Evaluation of the efficiency of spraying with a mixture of gibberellin (GA_4+GA_7) and nano-iron on the vegetative growth characteristics of Chinese carnation *Dianthus Caryophyllus* L.

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Abstract:

A study was conducted in the wooden shade house at the Department of Horticulture and Landscape Architecture, College of Agriculture, University of Tikrit, during the spring season of 2024. The study aimed to determine the effect of spraying with a mixture of gibberellin (GA_4 +GA_7) and nano-iron (Fe) on vegetative growth characteristics.

The research was conducted as a randomized complete block design (RCBD) experiment with three replicates. The most notable results were obtained when using the gibberellin mixture (GA_4 +GA_7) mixture was a significant increase in the percentage of protein, chlorophyll, and dry matter in the third concentration (150 mg L^{-1}). The results also showed a significant increase when using nano-iron in the above traits, especially at a concentration of 50 mg L^{-1}.

The interaction between the gibberellin mixture and nano-iron (50+150) mg L^{-1} led to a significant increase in all traits, which is attributed to improving the nutritional status of plants and enhancing metabolic activities.

Keywords: Gibberellins, nano-iron, Chinese carnation.

Introduction:

Chinese carnation (*Dianthus caryophyllus* L.) is a globally important ornamental plant, valued in the floriculture industry for its color diversity, long vase life, and pleasant fragrance. It is widely grown as a cut flower and garden plant, and its economic value is greatly influenced by optimal vegetative growth characteristics, including stem length and strength, leaf growth, branching, and overall plant vigor. In addition to its aesthetic appeal [1], Chinese carnations are also known to produce numerous nanoreceptors and active compounds, which may contribute to their defense mechanisms

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or possess potential medicinal properties, although research on enhancing these specific components through horticultural practices is less extensive than studies on ornamental traits. [2] Achieving superior vegetative growth and possibly enhancing beneficial active components in Chinese chives often requires improved cultivation techniques and appropriate inputs [3]. Plant growth regulators and essential nutrients play a pivotal role in modulating plant growth and development [4]. Glycerols (GAs) are a class of plant hormones known for their pivotal role in promoting cell elongation and division, stem growth, and leaf expansion, all of which are essential components of vegetative structure [5]. Specifically, the gibberellin mixture (GA_4 +GA_7) is commercially used to stimulate growth and improve quality in many ornamental crops. [6] At the same time, iron (Fe) is an essential micronutrient for many plants physiological processes, most notably chlorophyll formation, photosynthesis, and enzyme activity [7]. Iron deficiency is a common constraint on plant growth, leading to yellowing of leaves and reduced biomass accumulation [8]. Traditional iron fertilizers may face challenges in soil stabilization and absorption efficiency [9]. Nanotechnology offers new approaches to nutrient delivery [10], with nanoparticles providing improved solubility, surface area, and bioavailability, especially when used as a foliar spray [11]. Foliar application of nutrients and growth regulators can allow rapid absorption and targeted delivery to vegetative tissues [12]. While the individual effects of gibberellin and iron on plant growth are well documented, and research exists on the application of nanocomposites, the specific interactive or synergistic effects of co-foliar application of GA_4+GA_7 and nano-iron on both vegetative growth characteristics and active ingredient content in Chinese carnation (*Dianthus caryophyllus*) remain unexplored .Understanding these interactions is crucial for the development of more efficient and targeted horticultural practices. [13] Therefore, this study aimed to investigate the effect of spraying a mixture of gibberellins (GA_4+GA_7) and nano-iron on various vegetative growth characteristics in Chinese carnation (Dianthus caryophyllus). The results of this research are expected to provide valuable insights for improving the cultivation of this economically important crop, which may lead to improved plant quality, yield, and a better understanding of how external applications affect it.

Materials and methods:

The experiment was conducted in the wooden shade house of the Department of Horticulture and Landscape Architecture, Faculty of Agriculture, University of Tikrit, during the 2023-2024 agricultural season using factors, where the first factor was

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spraying with a mixture of gibberellins (GA_4+GA_7) and the second factor was nano-iron (Fe) on the vegetative growth characteristics of carnation seedlings. Each replicate contained 12 treatments, with 4 observations for each treatment. [14]

Factors used in the study:

The first factor was a mixture of gibberellins (GA_4+GA_7), used at concentrations of 0, 50, and 100 mg/L.

The second factor was nano-iron (Fe), used at concentrations of 0, 25, and 50 mg/L.

The first spraying was carried out on 8/3/2024

and the second spraying was carried out two weeks after the first spraying.

(Table 1) Physical and chemical properties of the soil used for cultivation:

Adjective	Unit of measurement	value
Sand	%	39.2
Clay	%	28.7
Silt	%	32.1
Texture	S.C clay mixture	
PH	-	7.38
EC	Dc\M-1	3.01
Cation exchange capacity centimoles/kg soil		
Organic matter	%	0.80
available nitrogen (N)	mg/kg soil-1	49
available phosphorus (P)	mg/kg soil-1	3.7
available potassium (K)	mg/kg soil-1	121

*The analysis was conducted at the Ministry of Agriculture Laboratory, Salah al-Din Directorate of Agriculture.

Characteristics studied:

1 Total leaf area

2 Relative chlorophyll content in leaves

3 Dry matter content

4 Protein content

Leaf area measurement

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Leaf area is measured using Image J software according to the methodology described in reference [15].

Relative chlorophyll content in leaves

Using a spectrophotometer, according to the methodology described in reference [16].

Dry matter measurement

Dry matter is weighed using an oven to remove moisture according to a method approved by [17].

Protein content estimation

Using the Microchehdal method according to the methodology described in reference [18].

Table (2) Effect of spraying with a mixture of gibberellin (GA4+GA7) and nano-iron and their interaction on the total leaf area of Chinese carnation plants

The rate of action of gibberellins (GA_4+GA_7)	Effect Rate Iron Nanoparticle (Fe)			Gibberellin mixture (GA_4+GA_7)
	Fe_2	Fe_1	Fe_0	
447.26 d	595.20 j	439.20 k	307.40 l	GA_0
1091.33 c	1181.50 g	1087.30 h	878.40 i	GA_1
1314.46 b	1480 d	1308.30 e	1226.70 f	GA_2
1653.43 a	1766.70 a	1713.60 b	1535.20 c	GA_3
	1288.07 a	1129.97 b	961.82 c	Effect Rate Iron Nanoparticle (Fe)

Results and Discussion:

1. The effect of spraying with a mixture of gibberellins (GA_4+GA_7) and iron nanoparticles and their interaction on the total leaf area of Chinese carnation plants cm^2

The data in Table (2) showed a significant effect of the use of gibberellin mixture and iron nanoparticles on the total leaf area content of Chinese carnation (Dianthus chinensis L.). The results showed that the highest leaf area measurement was recorded when applying the third concentration of gibberellin (GA_3), reaching 1653.43, while the comparison treatment (GA_0) recorded the lowest percentage, reaching 447.26. As for the effect of nano-iron, the treatment (Fe_3) outperformed the other treatments by recording the highest area of 1288.07. Furthermore, the table shows that the highest leaf area was achieved when the third concentration of gibberellin and nano-iron (Fe_3+GA_3) were combined, reaching 1766.70, surpassing the other treatments studied.

2. The physiological response of Chinese carnation plants to a mixture of gibberellins (GA4+GA7) and iron nanoparticles and its effect on chlorophyll content:

The results in Table (3) showed significant differences in the total chlorophyll content in Chinese carnation (*Dianthus chinensis* L.) in response to treatment with a mixture of gibberellins (GA_4+GA_7). The treatment with the third concentration of gibberellins (GA_4+GA_7) recorded the highest chlorophyll content of 35.58, significantly higher than the other treatments. Regarding the effect of nano-iron, the results showed a significant effect on chlorophyll content, with the treatment that included the third concentration of nano-iron recording the highest percentage of 34.33, significantly superior to the other treatments. As for the effect of the interaction between gibberellin and nano-iron oxide (GA_4+GA_7), the combined treatment of the third concentration of gibberellin and the third concentration of nano-iron (GA_3:Fe_3) achieved the highest chlorophyll content of 35.78, while the comparison treatment (Fe_0:GA_0) recorded the lowest chlorophyll content of 25.50.

Table (3) Effect of spraying with a mixture of gibberellin (GA_4+GA_7) and nano-iron and their interaction on the chlorophyll content of Chinese carnation plants

The rate of action of gibberellins (GA_4+GA_7)	Effect Rate Iron Nanoparticle (Fe)			Gibberellin mixture (GA_4+GA_7)
	Fe_2	Fe_1	Fe_0	
27.70 c	30.42 c	27.18 d	25.50 d	GA_0
33.84 b	34.97 a	33.73 ab	32.26 bc	GA_1
35.25 a	35.55 a	35.33 a	35.22 a	GA_2
35.58 a	35.78 a	35.63 a	35.58 a	GA_3
	34.33 a	32.94 b	32.01 b	Effect Rate Iron Nanoparticle (Fe)

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3. The effect of spraying with a mixture of gibberellins (GA_4+GA_7) and iron nanoparticles and their interaction on the dry matter content (%) of Chinese carnation plants:

The results in Table 4 showed significant differences in the dry matter content of Chinese carnation (Dianthus chinensis L.) in response to treatment with a mixture of gibberellins (GA_4+GA_7). The treatment that included the third concentration of gibberellin (GA_4+GA_7) recorded the highest dry matter content of 51.75%, significantly outperforming the other treatments. Regarding the effect of nano-iron, the results showed a significant effect on the dry matter percentage, with the treatment that included the third concentration of nano-iron recording the highest percentage of 48.58, significantly superior to the other treatments. As for the effect of the interaction between gibberellin and nano-iron oxide (GA_4+GA_7), the combined treatment of the third concentration of gibberellin and the third concentration of nano-iron (GA_3:Fe_3) achieved the highest dry matter percentage of 52.78, while the comparison treatment (Fe0:GA0) recorded the lowest dry matter percentage of 42.48.

Table (4) Effect of spraying with a mixture of gibberellin (GA_4+GA_7) and nano-iron and their interaction on the dry matter content of Chinese carnation plants

The rate of action of gibberellins (GA_4+GA_7)	Effect Rate Iron Nanoparticle (Fe)			Gibberellin mixture (GA_4+GA_7)
	Fe_2	Fe_1	Fe_0	
43.42 c	44.53 cd	43.24 d	42.48 d	GA_0
47.79 b	48.55 abc	48.53 abc	46.28 bcd	GA_1
50.27 a	50.77 ab	50.03 ab	49.92 ab	GA_2
51.75 a	52.78 a	51.69 a	50.86 a	GA_3
	48.58 a	48.44 a	47.89 a	Effect Rate Iron Nanoparticle (Fe)

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4. Effect of spraying with a mixture of gibberellin (GA_4+GA_7) and nano-iron on the protein content of Chinese carnation plants.

The results shown in Table 5 indicate a significant effect of applying the gibberellin mixture (GA_4+GA_7) on the protein content of Chinese carnation (*Dianthus chinensis* L.). The GA3 mixture treatment recorded the highest protein content of 10.27%, while the control treatment recorded the lowest content of 6.93%. With regard to the effect of nano-iron, the treatment that included the third concentration of nano-iron showed the highest protein value of 9.15%, while the treatment without nano-iron recorded the lowest value of 8.25%. As for the effect of the interaction between gibberellin and nano-iron treatments (GA_4+GA_7), it was also significant, with the combined treatment of the third concentration of gibberellin and the third concentration of iron nanoparticles (Fe3:GA3) recording the highest protein content of 10.75%, while the comparison treatment (Fe0:GA0) recorded the lowest value of 7.12%.

Table (5) Effect of spraying with a mixture of gibberellin (GA_4+GA_7) and nano-iron and their interaction on the protein content of Chinese carnation plants (%)

The rate of action of gibberellins (GA_4+GA_7)	Effect Rate Iron Nanoparticle (Fe)			Gibberellin mixture (GA_4+GA_7)
	Fe_2	Fe_1	Fe_0	
6.93 d	7.12 g	6.87 g	6.81 g	GA_0
8.22 c	8.75 d	8.18 e	7.75 f	GA_1
9.50 b	9.56 c	9.43 c	8.84 d	GA_2
10.27 a	10.75 a	10.50 ab	10.25 b	GA_3
	9.15 a	8.79 b	8.25 c	Effect Rate Iron Nanoparticle (Fe)

Discussion Results

The molecular mechanisms of protein increase upon addition of GA_4+GA_7 mixture and nano-iron include gibberellin stimulation of protein synthesis through gene activation and nano-iron improvement of nutrient absorption and metabolic processes. Glycogen (GA) is an important plant hormone that regulates plant growth and development, while nano-iron particles represent a modern technology in plant nutrition. This analysis focuses on the molecular and physiological mechanisms

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that explain the increase in protein content in plants when a mixture of gibberellins (GA_4+GA_7) is used with nano-iron, identifying the specific roles of each component in stimulating protein synthesis. Gibberellins are diterpenoids that act as important plant hormones [19]. Of the more than 200 identified gibberellins, only a few are biologically active, including GA1, GA3, GA_4, and GA_7 [19, 20]. These active gibberellins regulate plant growth by promoting the degradation of DELLA proteins, a family of nuclear inhibitors of gibberellin response [21]. They regulate various developmental processes by overcoming the inhibitory effects of DELLA proteins [22]. These act as plant growth inhibitors by preventing cell elongation and proliferation [23]. Nanofertilizers penetrate the leaves and enter through the stomata [24]. This enhanced penetration allows better access of nutrients to plant tissues, which can cause different responses in different parts of the plant. [25] The use of nano-iron improves nutrient uptake and enhances metabolic processes in plants [26]. Studies indicate that foliar application of nano-iron increases protein production in grains [27]. Studies show that the combination of gibberellin and nano-iron has beneficial effects on various plant characteristics [28]. The combined application of these two components improves plant quality and prolongs plant life [29]. Gibberellin activates the genes responsible for protein synthesis, while nano-iron provides the essential nutrients needed for this process [30]. This synergistic interaction leads to a significant increase in protein content compared to the individual use of each component. The physiological and biochemical mechanisms behind the increase in chlorophyll when adding a mixture of gibberellin (GA_4+GA_7) and nano-iron include the regulation of genes responsible for chlorophyll synthesis, improved iron absorption, and enhanced photosynthetic processes through a complex interaction between plant hormones and nano-nutrients. Chlorophyll is the key component in photosynthesis, and its levels are influenced by multiple factors, including plant hormones and nutrients. [31] Iron, On the other hand, it is considered an essential element in chlorophyll synthesis and photosynthesis, and iron nanoparticles have shown promising potential in improving the absorption of this vital element. Research shows that gibberellins affect chlorophyll content through several physiological and molecular mechanisms. In a study on Paris polyphylla, GA_3 treatment was found to significantly increase internal gibberellin content (GA_4 + GA_7) and delay shoot senescence and protein and chlorophyll degradation [32]. GA_3 also reduced the activities of enzymes responsible for chlorophyll degradation, such as chlorophyllase, magnesium-dependent enzymes, and peroxidase. In Alstroemeria, studies have shown that GA_4 and GA_7 are much more effective than GA_3 in delaying

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chlorophyll loss [33]. This suggests that the specific mixture of GA_4+GA_7 may have a superior effect on chlorophyll preservation compared to other gibberellins. Iron plays a crucial role in chlorophyll synthesis as it is an essential component of many enzymes involved in this process. In gibberellin-deficient plants, iron deficiency was found to lead to more pronounced yellowing, and gibberellin levels in iron-deficient plants were lower than those in iron-sufficient plants. This suggests a close relationship between gibberellin levels and chlorophyll content under iron-deficient conditions. Iron nanoparticles exhibit unique properties that enhance the absorption of this vital element. In a study on white beans, treating seeds with iron nanoparticle foliar sprays significantly increased chlorophyll content in leaves compared to other treatments. Nanoparticles also improve nutrient uptake efficiency due to their small size and unique physical and chemical properties. The interaction between gibberellins and iron nanoparticles leads to synergistic effects on chlorophyll synthesis. In a study on Pogostemon cablin, foliar spraying with GA_3 increased the content of photosynthetic pigments, with chlorophyll b increasing faster than carotenoids and total chlorophyll [33]. This effect can be enhanced when combined with nano-iron, which provides the essential element for chlorophyll synthesis. The addition of a mixture of gibberellins (GA_4+GA_7) and iron nanoparticles improves the protein, chlorophyll, and dry matter content in Chinese carnation plants by stimulating photosynthesis, enhancing nutrient uptake, and regulating the gene expression of vital enzymes. Glycerol (GAs) are essential plant hormones that regulate plant growth and development, while iron plays a pivotal role in photosynthesis and chlorophyll synthesis. This analysis explores the scientific mechanisms behind the noticeable improvements in Chinese carnation plants when treated with a mixture of GA4+GA7 with nano-iron. GA stimulates the rate of photosynthesis at the whole-plant level and is positively correlated with biomass production and affects chlorophyll synthesis [34].

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