

Effect of Combined-Stimulants and Phenylalanine on Growth, Yield, Constituents, and Antioxidant Activity of Celery Leaves

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Abstract

Experiment was conducted during agricultural season 2022-2023 to study the effect of spraying Combined-Stimulants (contain Kinetin 0.05%, GA3 0.001% and IBA 0.001%) at a concentration 0, 1 and 2 mL⁻¹ and amino acid Phenylalanine at a concentration 25 and 50 g·L⁻¹ and their combinations on some parameters of growth, yield and antioxidant compounds and activities of celery leaves at 2st cuts, treatments were distributed in a factorial experiment according to randomized complete block design (R.C.B.D) with three replicates, and means were compared according with least significant difference test (L.S.D) at probability level 0.05.

The results showed a significant superiority of spraying 2 mL⁻¹ combined-Stimulants + 100 mg·L⁻¹ Phenylalanine at 1cut on leaves No., branch No., leaves content of chlorophyll pigments, dry matter, leaves oil, and fresh yield of leaves per unit area. While, treat of spraying 2 mL⁻¹ combined-Stimulants + 50 mg·L⁻¹ Phenylalanine on 2cut was suggested significantly superior on leaves content of ascorbic acid, total tannins, total flavonoids, total phenols, free radical scavenging activity and antioxidant activity reached 13.8 mg.100g FW, 1.85 mg.TAN/g DW, 4.22 mg.RUT/g DW, 22.8 mg.GAE/g DW, 80.2 mg.ASC/g DW and 50.2% respectively.

Keywords: celery; Combined-Stimulants; Phenylalanine; Oxidative Activity

Introduction

Celery (*Apium graveolens var. secalinum*) was belonged to Umbelliferae family. Its native habitat to Mediterranean basin [1]. Its fresh and dried green leaves are used in food industry, especially pastries. The largest part of global production is consumed in various authorities around the world. Its low contain of calories and high percentage of fibers also useful in preventing constipation by increasing bowel movement, there are many medical benefits from using celery leaves such as laxative, analgesic, digestion, neutralizing acidity, kidney function tonic, diuretic to remove gallstones and bladder, anti-inflammation for many infections such as rheumatism, gout, asthma and bronchitis [2], anti-cirrhosis of the spleen and liver [3], antihypertensive and cholesterol [4], hypocholesterolemia [5], and a stimulant for the immune system. The celery leaves oil contains essential and aromatic fatty acids that gives a distinct taste and comfort [6], leaves also contain many vitamins like C, K, and A, and a group of carotenoid compounds, especially flavonoids such as apigenin and luteolin, which have anticancer effects [7], in addition to their content of nutrients such as calcium, phosphorus, and potassium.

The treating plants with growth regulators are one of important agricultural techniques to increasing growth and yield in quantity and quality, as it has an important role in plant growth and development by directing cell activity towards division and elongation, which enhances metabolic processes of production dry matter and bioactive compounds. Combined-Stimulants consists of three important growth regulators (cytokinin, gibberellins and auxin) that controlling to stages and nature of plant growth and its production. Results of experiment [8] showed that there was a significant effect of spraying Coleus plants with Kinetin at concentration 100 ppm on leaves contents of chlorophyll pigments, total phenolics, total flavonoids and total tannins compared with control treat. [9] were found that spraying carrot plants with auxin (Indole Butyric Acid) at a concentration of 100 and 150 μM resulted in significant differences in plant height and fresh weight of shoots compared to plants sprayed with distilled water only. [10] concluded that spraying alfalfa plants with GA3 at concentration 100 ppm at different intervals significantly increased growth and yield parameters as compared to control when harvest after 50, 80, 110 and 140 days, respectively.

Phenylalanine is Phenylalanine is aromatic amino acid that participates in many metabolic processes in higher plants, particularly production of secondary metabolite compounds at direct or indirect ways such as phenols, flavonoids, tannins and terpenes in the shikimic acid cycle, its perhaps partake as source of nitrogen for building proteins or enzymes [11]. Researches [12] were found that foliar application of roselle plants by phenylalanine gives an increase on growth parameters (branch number, chlorophyll pigments and dry weight) and anthocyanin compounds. The [13] shown that treated *Trigonella foenum-graecum* L. with phenylalanine increased significantly growth and yield characters, also [14] noted for set significant effects on vegetative growth parameters, yield and anthocyanin pigments of spraying phenylalanine on black rice.

In view of importance of celery plant nutritionally and medicinally, and for purpose of increasing the productivity of area unit, this experiment was conducted to measure the effect of spraying combination-stimulants and Phenylalanine on growth and yield of fresh leaves, oil, total constituent compounds and antioxidant activity of leaves celery plant.

Material and Methods

The experiment was conducted at during agricultural season 2022-2023 to demonstrate the effect of spraying combination-stimulants and phenylalanine on some parameters of growth, yield, constituents compounds and antioxidant activity in 2cuts of celery leaves, experiment consist of two factors, first was combination-stimulants which contain of three phytohormones, cytokinins (Kinetin 0.05%), gibberellins (GA3 0.001%) and auxins (Indole Butyric Acid 0.001%) at concentration 0, 1 and 2 $\text{mL}\cdot\text{L}^{-1}$, second factor aromatic amino acid phenylalanine at concentration 25 and 50 $\text{gm}\cdot\text{L}^{-1}$ and their combinations between them, treatments were dispensed in a factorial experiment according to randomized complete block design (R.C.B.D) with three replicates, and means were tested according with least significant difference test (L.S.D) at probability level 0.05 [15].

The experimental field was prepared by plowing, leveling, sampling to analysis Chemophysical properties of soil (Table 1), adding Tri-superphosphate fertilizer (NPK 20:20:20) at level 80 $\text{kg}\cdot\text{h}^{-1}$, then dividing into three blocks ($1\text{*}22 \text{ m}$), each block was divided into 9 experimental units ($1\text{*}2 \text{ m}$), distance between them 0.5 m, seeds were sown at 1 Nov 2022 on lines, agriculture distance set ($10\text{*}10 \text{ cm}$) and agriculture density was 855000 plant. h^{-1} , The process of foliar application were carried out twice 30 and 60 days after planting, plants cuttings done (1st and 2st cut) after 45 and 75 days after planting respectively.

Sample depth (cm)	pH	Organic Matter (%)	Ec dsm^{-1}	Total Nitrogen (%)	Available Phosphor mg kg^{-1}	Available Potassium mg kg^{-1}	Silt (%)	Clay (%)	Sand (%)	Soil Texture
0-30	7.5	2.73	2.1	3.2	8.9	260	45.2	28.6	26.2	Silt-Clay

Table 1: Chemophysical properties of soil.

The experimental measures for 2cuts consist of some growth indicators as leaves number, branch number, total chlorophyll pigments and dry weight. Yield parameters as fresh leaves production and oil percentage [16]. The constituent compounds determined depend on: total Ascorbic acid by titration with 2,6-dichlorophenol Indophenol reagent [17], total tannins by method of [18], which taking 1 ml of methanolic extract, adding 0.5 ml of Folin-Ciocalteu reagent, 1 ml of sodium carbonate solution, and 8 ml of distilled water, then incubated for 30 minutes at room temperature, then taken optical density at wavelength of 725 nm, calibrate readings on tannic acid standard curve, and adjust the calibration results by multiplying them by a coefficient of 7.061. While aluminum chloride colorimetric method was used to estimate total flavonoids by taking optical absorption at wavelength 510 nm and calibrating readings on catechin standard curve [19], and total phenolics by a Folin-Ciocalteu reagent method and recording optical absorption of spectrophotometer at 765 nm then equilibriums with gallic acid standard curve [20]. The antioxidant activity was estimated by measuring free radical scavenging activity ABTS method and taking optical absorption at 734 nm then balancing with gallic acid standard curve [21], and antioxidant activity with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical reagent and recording optical absorption of spectrophotometer at 517 nm [22].

Results and Discussion

Growth and Yield Parameters

The noted from results of statistical analysis in Table 2 that there was a significant effect of experimental treatments on growth and yield parameters in 2cuts of celery, The spraying treatment C-S² + Phe¹⁰⁰ was significantly superior and recorded the highest averages at 2cuts on traits, leaves number, branches number, leaves content of total chlorophyll pigments, plant percentage of dry weight, fresh leaves yield, and leaves oil percentage reached 36.6, 33.3 leaves.plant⁻¹, 25.4, 19.9 branch.plant⁻¹, 118.2, 98.7 mg 100g⁻¹ FW, 18.2, 13.4%, 1.356, 0.998 Kg.m², and 4.35, 3.41% respectively, compared with control treatment (spraying distill water only) which recorded the lowest averages reached 19.3, 15.6 leaves.plant⁻¹, 11.3, 8.2 branch.plant⁻¹, 87.2, 75.6 mg 100g⁻¹ FW, 10.9, 8.5%, 0.891, 0.695 Kg.m², and 3.61, 2.82% respectively for 2cuts.

Treatments	LN		BN		Ch		DW		FLY		Oil	
	1cut	2 cut	1cut	2 cut	1cut	2 cut	1cut	2 cut	1cut	2 cut	1cut	2 cut
Control	19.3	15.6	11.3	8.2	87.2	75.6	10.9	8.5	0.891	0.695	3.61	2.82
C-S ¹	23.5	22.7	15.6	11.9	93.2	79.5	11.7	9.6	0.933	0.766	3.77	3.09
C-S ²	31.4	28.6	18.9	15.2	98.5	85.4	12.6	10.1	1.025	0.822	3.85	3.17
Phe ⁵⁰	20.7	17.9	13.4	10.3	90.1	81.7	13.4	10.8	1.087	0.876	3.81	3.22
Phe ¹⁰⁰	24.1	22.4	16.8	13.4	92.6	82.3	14.5	11.4	1.128	0.887	3.91	3.28
C-S ¹ + Phe ⁵⁰	27.5	25.8	19.5	16.2	105.2	88.4	15.7	11.7	1.166	0.908	4.05	3.33
C-S ¹ + Phe ¹⁰⁰	30.9	28.7	21.1	17.8	108.4	92.8	16.4	12.5	1.191	0.933	4.21	3.35
C-S ² + Phe ⁵⁰	32.3	29.6	22.6	18.9	112.5	95.5	17.7	12.9	1.208	0.958	4.29	3.38
C-S ² + Phe ¹⁰⁰	36.6	33.3	25.4	19.9	118.2	98.7	18.2	13.4	1.356	0.998	4.35	3.41
LSD _(P≤0.05)												

Parameters: Leaves No. (LN), Branch No. (BN), Chlorophyll mg 100g⁻¹ FW (Ch), Dry Weight % (DW), Fresh Leaves Yield Kg.m² (FLY), and Oil (%).

Table 2: Effect of Combined-Stimulants and Phenylalanine on Some Growth and Yield Parameters of Celery Leaves.

The reason for this may be attributed to the role of treat components in growth and yield indicators, especially kinetin, which works to prevent apical dominance, which increases the growth of lateral buds and leaves number, also kinetin helps to accumulate chlorophyll in leaves tissues, which increased chlorophyll pigments and dry weight percentage, In addition to the role of gibberellin and auxin, which work to increase the processes of elongation and cell division, accelerate the respiration process, activate enzymes, and form RNA and protein, enhancing the effectiveness of dry matter photosynthesis. Phenylalanine also contributes to an initiating compound to a product of compounds similar effects of auxin, such as phenylpyruvate, or it is a source of plant supply with nitrogen, which is an essential and macronutrient for plants to the production of protein, enzymes, and genetic material.

Antioxidant Activity Parameters

The results in Table 3 show that there was a significant effect of experimental treatments on antioxidant activity parameters in 2cuts of celery leaves, The spraying treatment C-S² + Phe⁵⁰ was significantly superior and recorded the highest averages at 2cuts on traits, leaves contain of ascorbic acid, total tannins, total flavonoids, total phenolics, total antioxidant capacity mg, and radical scavenging activity reached 9.2, 13.8 mg.100g FW, 1.24, 1.85 mg.TAN/g DW, 3.51, 4.22 mg.RUT/g DW, 19.4, 25.8 mg.GAE/g DW, 69.7, 80.2 mg ASC/g DW (TCA), and 41.2, 50.2 %(DPPH) respectively, compared with control treatment (spraying distill water only) which recorded the lowest averages reached 6.2, 8.5 mg.100g FW, 0.82, 1.33 mg.TAN/g DW, 2.89, 3.08 mg.RUT/g DW, 11.3, 13.3 mg.GAE/g DW, 50.5, 60.7 mg ASC/g DW, and 29.3, 37.7 % respectively for 2cuts. The superiority of this treatment may be mainly due to the occurrence of abiotic stress, done by the process of cutting the vegetative parts of plants, which led to stimulation of the plant's defense system to maintain the completion of its life cycle, and this led to the consumption of the dry matter produced from photosynthesis in increasing the production of secondary compounds, especially tannins, flavonoids, phenols, along with ascorbic acid, which participates in these compounds as an antioxidant compound to controlling free radicals resulting from bio-processes, especially increased respiration as a result of environmental stress, these events are supported by the amino acid phenylalanine, due to its participation as an initiating compound in a shikimic acid pathway, which results in the above compounds as well, the role of growth regulators that enhance the activity of photosynthesis is to produce primary metabolite compounds, which is accompanied by increased production of secondary metabolite compounds.

Treatments	AscA		TT		TF		TP		TAC		DPPH	
	1cut	2 cut										
Control	6.2	8.5	0.82	1.33	2.89	3.08	11.3	13.3	50.5	60.7	29.3	37.7
C-S ¹	6.5	8.9	0.89	1.42	2.95	3.19	11.9	15.1	51.6	62.3	30.2	39.4
C-S ²	6.9	9.2	0.95	1.46	3.05	3.32	12.7	17.2	54.2	64.8	33.1	40.2
Phe ⁵⁰	7.2	9.7	1.04	1.59	3.11	3.58	14.6	19.6	57.4	68.9	35.7	43.5
Phe ¹⁰⁰	7.7	10.1	1.11	1.71	3.17	3.71	16.4	21.5	61.3	73.5	36.3	45.7
C-S ¹ + Phe ⁵⁰	8.3	12.3	1.21	1.79	3.43	3.91	17.1	23.1	67.4	77.7	38.5	47.1
C-S ¹ + Phe ¹⁰⁰	7.9	10.8	1.17	1.71	3.25	3.75	16.2	21.9	65.5	75.6	36.4	46.6
C-S ² + Phe ⁵⁰	9.2	13.8	1.24	1.85	3.51	4.22	19.4	25.8	69.7	80.2	41.2	50.2
C-S ² + Phe ¹⁰⁰	8.5	12.5	1.19	1.77	3.33	3.82	17.3	22.4	67.3	78.1	38.4	47.3
LSD _(P≤0.05)												

Parameters: Ascorbic Acid mg.100g FW (AscA), Total Tannins mg.TAN/g DW (TT), Total Flavonoids mg.RUT/g DW (TF), Total Phenolics mg.GAE/g DW (TP), Total Antioxidant Capacity mg ASC/g DW (TCA), Radical Scavenging Activity %(DPPH).

Table 3: Effect of Combined-Stimulants and Phenylalanine on Some Antioxidant Activity Parameters of Celery Leaves.

Conclusion

It is noted from the results obtained that the amino acid phenylalanine is a compound with significant effectiveness in resisting abiotic stress and is effective in increasing secondary and antioxidant compounds. Growth regulators also contributed to enhancing the role of phenylalanine in increasing growth and yield indicators.

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