



EFFECT OF BRASSINOLIDE HORMONE ON SOME MORPHOLOGICAL AND CHEMICAL CHARACTERISTICS AND ACTIVE COMPOUNDS OF *ERUCA SATIVA* MILL PLANT

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ABSTRACT

A pots experiment was conducted in the greenhouse of the Department of Biology, College of Education for Pure Sciences Ibn Al-Haitham, University of Baghdad, for the growing season 2022-2023 to find out the effect of the concentrations of the growth regulator brassinolide on some morphological characteristics, the concentrations of macrolelements (nitrogen, potassium, phosphorus) and active compounds of the plant *Eruca sativa* Mill. The study was designed based on the Complete Randomized Design (CRD) and three replicates of 12 pots each, so that the number of pots was 36. Four concentrations of the growth regulator brassinolide (0, 1.5, 2.5, and 3.5) mg L⁻¹ were used. The results showed that spraying with the brassinolide growth regulator increased all the average characteristics studied, such as plant height, total chlorophyll index by Soil Plant Analysis Development (SPAD) in the leaves, nitrogen concentration and potassium concentration, especially at a concentration of 2.5 mg L⁻¹, while it had no significant effect on phosphorus concentration. The results also displayed a significant effect in all active compounds of the plant, especially at a concentration of 2.5 mg L⁻¹, while the results of the examination and chromatographic diagnosis using High Performance Liquid Chromatography technique gave the diagnosis of nine active compounds in the *E. sativa* plant, namely: Progoitrin, Glucoalyssin, Gluconapin, 4-OH-Glucobrassicin, Glucoerucin, Glucobrassicin, Methoxybrassicin, Gluconasturtin and Neoglucobrassicin.

Keywords: Brassinolide, *Eruca sativa*, Morphological, Chlorophyll, Active compounds.

* This article is taken from the doctoral dissertation of the first researcher.



The *Eruca sativa* plant belongs to the Brassicaceae family and returns to the plants angiosperms and is one of the dicotyledons; order Brassicales and *E. sativa* is one of the vegetable crops of nutritional, medical, and economic importance (Blamey & Grey-Wilson, 1989). Studies have indicated that *Eruca sativa* contains glycosides, essential oils, alkaloids, terpenoids, tannins, phenolic compounds, and flavonoids (Abdul-Jalil, 2016).

The brassinolide hormone is a steroid plant compound is one of the latest plant hormones discovered. At the present belongs to the family of that is the brassinosteroid. Brassinosteroids were first extracted in the Brassicaceae family, previously thought to be found only in the Animalia kingdom (Islam, 2014; Hayat & Ahmad, 2011). Recent studies have proven that brassinolide plays an important role in the growth and development of plants (Müssig, 2005) increasing morphological and chemical properties and the active ingredient in plants (Al-Hassany & Al Shammari, 2019).

It also plays a role in increasing plant fertility and accelerating the flowering process (Khashan & Al-Hilfy, 2020). Brassinosteroids are a group of naturally occurring steroid lactones found in many plant species (Pullman *et al.*, 2003).

Due to the importance of watercress for medicinal, therapeutic, and nutritional purposes and the lack of studies on this factor, the study was conducted with the aim of determining the effect of spraying the growth regulator brassinolide on the improvement of growth characteristics and some chemical properties of the *E. sativa* plant.

MATERIALS AND METHODS

A pot experiment was conducted in the glasshouse of the Department of Biology, College of Education for Pure Sciences Ibn Al-Haitham, University of Baghdad for the growing season 2022-2023, and the study was designed according to CRD with three replicates, each replicate 12 pots, so the number of pots were 36.

All pots were fertilized with neutral nitrogen, phosphorus, and potassium (NPK) fertilizer before cultivation, seeds were sown on 30/10/2022 after sieving and testing germination rate (98%) and emergence started 5 days after cultivation on 3/11/2022. Thinning was performed 15 days after germination and all plant treatment measures such as irrigation, hoeing, and weeding were performed as needed until the end of the trial. The growth regulator brassinolide was applied at concentrations of (0, 1.5, 2.5, and 3.5) mg L⁻¹.



Table (1): Some chemical and physical characteristics of the experiment soil before cultivation.

Properties	Value	Unit
Soil texture	clay-silt	---
Sand	233	g kg ⁻¹ soil
Silt	470	g kg ⁻¹ soil
Clay	320	g kg ⁻¹ soil
pH	6.8	---
Electrical conductivity (EC)	2.60	Desimens m ⁻¹
Available nitrogen	0.69	mg kg ⁻¹ soil
Available phosphorus	20.50	mg kg ⁻¹ soil
Potassium	360	mg kg ⁻¹ soil

The soil was prepared before cultivation by mixing, leveling and air drying. It was then sieved through a sieve with a pore diameter of 2 mm and filled into plastic pots with 5 kg of soil per pot. The samples were then digested and dried in an electric oven. Each sample was crushed with an electric grinder and 0.2 g of it was taken and digested according to the method proposed by **Cresser & Parsons (1979)** and the following characteristics were analyzed:

1. Plant height (cm): The plant height of three random plants per pot was measured from the soil surface to the highest point of the plant by means of a ruler.
2. The total chlorophyll index SPAD in the leaves of three vegetative growth plants per pot was randomly measured by a Japanese origin SPAD device.
3. Determination of total nitrogen content in plants (mg plant⁻¹): Estimated according to the Kjeldahl method **Jackson (2005)**.
4. Determination of total phosphorus content in plants (mg plant⁻¹): Estimated according to method **Sparks et al. (2020)**.
5. Determination of total potassium content in plants (mg plant⁻¹): Estimated according to method **Chapman & Pratt (1961)**.
6. Diagnosis of active compounds using HPLC :Diagnosis is made by **Chen et al. (1993)** method.



STATISTICAL ANALYSIS

The statistical program SAS (2012) was utilized in data examination to concentrate on the impact of various variables on the studied characteristics, and the significant differences between the mean were compared with the test of the least significant difference (LSD) at a probability levels of 0.05 (SAS, 2012).

RESULTS AND DISCUSSION

Plant height (cm):

The results of Table (2) show the effect of brassinolide on plant height and the presence of significant differences in mean plant height by the effect of different concentrations of brassinolide, with the concentration of 2.5 mg L⁻¹ having the highest mean value for this trait of 52. The reason for this is the role of brassinolide acting on cell elongation and division and the elasticity of the cell wall, which has a positive effect on the average plant height, as it plays a role in genetic coding against plant stunting (Islam, 2014). These results are consistent with the results of Al-Aubadi *et al.* (2011); Al-Halfi (2018); Sura & Al-Hilfy (2022).

Total chlorophyll index SPAD:

The results showed the effect of brassinolide on the total chlorophyll index SPAD of significant differences in the mean content of total chlorophyll in leaves, where the concentration of 2.5 mg L⁻¹ presented the highest mean value for this trait, namely 39.35 SPAD compared to the control treatment (Table 2).

The increase in total chlorophyll index SPAD in the leaves of the *E. sativa* plant due to the addition of brassinolide can be attributed to the role of brassinolide in inhibiting the enzyme chlorophyllase, which is responsible for the degradation of chlorophyll, leading to an increase in chlorophyll in the leaves (Fariduddin *et al.*, 2003) and this result is in agreement with the findings of Kandil *et al.* (2007); Al-Jumaily & Al-Esawi (2016); Al-Shimary & Al-Jboury (2017a); Amarasinghe *et al.* (2022).

Nitrogen concentration in the vegetative system of the plant (%):

The results of Table 2 show the effect of brassinolide on nitrogen concentration in % and there are significant differences in the mean nitrogen concentration in *E. sativa* plant with the effect of different concentrations of brassinolide. The concentration of 2.5 mg L⁻¹ gave the highest value of 4.73% compared to not spraying with brassinolide. It can be attributed to the role of brassinolide in increasing root growth (Al-Halfi, 2018), which improves plant efficiency and capability to absorb nutrients, including nitrogen (Bera *et al.*, 2008). The reason is also due to the role of brassinolide, which encodes the genes responsible for increasing the production of gibberellin in the root tips and increases the absorption of elements, since brassinolide is a steroid compound and steroids have the ability to bind to polyamines and increase the osmotic cell in favor of the entry of water and nutrients, as well



as the compound glucobrassicin is one of the brassinosteroids and this compound is metabolized to auxin, which increases the growth of the root system and then increases the absorption capacity of elements, including nitrogen (Hayat & Ahmad, 2011; Ross & Quittenden, 2016) and is consistent with the results of (Al-Shimary & Al-Jboury, 2017b) as well as agree with (Al-Halfi, 2018; Al-Saadi & Al-Muntafji, 2016, Khashan & Al Hilfy, 2019).

Phosphorus concentration in the vegetative system of the plant (%):

The results of Table 2 showed that there were no significant differences in the mean phosphorus concentration in the *E.sativa* plant with the effect of different concentrations of brassinolide.

Potassium concentration in the vegetative system of the plant (%):

In the results, the effect of brassinolide on potassium concentration was found that there were significant differences in the mean concentration of potassium in *E.sativa* plant due to the effect of different concentrations of brassinolide, as the mean property increased with increasing concentration from 0 to 3.5 mg L⁻¹, as the concentration of 2.5 mg L⁻¹ gave the highest value of 4.34% related to the lack of spraying with brassinolide (Table 2).

The superiority of the sprayed plants with a concentration of 2.5 mg L⁻¹ is due to the role of brassinolide in increasing the length of the root and its soft and dry weight (Chapman & Pratt 1961), which increases the efficiency of the plant in the absorption of nutrients, including potassium, and positively reflects the work of brassinolide in increasing the concentration of potassium in the vegetative system (Fariduddin et al., 2003). This is also due to the role of brassinolide, which encodes the genes responsible for increasing the production of gibberellin in the root tips and increasing the uptake of elements. Brassinolide is a steroid compound, and steroids have the ability to bind to polyamines and increase the osmolality of cells in favor of the entry of water and nutrients.

Table (2): Effect of brassinolide on plant high, total chlorophyll index SPAD, nitrogen, phosphorus and potassium concentrations.

Brassinolide mg L ⁻¹	Plant height cm	Total chlorophyll index SPAD	Nitrogen concentrat ion (%)	Phosphoru s concentrat ion (%)	Potassium concentrat ion (%)
0	31.67	36.07	3.23	0.417	3.10
1.5	49.00	38.07	4.23	0.453	3.85
2.5	52.33	39.35	4.73	0.497	4.34
3.5	38.67	37.80	3.93	0.443	3.42
LSD p≤0.05	5.32	2.81	0.72	NS	0.65



Effect of brassinolide on some active compounds in watercress plant

The results of Table 3 showed that the watercress plant contains nine active compounds with the effect of different concentrations of brassinolide, namely: (Progoitrin, Glucoalyssin, Gluconapin, 4-OH-Glucobrassicin, Glucoerucin, Glucobrassicin, 4.Methoxybrassicin, Gluconasturtin, Neoglucobrassicin).

The concentration of 2.5 mg L^{-1} presented the highest value of 15.87%, 12.42%, 4.94%, 5.45%, 20.33%, 19.27%, 6.47%, 11.29%, and 8.29% compared to no spraying with brassinolide, so the plants sprayed with concentration above 2.5 mg L^{-1} increased the concentration of the active ingredients. The reason for this, as already mentioned, is the role of brassinolide in increasing the vegetative growth of the plant, resulting in primary and by-products of metabolism represented by medically active compounds in volatile oil and may return to the role of the hormone, which is mainly a derivative of secondary metabolism in medicinal plants and participates as a product of oil compounds from the metabolism of sterols (Dutta, 2003). The hormone also has a role in increasing vegetative and reproductive growth through increasing cell divisions and coding the synthesis of nucleic acids and cyclines, which are phosphorylated proteins that have high activity in increasing cell division, especially the enzyme Cyclin Dependent Kinase (CDK) (Hayat & Ahmad, 2011) and agree with the results Al-Halfi (2018).

Table (3): Effect of brassinolide on traction plant active compounds.

Brassinolide mg L ⁻¹	Progoitrin (%)	Glucosyl sin (%)	Gluconapi n (%)	4-OH- Glucobras sacin (%)	Glucocer in (%)
0	7.41	5.28	3.25	3.99	10.26
1.5	12.83	11.79	4.56	5.11	18.71
2.5	15.87	12.42	4.94	5.45	20.33
3.5	10.83	11.08	3.92	4.45	11.79
LSD α 0.05	3.76	2.59	0.71	0.97	3.86
Brassinolide mg L ⁻¹	Glucobrassicin %	4.Methoxybrassic in %	Gluconasturtin %	Neoglucobrassicin %	
0	11.38	4.30	5.54	4.48	
1.5	17.92	6.22	9.29	5.00	
2.5	19.27	6.47	11.29	8.29	
3.5	13.59	5.67	6.82	5.11	
LSD α 0.05	2.72	1.22	2.76	1.76	

CONCLUSION

We can conclude that brassinolide has an important role in stimulating the growth of the *Eruca sativa* plant. Spraying brassinolide has shown a positive effect on *Eruca sativa* growth, improving morphological and chemical qualities with concentrations exceeding 2.5 mg L⁻¹ by providing it the highest values in most of the studied characteristics.

COMPETING INTERESTS:

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