the other treatments (Mahgoub *et al.*, 2011).

Realizing the potentiality and importance of amino acids and vitamins on growth, development and quality improvements in other crops the present investigation has been formulated to improve the total chlorophyll content of turmeric leaves.

Materials and Methods

The present investigation was carried out at the Horticultural Research Station, Mondouri, Faculty of Horticulture, Bidhan Chandra Krishi Vishwavidyalaya, Nadia, West Bengal during two successive seasons of 2023-24 and 2024-25 to study the effect of foliar application of different amino acids at various concentrations on total chlorophyll content of turmeric leaves. The soil of the experimental field was Gangetic alluvial (Entisol) with sandy clay loam texture and good water holding capacity.

The experiments were designed in completely randomised block design with three replications. The first experiment consisting of 21 treatments with five amino acids including glutamine, lysine, glycine, phenyl alanine and aspartic acid at four concentrations (50, 100, 150 and 200 ppm) each. The experiment comprised with vitamins such as ascorbic acid, thiamine, folate and niacin each with four different concentrations of 25, 50, 75 and 100 ppm. The amino acids and vitamins were applied as foliar spray after dissolving in water at 60, 90 and 120 days after planting in both seasons. Application of FYM @ 25 t ha⁻¹ and recommended dose of fertilisers (NPK - 150:60:150 kg ha⁻¹) were applied during land preparation and crop growth period for all treatments including control. Irrigation and mulching were done with paddy straw immediately after planting of rhizomes. Intercultural operations like weeding, earthing up and irrigation were taken up based on requirement of the crop.

Treatment details of experiment - 1

T ₁ - Glutamine @ 50 ppm	T ₁₂ - Glycine @ 200 ppm
T ₂ - Glutamine @ 100 ppm	T ₁₃ - Aspartic acid @ 50 ppm
T ₃ - Glutamine @ 150 ppm	T ₁₄ - Aspartic acid @ 100 ppm
T ₄ - Glutamine @ 200 ppm	T ₁₅ - Aspartic acid @ 150 ppm
T ₅ - Lysine @ 50 ppm	T ₁₆ - Aspartic acid @ 200 ppm
T ₆ - Lysine @ 100 ppm	T ₁₇ - Phenylalanine @ 50 ppm
T ₇ - Lysine @ 150 ppm	T ₁₈ - Phenylalanine @ 100 ppm
T ₈ - Lysine @ 200 ppm	T ₁₉ - Phenylalanine @ 150 ppm
T ₉ - Glycine @ 50 ppm	T ₂₀ - Phenylalanine @ 200 ppm
T ₁₀ - Glycine @ 100 ppm	T ₂₁ - Control (Water spray)
T ₁₁ - Glycine @ 150 ppm	

Treatment details of experiment - 2

T ₁ - Ascorbic acid @ 25 ppm	T ₁₀ - Folate @ 50 ppm
T ₂ - Ascorbic acid @ 50 ppm	T ₁₁ - Folate @ 75 ppm

T ₃ - Ascorbic acid @ 75 ppm	T ₁₂ - Folate @ 100 ppm
T ₄ - Ascorbic acid @ 100 ppm	T ₁₃ - Niacin @ 25 ppm
T ₅ - Thiamine @ 25 ppm	T ₁₄ - Niacin @ 50 ppm
T ₆ - Thiamine @ 50 ppm	T ₁₅ - Niacin @ 75 ppm
T ₇ - Thiamine @ 75 ppm	T ₁₆ - Niacin @ 100 ppm
T ₈ - Thiamine @ 100 ppm	T ₁₇ - Control (Water spray)
T ₉ - Folate @ 25 ppm	

Data recorded

A random sample of turmeric leaves were collected at 150 days after planting from the experimental field for analysis of chlorophyll. Fresh leaf tissue weighing about one gram was smashed and put in a container with 10 ml of 80% acetone. After the mixture was well combined, it was placed in a cool, dark chamber for two days to allow complete pigment extraction. The extract was strained after incubation and then centrifuged for five minutes at 5000 rpm. After the supernatant was meticulously gathered, one ml was put into a test tube. To create the initial dilution, 9 ml of 80% acetone were added. A second dilution was prepared by taking one ml of this solution and diluting it to 5 ml with 80% acetone. The final solution optical density (OD) was measured at 663 nm and 645 nm using UV-Visible spectrophotometer against 80% acetone as blank (Moran, 1982) [27].

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = 20.2 \times \text{OD}_{645\text{nm}} + 8.02 \times \text{OD}_{663\text{nm}} (\text{V/W} \times 1000)$$

Statistical analysis

The data obtained for each character was analysed statistically by the variance method (Gomez and Gomez, 1984) [15]. Significance of different sources of variation was evaluated by Error Mean Square using Fisher-Sen decor's 'F' test at probability level of 0.05. For computation of critical differences (CD) at 5% level of significance, Fisher and Yates (1953) [13] table was consulted.

Results and Discussion

Effect of foliar application of amino acids on total chlorophyll content of turmeric leaves

The total chlorophyll content of turmeric leaves varied significantly influenced with the application of various concentrations of amino acids. As per pooled analysis (Table. 1), highest chlorophyll content was observed in the treatment glutamine 150 ppm (4.49 mg g⁻¹), which was *at par* with glycine 150 ppm (4.41 mg g⁻¹), glutamine 200 ppm (4.40 mg g⁻¹) and lysine 150 ppm (4.37 mg g⁻¹), whereas the lowest chlorophyll content in control (3.55 mg g⁻¹). The increasing concentration of aspartic acid and phenyl alanine leads to increasing trend in chlorophyll content but in case of glutamine, lysine and glycine it was increased up to 150 ppm concentration. Proteins were associated with the chlorophylls, particularly proteins of light harvesting complexes in chloroplast thylakoid membranes. From the abovementioned results, it could be concluded that foliar application of the amino acids might be play an important role in many metabolic and physiological processes, through affecting the metabolism of photosynthesis process which led to increase in total chlorophyll and mineral content. Two main fundamental metabolites in the process of formation of vegetable tissue and

chlorophyll synthesis are glycine and glutamic acid which accounted for increasing chlorophyll content in plant which led to higher photosynthesis, indirectly. Aziz *et al.* (2009) [8] reported that the leaves with chlorophyll contents in snapdragon increased by increasing amino acid concentration. They attributed these improvements to the role of some amino acids, such as glycine in the biosynthetic pathway leading to chlorophyll formation. In other words, amino acids such as glycine initiate chlorophyll formation pathway through succinyl CoA (Krebs cycle intermediate) (Aziz *et al.*, 2009) [8]. These results are in conformity with the findings of Amin *et al.* (2011) [5] and Hegazi *et al.* (2016) [18]. They also observed higher chlorophyll content with glutamine 200 ppm application in onion and garlic. Increasing chlorophyll content was in harmony with the findings of Hassan *et al.* (2020) [17] and Mira *et al.* (2024) [26] in cassava and grapevine, respectively with increasing in growth, yield and quality parameters observed by Abd-Elkader *et al.* (2020) [2] and Mosa *et al.* (2020) [28] with glycine application. Enhancement of chlorophyll in groundnut with aspartic acid also observed by Sadak *et al.* (2023) [36].

Effect of foliar application of vitamins on total chlorophyll content of turmeric leaves

The chlorophyll content of turmeric leaves varied significantly among the treatments. As per pooled analysis (Table. 2), highest chlorophyll content was observed in ascorbic acid 100 ppm (4.38 mg g⁻¹), which is statistically *at par* with folate 100 ppm (4.32 mg g⁻¹), thiamine 75 and 100 ppm (4.27 and 4.24 mg g⁻¹), ascorbic acid 50 ppm (4.21 mg g⁻¹). The lowest chlorophyll content recorded in control (3.52 mg g⁻¹), followed by niacin 25 ppm (3.80 mg g⁻¹). The increase in photosynthetic pigments in turmeric leaves may be due to the role of antioxidants in protecting chloroplast membranes from photooxidation and help to provide an optimal environment for the photosynthetic machinery. The increased chlorophyll content with ascorbic acid application were in accordance with the findings of Nassar *et al.* (2019) [29] in basil plants and in potato plants by Youssif (2017) [39]. Foliar spraying of faba bean (*Vicia faba*) plants with 30 mg l⁻¹ folic acid resulted in significant improvements in shoot height, chlorophyll and nutritional content in the leaves (Al-Maliky *et al.*, 2019) [4]. The increased chlorophyll content with increasing concentrations of vitamins might have produced more photosynthates resulting in higher growth rates as higher dry matter production is a prerequisite for higher rhizome yield. The enhancement in the content of the chlorophylls could have possibly led to the improvement of light-capturing efficiency which might have resulted in higher photosynthetic rate manifested in terms of improved plant fresh mass and growth. Increasing plant pigments chlorophyll% by using ascorbic acid may be due to the its role in increasing the rates of photochemical reduction (Kumar and Bhushan, 1978) [21]. Khurshid *et al.* (2023) [20] reported that wheat foliar administered with nicotinic acid significantly increasing the growth of roots, shoots and the spikes also recorded the highest SPAD value for leaf chlorophyll content. Applying thiamine to fenugreek improved its vegetative growth, chlorophyll a & b, carotenoids, phenolics and antioxidant activity particularly at 750 and 500 ppm (Aminifard *et al.*, 2018) [6] and similar results were found by Fallahi *et al.* (2018) [11] in basil plants.

Table 1: Influence of amino acids on total chlorophyll content of turmeric leaves

Treatments	Total chlorophyll content (mg g ⁻¹)		
	2023-24	2024-25	Pooled
Glutamine -50ppm	4.05	4.08	4.07
Glutamine-100ppm	4.19	4.25	4.22
Glutamine-150ppm	4.47	4.51	4.49
Glutamine-200ppm	4.41	4.39	4.40
Lysine -50ppm	3.97	3.99	3.98
Lysine -100ppm	4.07	4.11	4.09
Lysine -150ppm	4.31	4.42	4.37
Lysine -200ppm	4.22	4.30	4.26
Glycine -50ppm	4.04	4.02	4.03
Glycine -100ppm	4.12	4.15	4.14
Glycine -150ppm	4.34	4.47	4.41
Glycine -200ppm	4.26	4.33	4.30
Aspartic acid-50ppm	3.99	3.85	3.92
Aspartic acid-100ppm	3.91	3.81	3.86
Aspartic acid-150ppm	4.10	4.18	4.14
Aspartic acid-200ppm	4.19	4.26	4.23
Phenylalanine -50ppm	3.93	3.83	3.88
Phenylalanine -100ppm	4.17	4.21	4.19
Phenylalanine -150ppm	4.02	4.07	4.05
Phenylalanine -200ppm	4.25	4.36	4.31
Control (Water spray)	3.55	3.54	3.55
S.Em. (±)	0.059	0.059	0.057
C.D. (P=0.05)	0.168	0.170	0.162

Table 2: Influence of vitamins on total chlorophyll content of turmeric leaves

Treatments	Total chlorophyll content (mg g ⁻¹)		
	2023-24	2024-25	Pooled
Ascorbic acid-25ppm	3.90	3.89	3.90
Ascorbic acid-50ppm	4.17	4.25	4.21
Ascorbic acid-75ppm	4.04	4.10	4.07
Ascorbic acid-100ppm	4.36	4.40	4.38
Thiamine-25ppm	3.96	3.92	3.94
Thiamine-50ppm	4.08	4.15	4.12
Thiamine-75ppm	4.23	4.31	4.27
Thiamine-100ppm	4.19	4.28	4.24
Folate-25ppm	3.95	4.01	3.98
Folate-50ppm	3.87	3.94	3.91
Folate-75ppm	4.14	4.19	4.17
Folate-100ppm	4.28	4.35	4.32
Niacin-25ppm	3.80	3.79	3.80
Niacin-50ppm	4.01	4.05	4.03
Niacin-75ppm	3.84	3.85	3.85
Niacin-100ppm	4.11	4.22	4.17
Control (Water spray)	3.50	3.53	3.52
S.Em. (±)	0.062	0.063	0.062
C.D. (P=0.05)	0.179	0.181	0.179

Conclusion

The present investigation demonstrated that application of amino acids and vitamins promptly influenced the total chlorophyll content of turmeric leaves which plays a vital role in photosynthetic rate and physiological process of the plant. The chlorophyll content was increased with enhancing the concentrations of amino acids and vitamins up to high level, thereby helps in plant growth, development and rhizome production. So, we can conclude that glutamine 150 ppm and ascorbic acid 100 ppm recorded the highest total chlorophyll content among the other treatments.

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Conflict of Interest

The authors declare that they do not find any relevant competing interests.

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