Effect of hornwort extract and spraying with thiamine and phenylalanine on some vegetative growth indicators of Damask rose (*Rosa domascena* Mill.)

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Abstract

The experiment was conducted in the plant canopy of the Department of Horticulture and Landscape Engineering at the College of Agriculture - University of Kerbala for the period from 1/3/2024 to 1/7/2024 to study the effect of adding hornwort extract and spraying with thiamine and phenylalanine on the vegetative traits of Damask rose seedlings (Sultani variety). The experiment was conducted using a randomized complete block design (R.C.B.D) according to the factorial experiment arrangement with three replications. The factors included hornwort extract at two concentrations (0 and 8 g L^{-1}). Thiamine spray at three concentrations (0, 75 and 150 mg L^{-1}). Phenylalanine spray at three concentrations (0, 75 and 150 mg L^{-1}). The results showed that the treatment of the extract of the hornwort at a concentration of (8 g L^{-1}) was outperforming in the average increase of seedling height (36.859 cm), seedling diameter (4.690 mm), number of leaves (38.830 leaf seedling^{-1}) and leaf area (29.879 cm^2). The results showed significant differences between the thiamine spray concentrations, as the concentration of (150 mg L^{-1}) outperforming the aforementioned traits, as it recorded averages of (42.722 cm, 5.436 mm, 50.167 leaf seedling^{-1}, 35.571 cm^2) respectively. The results also showed significant differences between the phenylalanine spray concentrations, as the concentration of (150 mg L^{-1}) outperforming the same traits, with averages of (37.922 cm, 4.584 mm, 40.522 leaf seedling^{-1}, 30.262 cm^2) respectively. The triple intervention achieved the best results, indicating the efficiency of the combined use of the three factors.

Keywords: *Rosa domascena* Mill., *Ceratophyllum demersum* L., Thiamine, Phenylalanine.

Introduction

Damask rose (*Rosa domascena* Mill., commonly known as Damask rose, is widely recognized as one of the most important species of ornamental and cut flowers . The genus Rosa belongs to the family Rosaceae and is cultivated in all temperate regions, except in hot climates. It is commonly grown in areas of Asia, the Middle East, and

North America [1]. The genus Rosa includes around 200 species and more than 1800 cultivars [2]. Among these, *Rosa domascena* is regarded as one of the most important species. It is believed to have originated in the western regions of the Middle East, specifically in the area of Syria [3]. Damask rose is cultivated for the aesthetic appeal of its flowers, which are characterized by their vivid colors and extended blooming period. It is commonly grown in both private and public gardens as well as in green spaces surrounding buildings [4]. The rose also has numerous and significant industrial applications. Its essential oil, obtained through the distillation of petals, is widely used in various fields, such as in perfuming soaps and cosmetics, and as a flavoring agent in food products like tea [5] . The importance of the essential oil extracted from Damask rose lies in its high content of monoterpenes such as citronellol, trans-geraniol, linalool, phenylethyl alcohol, as well as hydrocarbons like nonadecene and nonadecane.

Many researchers have turned to the use of novel approaches aimed at providing plants with essential nutrients. Among these approaches is the use of plant extracts, which are characterized by their availability, ease of application, and their environmentally friendly nature. One such extract is *Ceratophyllum demersum* (hornwort) extract, which is distinguished by its rich content of essential elements such as calcium (Ca), phosphorus (P), nitrogen (N), and several amino acids that are vital for plant growth and development [6]. In order to achieve an increase in the production of this plant, it is necessary to follow modern scientific methods that are capable of achieving this, as the use of vitamins and amino acids in the growth and development of the plant has become one of the standard methods in modern agriculture, due to the ability of these materials to modify growth and flowering through their effect on the physiological processes within the plant [7].

The use of amino acids and vitamins also plays an important role in plant growth and development, due to their role in vital processes within the plant. Among these vitamins is thiamine, a water-soluble vitamin essential for enzyme biosynthesis and a key player in carbohydrate metabolism [8].

Phenylalanine is considered one of the most important primary amino acids involved in plant growth and development, as it serves as a key precursor for numerous cellular bioactive compounds such as phenylpropanoids, flavonoids, lignin, tannins, salicylates, and anthocyanins, all of which contribute to improved plant growth [9]. In a study conducted by [10], it was shown that foliar application of thiamine at a concentration of 100 mg L^{-1} led to a significant increase in the leaf area of Alaria plants. Similarly, findings by [11] demonstrated that spraying thiamine at a concentration of 150 mg L^{-1} resulted in the best outcomes in terms of Regal geranium plant height. Based on the above, the current study aims to investigate the integrated effects of hornwort extract, thiamine, and phenylalanine on the vegetative growth of Damask rose seedlings and to assess how these treatments contribute to the enhancement of vegetative growth traits.

Materials and Methods

The experiment was carried out in the plant canopy of the Department of Horticulture and Landscape Engineering/College of Agriculture, University of Kerbala, for the period from 1/3/2024 to 1/7/2024 to study the effect of adding hornwort extract and spraying with thiamine and phenylalanine on the vegetative traits of Damask rose seedlings, (Sultani variety). A total of 270 one-year-old seedlings were selected, exhibiting as much uniformity as possible in size and vegetative growth. These seedlings were initially planted in black polyethylene plastic bags with a capacity of 2 kg. On February 20, 2024, they were transplanted into 10 kg pots filled with a mixture of sandy soil and peat moss in a 1:3 ratio. The experiment was conducted using a Randomized Complete Block Design (R.C.B.D.) in a factorial arrangement involving three factors. The first factor consisted of the application of hornwort extract at two concentrations (0 and 8 g L^{-1}), applied four times at 15-day intervals starting from March 1, 2024, until April 15, 2024. Upon analysis, the extract was found to be rich in various nutrients, amino acids, and vitamins in varying concentrations. These included phosphorus (0.268%), potassium (0.392%), calcium (3.731%), indole-3-acetic acid (124.6 ppm), gibberellins (30.28 ppm), cytokinins (180.88 ppm), and the amino acids serine (430.57 ppm), glycine (528.15 ppm), threonine (9266.15 ppm), valine (898.86 ppm), tryptophan (151.37 ppm), and phenylalanine (654.72 ppm), in addition to vitamin C (361.91 ppm).

The second factor involved foliar thiamine at spraying with three concentrations (0, 75, and 150 mg L^{-1}), while the third factor included foliar application of phenylalanine at three concentrations (0, 75, and 150 mg L^{-1}). The experiment comprised three replications, each containing 18 seedlings, with five seedlings per experimental unit. The seedlings were sprayed four times until complete wetting, at 15-day intervals from March 1, 2024, to April 15, 2024. To enhance the efficiency of nutrient absorption, seedlings were irrigated one day prior to each spraying, as moisture contributes to cell turgidity and stomatal opening. Moreover, pre-spray irrigation helps reduce solute concentration in the leaf, thereby increasing the permeability of foliar solution ions into leaf tissues [12]. Spraying was carried out early in the morning using a 2-liter hand sprayer. In all treatments, a small amount of detergent (Zahi) was added at a concentration of one drop per seedling to reduce the surface tension of water molecules and ensure complete wetting of the vegetative parts. All agronomic practices, such as irrigation and weeding (removal of weeds) within the pots and between replications, were performed equally across all treatments as needed. Measurements were recorded at the end of the study on July 1, 2024.

Data recorded

Average seedling height increase (cm)

Seedling height increase was measured by finding the difference in stem height before and after the experimental treatments. A tape measure was used to measure the length of the stem from the soil surface to the top of the main stem. The average was calculated for each experimental unit for the treatments used in the study and for each replication.

Average increase in seedling diameter (mm)

The increase in stem diameter was measured before and after the experimental treatments using a digital scale (vernier), and the average was extracted in each experimental unit for the treatments used in the study and for each replication.

Average increase in the number of leaves (leaf seedling^{-1})

The number of leaves per seedling was calculated before and after the experimental treatments. The average number of leaves in each experimental unit was calculated for the treatments used in the study and for each replication.

Average increase in the leaf area (cm²)

Leaf area for each treatment and replication was calculated using the ImageJ software under the Windows 7 operating system [13] .

Statistical analysis

The results were statistically analyzed according to the analysis of variance (ANOVA) as per the randomized complete block design (R.C.B.D.), the least significant difference ($LSD_{0.05}$) test was used to compare and separate the means [14], this is done using statistical analysis software GenStat12.

Results and Discussion

Average seedling height increase (cm)

Table (1) indicates a significant effect of the concentrations of the hornwort extract on the average seedling height increase. The treatment with a concentration of (8 g L^{-1}) achieved the highest average of 36.859 cm, compared to the control treatment (0 g L^{-1}), which recorded the lowest average of (33.244 cm), an increase of 10.8%. The results also showed significant differences between the thiamine concentrations. The treatment with a concentration of (150 mg L^{-1}) achieved the highest average of (42.722 cm), while the control treatment (0 g L^{-1}) achieved the lowest average of 26.667 cm, an increase of 60.20%. The results of the same table showed that phenylalanine concentrations had a significant effect on this trait, as the treatment with a concentration of (150 mg L^{-1}) gave the highest average of (37.922 cm), while the control treatment recorded the lowest average of (32.133 cm), with an increase percentage of 118.0%.

The bilateral interactions between the study factors showed a significant effect on the average increase in seedling height, as the highest interaction was recorded when treating the hornwort extract at a concentration of (8 g L^{-1}) and thiamine at a concentration of (150 mg L^{-1}) with the highest interaction of (44.933 cm). In contrast, the control treatment for both factors recorded the lowest interaction of (25.067 cm) with an increase percentage of 79.25%. The results also showed a significant interaction between the hornwort extract and phenylalanine, as the treatment with the hornwort extract at a concentration of (8 g L^{-1}) and the treatment with phenylalanine at a concentration of (150 mg L^{-1}) achieved the highest interaction of (39.800 cm). In contrast, the control treatment for both factors recorded the lowest interaction of (30.556 cm) with an increase percentage of 30.25%. As for the interaction between thiamine and

phenylalanine, the treatment of thiamine at a concentration of (150 mg L^{-1}) with the treatment of phenylalanine at a concentration of (150 mg L^{-1}) gave the highest interaction of (46.500 cm), while the lowest interaction of (23.600 cm) was in the control treatment of both factors, with an increase percentage of 97.03%. The results also revealed a significant three-way interaction among the studied factors in the average increase of this trait. The combination of hornwort extract at a concentration of (8 g L^{-1}), thiamine at (150 mg L^{-1}), and phenylalanine at (150 mg L^{-1}) recorded the highest interaction of 48.800 cm, whereas the control treatment for these factors showed the lowest of 22.000 cm, representing an increase of 121.81%.

Table (1): Effect of hornwort extract, spraying with thiamine and phenylalanine, and their interaction on the average increase in seedling height (cm)

Hornwort extract concentrations (g L^{-1})	Thiamine concentrations (mg L^{-1})	Phenylalanine concentrations (mg L^{-1})			Hornwort extract × Thiamine
		0	75	150	
0	0	22.000	25.000	28.200	25.067
	75	32.533	34.200	35.733	34.156
	150	37.133	40.200	44.200	40.511
8	0	25.200	28.533	31.067	28.267
	75	35.533	37.067	39.533	37.378
	150	40.800	45.600	48.400	44.933
L.S.D $_{0.05}$		0.8075			0.4662
Means (Phenylalanine)		32.133	35.100	37.922	Means (Hornwort extract)
L.S.D $_{0.05}$		0.3297			
Hornwort extract × Phenylalanine					
0		30.556	33.133	36.044	33.244
8		33.711	37.067	39.800	36.859
L.S.D $_{0.05}$		0.4662			0.2692
Thiamine × Phenylalanine					Means (Thiamine)
0		23.600	26.767	29.633	26.667
75		34.033	35.633	37.633	35.767
150		38.767	42.900	46.500	42.722
L.S.D $_{0.05}$		0.5710			0.3297

Average increase in seedling diameter (mm)

Table (2) shows a significant effect between the concentrations of the hornwort extract on the average increase in seedling diameter, as the treatment with a concentration of (8 g L^{-1}) achieved the highest average of (4.690 mm) compared to the control treatment (0 g L^{-1}) which recorded the lowest average of (3.900 mm) and an increase percentage of 20.25%. The results also showed significant differences between the

thiamine concentrations, as the treatment with a concentration of 150 mg L^{-1}) achieved the highest average of (5.436 mm) while the lowest average of (3.018 mm) in the control treatment (0 g L^{-1}), and an increase of 80.11%. The results of the same table showed that phenylalanine concentrations had a significant effect on this trait, as the treatment with a concentration of (150 mg L^{-1}) gave the highest average of (4.584 mm), while the control treatment recorded the lowest average of (3.916 mm), with an increase percentage of 517.0%.

The bilateral interactions between the study factors showed a significant effect on the average increase in seedling height, as the highest interaction was recorded when treating the hornwort extract at a concentration of (8 g L^{-1}) and thiamine at a concentration of (150 mg L^{-1}) with the highest interaction of (5.729 mm). In contrast, the control treatment for both factors recorded the lowest interaction of (2.535 mm) with an increase percentage of 125.9%. The results also showed a significant interaction between the hornwort extract and phenylalanine, as the treatment with the hornwort extract at a concentration of (8 g L^{-1}) and the treatment with phenylalanine at a concentration of (150 mg L^{-1}) achieved the highest interaction of (4.922 mm). In contrast, the control treatment for both factors recorded the lowest interaction of (3.455 mm) with an increase percentage of 42.4%. As for the interaction between thiamine and phenylalanine, the treatment of thiamine at a concentration of (150 mg L^{-1}) with the treatment of phenylalanine at a concentration of (150 mg L^{-1}) gave the highest interaction of (5.678 mm), while the lowest interaction of (2.615 mm) was in the control treatment for both factors, with an increase percentage of 117.13%. The results indicated a significant three-way interaction among the studied factors on the average increase of this trait, as the hornwort extract at a concentration of (8 g L^{-1}), thiamine at a concentration of (150 mg L^{-1}), and phenylalanine at a concentration of (150 mg L^{-1}) achieved the highest interaction of (5.886 mm), while the control treatment of these factors recorded the lowest interaction of (2.434 mm) with an increase percentage of 141.8%.

Table (2): Effect of hornwort extract, spraying with thiamine and phenylalanine, and their interaction on the average increase in seedling diameter (mm)

Hornwort extract concentrations (g L^{-1})	Thiamine concentrations (mg L^{-1})	Phenylalanine concentrations (mg L^{-1})			Hornwort extract × Thiamine
		0	75	150	
0	0	2.434	2.498	2.674	2.535
	75	2.969	4.503	4.596	4.023
	150	4.963	4.994	5.469	5.142
8	0	2.795	3.754	3.953	3.500
	75	4.763	4.828	4.927	4.839
	150	5.575	5.727	5.886	5.729
L.S.D $_{0.05}$		0.0926			0.0534
Means (Phenylalanine)		3.916	4.384	4.584	Means (Hornwort extract)
L.S.D $_{0.05}$		0.0378			

Hornwort extract × Phenylalanine				
0	3.455	3.998	4.246	3.900
8	4.378	4.770	4.922	4.690
L.S.D $_{0.05}$	0.0534			0.0308
Thiamine × Phenylalanine				Means (Thiamine)
0	2.615	3.126	3.313	3.018
75	3.866	4.666	4.761	4.431
150	5.269	5.360	5.678	5.436
L.S.D $_{0.05}$	0.0655			0.0378

Average increase in the number of leaves (leaf seedlings^{-1})

Table (3) shows a significant effect between the concentrations of the hornwort extract on the average increase in number of leaves, as the treatment with a concentration of (8 g L^{-1}) achieved the highest average of (38.830 leaf seedlings^{-1}) compared to the control treatment (0 g L^{-1}) which recorded the lowest average of (35.319 leaf seedlings^{-1}) and an increase percentage of 9.9%. The results also showed significant differences between the thiamine concentrations, as the treatment with a concentration of (150 mg L^{-1}) achieved the highest average of (50.167 leaf seedlings^{-1}) while the lowest average of (23.356 leaf seedlings^{-1}) in the control treatment (0 g L^{-1}) and an increase of 114.79%. The results of the same table showed that the concentrations of phenylalanine had a significant effect on this trait, as the treatment with a concentration of (150 mg L^{-1}) gave the highest average of (40.522 leaf seedlings^{-1}), while the control treatment recorded the lowest average of (33.589 leaf seedlings^{-1}), with an increase percentage of 20.6%.

The results also showed that the bilateral interactions between the study factors significantly affected the average increase in seedling height, as the highest interaction was recorded when treating the hornwort extract at a concentration of (8 g L^{-1}) and thiamine at a concentration of (150 mg L^{-1}), with the highest interaction of (51.711 leaf seedlings^{-1}), while the control treatment for both factors recorded the lowest interaction of (22.333 leaf seedlings^{-1}) and an increase percentage of 131.54%. In contrast the results showed no significant interaction between hornwort extract and phenylalanine. As for the interaction between thiamine and phenylalanine, the treatment of thiamine at a concentration of (150 mg L^{-1}) with the treatment of phenylalanine at a concentration of (150 mg L^{-1}) gave the highest interaction of (53.700 leaf seedlings^{-1}), while the lowest interaction of (20.100 leaf seedlings^{-1}) was in the control treatment for both factors, with an increase percentage of 167.16%. The results showed a significant three-way interaction between the factors on the average increase of this trait, as the hornwort extract at a concentration of (8 g L^{-1}), thiamine at a concentration of (150 mg L^{-1}), and phenylalanine at a concentration of (150 mg L^{-1}) achieved the highest interaction of (55.800 leaf seedlings^{-1}). In contrast, the control treatment of these factors recorded the lowest interaction of (19.000 leaf seedlings^{-1}) with an increase percentage of 193.68%.

Table (3): Effect of hornwort extract, spraying with thiamine and phenylalanine, and their interaction on the average increase in number of leaves (leaf seedling^{-1})

Hornwort extract concentrations (g L^{-1})	Thiamine concentrations (mg L^{-1})	Phenylalanine concentrations (mg L^{-1})			Hornwort extract × Thiamine
		0	75	150	
0	0	19.000	22.000	26.000	22.333
	75	31.200	35.400	38.400	35.000
	150	45.533	48.733	51.600	48.622
8	0	21.200	25.200	26.733	24.378
	75	36.200	40.400	44.600	40.400
	150	48.400	50.933	55.800	51.711
L.S.D $_{0.05}$		0.4098			0.2366
Means (Phenylalanine)		33.589	37.111	40.522	Means (Hornwort extract)
L.S.D $_{0.05}$		0.1673			
Hornwort extract × Phenylalanine					
0		31.911	35.378	38.667	35.319
8		35.267	38.844	42.378	38.830
L.S.D $_{0.05}$		N.S			0.1366
Thiamine × Phenylalanine					Means (Thiamine)
0		20.100	23.600	26.367	23.356
75		33.700	37.900	41.500	37.700
150		46.967	49.833	53.700	50.167
L.S.D $_{0.05}$		0.2898			0.1673

Average increase in the leaf area (cm²)

The results presented in (Table 4) indicate that there is a significant effect between the concentrations of the hornwort extract on the average increase in leaf area, as the treatment with a concentration of (8 g L^{-1}) achieved the highest average of (29.879 cm^2) compared to the control treatment (0 g L^{-1}) which recorded the lowest average of (27.616 cm^2) and an increase percentage of 8.19%. The results also showed significant differences between the thiamine concentrations, as the treatment with a concentration of (150 mg L^{-1}) achieved the highest average of (35.571 cm^2), while the lowest average of (22.680 cm^2) in the control treatment (0 g L^{-1}), and an increase of 56.83%. The results of the same table showed that phenylalanine concentrations had a significant effect on this trait, as the treatment with a concentration of (150 mg L^{-1}) gave the highest average of (30.262 cm^2), while the control treatment recorded the lowest average of (27.197 cm^2), with an increase percentage of 11.26%.

It was also noted through the results (Table 4) that the bilateral interactions between the study factors had a significant effect on the average increase in seedling height, as the highest interaction was recorded when treating the extract of the hornwort at a concentration of (8 g L^{-1}) and thiamine at a concentration of (150 mg L^{-1}), with the highest

interaction of (36.887 cm^2), while the control treatment for both factors recorded the lowest interaction of (21.584 cm^2) and an increase percentage of 70.89%. In contrast the results showed no significant interaction between hornwort extract and phenylalanine. As for the interaction between thiamine and phenylalanine, the treatment with thiamine at a concentration of (150 mg L^{-1}) with the phenylalanine treatment at a concentration of (150 mg L^{-1}) gave the highest interaction of (37.410 cm^2), while the lowest interaction of (21.097 cm^2) in the control treatment for both factors with an increase percentage of 77.32%. In addition to the presence of a significant three-way interaction between the factors on the average increase of this trait, the extract of the champagne at a concentration of (8 g L^{-1}), thiamine at a concentration of (150 mg L^{-1}), and phenylalanine at a concentration of (150 mg L^{-1}) achieved the highest interaction of (38.884 cm^2). In contrast, the control treatment of these factors recorded the lowest interaction of (19.891 cm^2) and an increase percentage of 95.48%.

Table (4): Effect of hornwort extract, spraying with thiamine and phenylalanine, and their interaction on the average increase in leaf area (cm^2)

Hornwort extract concentrations (g L^{-1})	**Thiamine concentrations (mg L^{-1})**	**Phenylalanine concentrations (mg L^{-1})**			**Hornwort extract × Thiamine**
		0	75	150	
0	0	19.891	21.917	22.944	21.584
	75	26.004	27.015	28.015	27.011
	150	32.576	34.252	35.935	34.255
8	0	22.303	24.004	25.020	23.776
	75	27.066	29.088	30.771	28.975
	150	35.345	36.432	38.884	36.887
L.S.D $_{0.05}$		0.5890			0.3400
Means (Phenylalanine)		27.197	28.784	30.262	**Means (Hornwort extract)**
L.S.D $_{0.05}$		0.2404			
Hornwort extract × Phenylalanine					
0		26.157	27.728	28.965	27.616
8		28.238	29.238	31.238	29.879
L.S.D $_{0.05}$		N.S			0.1963
Thiamine × Phenylalanine					Means (Thiamine)
0		21.097	22.960	23.982	22.680
75		26.535	28.051	29.393	27.993
150		33.960	35.342	37.410	35.571
L.S.D $_{0.05}$		0.4165			0.2404

Based on the results presented (Tables 1, 2, 3, and 4), which included vegetative growth indicators such as plant height, number of leaves, leaf area, and stem diameter, respectively, it is evident that hornwort extract (*Ceratophyllum demersum*) had a

significant effect on these parameters, leading to a notable increase in all traits. This effect can be attributed to the extract's content of essential macronutrients such as nitrogen, potassium, and phosphorus. Nitrogen is considered one of the key elements required for plant growth and development, as it is a fundamental component of chlorophyll and proteins. The significance of chlorophyll lies in its role in determining and enhancing the rate of photosynthesis [15].

Nitrogen is considered one of the main constituents of organic compounds such as amino acids, nucleic acids, proteins, and the chlorophyll structure, all of which positively influence plant growth [16]. hornwort extract (*Ceratophyllum demersum*) also contains phosphorus, which plays a major and essential role in the formation of nucleic acids, amino acids, and enzyme cofactors that are involved in respiration and photosynthesis processes. This, in turn, reflects on the accumulation of organic compounds such as carbohydrates, proteins, vitamins, and other organic substances that are successfully synthesized from raw materials, especially water and carbon dioxide, by the plant. As a result, this leads to an increase in leaf number, plant height, and leaf area. In addition, the positive effect of hornwort extract on the vegetative growth parameters mentioned can also be attributed to its potassium content, which plays a major role as a stimulator and regulator of several vital processes. These include the biosynthesis of proteins, sugar transport, and the metabolism of carbon and nitrogen [17]. Potassium also contributes to the translocation of the products of photosynthesis to storage sites, thereby enhancing vegetative growth traits [18]. Furthermore, the presence of plant growth regulators and amino acids in hornwort extract contributes to the improvement of the plant's physiological and nutritional status. These substances, along with endogenous hormones within the plant, exert their physiological roles, thereby enhancing cell division in plants [19]. The extract also contains indole-3-acetic acid (IAA), which plays an active role in stimulating cell enlargement and elongation, as well as activating the production of amino acids and nucleic acids, including RNA. IAA contributes to the plasticity and elasticity of cell walls, increases cell volume, and facilitates water and nutrient uptake, all of which positively impact vegetative growth, moreover, the significant positive effect of hornwort extract on vegetative growth indicators can also be attributed to its content of various amino acids, including glutamic acid, valine, tryptophan, serine, and threonine, among others, which are crucial in enhancing photosynthetic activity [20]. Among the extract's components is vitamin C, which plays a vital role in cell division and elongation [21].

Among the components of hornwort extract are plant growth regulators, including gibberellins and auxins. Auxins function by increasing cell wall plasticity and elasticity, which leads to enhanced cell wall elongation. Additionally, auxins contribute to improving the efficiency of photosynthesis by stimulating the activity of enzymes and pigments involved in the photosynthetic process. The extract also contains auxins that activate genes responsible for cell division, thereby influencing plant growth and improving its vegetative traits, gibberellins, on the other hand, play a central role in promoting plant height by stimulating cell elongation in the sub-apical meristematic

region. Moreover, hornwort extract contains cytokinins, which promote cell division and elongation, as well as the initiation of leaf primordia [22,23].

The results also show that vegetative growth indicators increased with increasing concentrations of the amino acid phenylalanine. The primary reason is due to the role of phenylalanine in the biosynthesis of plant growth hormones, including gibberellins [24]. Studies have also shown that foliar spraying of the amino acid phenylalanine plays a role in improving plant growth during the vegetative and flowering periods [25]. Phenylalanine also plays a fundamental and important role in promoting plant growth through its role as a suitable substitute for nitrogen, which is important in building proteins and enzymes important for plant growth [26]. This is consistent with the findings of [27], in his study on basil plants, and is consistent with the results of [10], in his study to investigate the effect of phenylalanine on Iris plants. When spraying the plant with thiamine, a significant effect was observed on the leaf area. This is attributed to the role of thiamine in increasing the level of cytokinins and gibberellins present inside the plant and the growth factors that affect the meristem and enhance plant growth, in addition, it stimulates plant cells and their division [28]. This leads to an increase in the leaf area of the treated plants. This is consistent with [29], which demonstrated that thiamine foliar application had a significant effect on the growth and flowering of Zinnia plants.

The study's findings clearly showed that the combination of using hornwort extract at a concentration of 8 g L^{-1}, along with foliar spraying of phenylalanine at 150 mg L^{-1} and thiamine at 150 mg L^{-1}, had a significant positive impact on the vegetative traits observed. This included notable improvements in seedling height, stem diameter, leaf count, and leaf area. These enhancements can likely be attributed to the combined and supportive effects of all three components. The hornwort extract is known for being rich in organic compounds, amino acids, and growth-promoting substances, which probably helped with better nutrient absorption, thus aiding overall plant growth [19]. Phenylalanine, an essential aromatic amino acid, serves as a crucial building block in the production of phenolic compounds and plant hormones like auxins, which help stimulate vegetative growth [30]. On the other hand, thiamine (vitamin B1) is essential for cellular metabolism and energy production, encouraging cell division and the development of new tissues [31]. Together, this synergistic interaction boosted physiological and biosynthetic efficiency, leading to improved seedling vigor and increased vegetative biomass.

It is clear from the above that the use of Champlain extract, especially at a concentration of (8 g L^{-1}), led to a significant improvement in the vegetative traits of Damask rose seedlings. It was also found that spraying with thiamine and phenylalanine, each separately, had a significant effect, especially at the highest concentration (150 mg L^{-1}), indicating the role of these compounds in stimulating plant growth. It was also observed that the interactions between the three factors had the highest effect, indicating the existence of a complementary relationship between these treatments in improving the vegetative growth of seedlings.

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