



Effect of Different Concentrations of Brassinolide on Some Growth Parameters of Cucumber Plants (*Cucumis sativus* L.)

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Abstract

This study evaluated the physiological response of local cucumber (*Cucumis sativus* L.) seedlings to varying concentrations of brassinolide under controlled greenhouse conditions. Conducted at the Department of Life Sciences, Al-Mustansiriya University, the experiment utilized a completely randomized design featuring six treatments with seven replications. Plants were treated with foliar sprays of brassinolide at concentrations of 0 (control), 1, 2, 3, 4, and 5 mL/L 32 days after planting. Evaluation at 20 days post-spraying revealed significant dose-dependent increases across all measured vegetative parameters. Foliar application of 5 mL/L brassinolide yielded the highest performance, producing an average of 15 leaves per plant, a leaf area of 37 m², a shoot dry weight of 3.4 g, and a root dry weight of 3.1 g. These results demonstrate the efficacy of brassinolide in enhancing early vegetative development and biomass accumulation in cucumber crops.

Keywords: Plant growth regulators (PGRs); Leaf area; Shoot weight; Greenhouse cultivation

Received: July 2026; **Revised:** August 2026; **Accepted:** September 2026; **Published:** October 2026

1. Introduction

Cucumber (*Cucumis sativus*) is a high-value horticultural crop widely cultivated in Iraq and across the globe for its economic and nutritional importance. Optimizing early vegetative development, specifically leaf proliferation, photosynthetic surface expansion, and biomass partitioning between roots and shoots, is vital for maximizing light interception and overall yield potential (Dhall & Sharma, 2014; Słomnicka et al., 2024). Plant growth regulators (PGRs) play a crucial role in modern agriculture by modulating cell division, cell expansion, and metabolic pathways to improve crop vigor and stress resilience (Anwar et al., 2018; Nurrohman et al., 2025). Among endogenous plant hormones, brassinosteroids such as brassinolide represent a distinct class of polyhydroxylated steroidal hormones that are essential for plant growth and development (Clouse & Sasse, 1998; Khripach et al., 2000). Brassinolide regulates diverse physiological processes, including hypocotyl elongation, vascular differentiation, leaf expansion, root morphology, and photosynthetic pigment retention (Bishop & Koncz, 2002; Yang et al., 2019). Despite extensive global research on brassinosteroid signaling, evaluating specific concentration responses on local cultivars under regional greenhouse conditions remains vital for optimizing agronomic practices. This study investigates the impact of different foliar brassinolide concentrations on key vegetative growth parameters (leaf number, leaf area, shoot dry weight, and root dry weight) of a local Iraqi cucumber cultivar.

2. Materials and Methods

2.1 Experimental Site and Plant Material

The experiment was conducted in a greenhouse at the Department of Life Sciences, Al-Mustansiriya University. Soil was prepared using a loamy texture with a neutral pH of 7.2. Seeds of a local cucumber (*Cucumis sativus*) cultivar widely grown across Iraq were used for the experiment.

2.2 Experimental Design and Plant Maintenance

The study comprised six treatments with seven replications (totaling 42 pots, 30 cm in diameter). Seeds were sown directly into the pots, each containing soil. Standard agricultural practices and maintenance were consistently applied to ensure optimal growth conditions.

2.3 Treatment Application

Brassinolide (obtained from Aashi Chemical Mfg-Co-Ltd.) solutions were prepared in the plant physiology laboratory at concentrations of 0 (control), 1, 2, 3, 4, and 5 mL/L. At 32 days after planting, the plants were subjected to foliar spraying with their respective concentration treatments.

2.4 Data Collection and Growth Parameters

Growth parameters were recorded 20 days post-spraying:

1. Number of Leaves per Plant: Counted manually per plant.
2. Leaf Area per Plant (m²): Calculated using the formula:

Leaf Area per Plant = Length \Width

3. Dry Weight Determination: Shoots and roots were harvested separately and dried in an electric oven at 105°C for 42 hours until a constant dry weight was achieved, after which weights were measured using a sensitive balance.

2.5 Statistical Analysis

Data were evaluated using Analysis of Variance (ANOVA), and treatment means were compared using the Least Significant Difference (LSD) test at the $\alpha \leq 0.05$ probability level.

3. Results

The foliar application of brassinolide significantly influenced all evaluated growth parameters of cucumber seedlings 20 days after treatment.

3.1 Leaf Number and Leaf Area

A strong positive correlation was observed between increasing brassinolide concentration and leaf proliferation as well as leaf surface expansion. Control plants (0.0 mL/L) and plants treated with 1 mL/L exhibited the lowest leaf count (5 leaves). Incremental increases in concentration led to significant advancements, reaching a maximum of 15 leaves at 5 mL/L brassinolide, which significantly exceeded the LSD threshold of 3.02.

Leaf area expanded markedly with higher concentration levels. The control treatment produced a mean leaf area of 7.8 m², while application of 5 mL/L brassinolide increased the leaf area to 37 m². The difference between lower concentrations (3 mL/L) and higher concentrations (4 and 5 mL/L) was statistically significant based on the LSD value of 12.8.

Table (1) Effect of different concentrations of Brassinolide acid on the number of leaves and leaf area of cucumber seedlings (20 days after spraying)

Brassinolide concentration	Average of leaves number	Average of leaf area
0.0	5	7.8
1 mL/L	5	8
2 mL/L	8	9.6
3 mL/L	10	10
4 mL/L	12	23
5 mL/L	15	37
LSD	3.02	12.8

3.2 Shoot and Root Dry Weight

Shoot and root biomass accumulation exhibited a progressive increase corresponding to higher brassinolide applications. Shoot dry weight increased from 1.20 g in control plants to 3.4 g at 5 mL/L brassinolide. Treatments of 3 mL/L (2.5 g), 4 mL/L (2.6 g), and 5 mL/L (3.4 g) showed statistically significant improvements over the control (LSD = 0.76).

Root dry weight showed a similar trend, rising from 1.15 g in control plants to 3.1 g in plants treated with 5 mL/L brassinolide. Root weight gain was steady across treatments, with significant increases observed beginning at the 3 mL/L level (1.85 g) relative to the control (LSD = 0.33).

Table (2) Effect of different concentrations of brassinolide on shoots and roots of cucumber seedlings (20 days after spraying)

Brassinolide concentration	Average of shoot weight	Average of root weight
0.0	1.20	1.15
1 ml/L	1.31	1.2
2 ml/L	1.44	1.3
3 ml/L	2.5	1.85
4 ml/L	2.6	2.5
5 ml/L	3.4	3.1
LSD	0.76	0.33

4. Discussion

The observed increase in leaf number, leaf area, shoot weight, and root weight following foliar application of brassinolide aligns directly with established physiological functions of brassinosteroids (Bishop & Koncz, 2002; Clouse & Sasse, 1998). Brassinolide promotes gene expression required for wall-relaxing enzymes (such as xyloglucan endotransglucosylase/hydrolases and expansins), driving cell division and cell wall expansion in young, growing foliage (Duran et al., 2017; Nurrohman et al., 2025). The pronounced expansion in leaf area (from 7.8 m² to 37 m²) increases total light interception, boosting net photosynthetic rate, stomatal conductance, and carbon assimilation (Yang et al., 2019). The concurrent increase in root dry weight (from 1.15 g to 3.1 g) indicates that brassinolide enhances root architecture and root tip development. Brassinosteroid crosstalk with endogenous gibberellins and auxins stimulates root elongation and lateral root formation, improving the root system's capacity to absorb essential nutrients like potassium and nitrogen (Al-Halfi, 2026; Dhall & Sharma, 2014). This enhanced nutrient and water uptake creates a positive feedback loop that supports shoot biomass accumulation (3.4 g at 5 mL/L compared to 1.20 g in the control). Overall, the synchronized enhancement of root and shoot systems demonstrates that higher concentrations of brassinolide (up to 5 mL/L) effectively stimulate vegetative growth performance in local cucumber cultivars under greenhouse conditions.

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