Synergistic Effects of Brassinolide and applied Manvert Soil on Growth and Chemical Content of *Ziziphus spina-christi* Cultivars

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Abstract:
This study evaluated the effects of foliar-applied Brassinolide (0 and 2 mg L^{-1}) and soil-applied Manvert Soil Best (0 and 3.5 mL L^{-1}) on the growth and biochemical composition of three Ziziphus cultivars: Tufahe, Bembawi, and Armuti, during the 2025 growing season at the Horticulture and Landscape Engineering Department, University of Diyala. Seedlings were treated ten times at 14-day intervals. The experiment was conducted as a factorial trial in a Randomized Complete Block Design (RCBD) with three replicates. Results showed that the Pear cultivar exhibited superior performance in leaf number (547 leaves per plant), branch number (49.99 per plant), leaf phosphorus (0.322%), leaf magnesium (185.4 mg L^{-1}), and saponin contents JUJUVOSIDE—A (415.2 mg L^{-1}) and CHRISTININ—C (424.6 mg L^{-1}) compared to Tufahe_and Bembawi. Combined application of Brassinolide and Manvert Soil Best significantly enhanced all measured traits compared to the control, and both two-way and three-way interactions among factors were significant for all parameters.

Keywords: Cultivars, *Ziziphus spina-christi*, Brassinolide, Manvert Soil Best, Saponins

Introduction

Jujube (*Ziziphus spina-christi*) belongs to the family Rhamnaceae and the genus Zizyphus, which includes evergreen trees and shrubs that are either erect or climbing, and only rarely herbaceous in form. Jujube is cultivated for multiple purposes, including fresh consumption, medicinal uses, hedging, and ornamental planting, owing to its high nutritional, economic, and ecological value (1,2). The species Ziziphus comprises several cultivars, among which Bembawi and Malasi are the most prominent, whereas *Ziziphus mauritiana* includes economically important cultivars such as Apple and Olive (3).

In recent years, research has increasingly focused on foliar nutrition due to its effectiveness in enhancing crop productivity and quality by rapidly supplying deficient nutrients, either because of their limited availability in soil or restricted root uptake (4). Plant responses to foliar fertilization depend on fertilizer type, formulation, concentration, application frequency, and the physiological growth stage of the plant (5).

Plant growth regulators (PGRs) are chemical compounds applied at low concentrations to induce significant physiological changes in plant growth, either through stimulation or inhibition (6). Among these regulators, **Brassinolide** plays a vital role, as it is absorbed by plant tissues and transported to its site of action, where it binds to specific receptors and activates secondary signaling

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pathways that regulate cellular activity. Brassinolide belongs to the steroidal hormone group, which is essential for plant growth and developmental processes (7). Soil fertilization remains a fundamental component of modern agricultural production, as it supplies essential mineral nutrients required for optimal crop growth and yield. Even high-yielding cultivars may fail to reach their genetic potential without balanced nutrient management (8). Manvert Soil Best is widely used as a soil conditioner to mitigate salinity stress and improve crop performance. Previous studies emphasize the importance of applying chemical, biological, and physical treatments to seedlings prior to exposure to salinity stress in order to enhance plant tolerance (9). Accordingly, the present study was conducted to evaluate the role of Brassinolide in enhancing the growth performance of jujube cultivars grown under the soil conditions of the University of Diyala, to assess the effectiveness of Manvert Soil Best in alleviating salinity stress, and to identify the cultivar with the highest tolerance and growth capacity under saline soil conditions.

Materials and Methods

The experiment was conducted in the orchard of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Diyala, during the autumn growing season of 2025 to evaluate the effects of foliar application of the plant growth regulator Brassinolide and the salinity stress–alleviating agent Manvert Soil Best on the growth of seedlings of three Ziziphus cultivars, namely Tufahe, Bembawi, and Armuti. Sixty uniform, one-year-old seedlings with similar vegetative growth were planted at a spacing of 6 × 6 m per seedling. The experiment included three factors: cultivars, foliar application of Brassinolide at two levels (0 and 2 mg L^{-1}), and soil application of Manvert Soil Best at two concentrations (0 and 3.5 mL L^{-1}). Seedlings were treated ten times at 14-day intervals throughout the growing season. Brassinolide application started on 28 April 2025, followed by Manvert Soil Best application two days later, with the final treatment applied on 2 September 2025. The experiment was arranged in a randomized complete block design (RCBD) as a three-factor factorial experiment with three replications. Data were statistically analyzed using SAS software, and mean comparisons were performed according to Duncan's multiple range test at a probability level of 0.05.

Studied Traits

- Rate of increase in number of leaves (leaf $plant^{-1}$). determined according to (10)
- Rate of increase in number of main branches (branch $plant^{-1}$).
- Leaf phosphorus concentration (%), determined according to (11).
- Leaf magnesium concentration (%), determined according to (12).
- Leaf saponin content (Jujuvoside-A and Christinin-C), determined according to (13).

Results and Discussion

- Rate of increase in number of leaves

Results in table 1 indicate significant differences among the Tufahe, Bembawi and Armuti cultivars in the rate of increase in leaf number. The pear cultivar significantly outperformed the other cultivars, recording 574.0 leaves $plant^{-1}$, whereas the Bombay cultivar produced the lowest

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leaf number (536.8 leaves plant^{-1}). Foliar application of Brassinolide at 2 mg L^{-1} increased the number of leaves to 639.6 leaves plant^{-1}, followed by the non-sprayed treatment, which produced 468.8 leaves plant^{-1}. Similarly, soil application of Manvert Soil Best at 3.5 mL L^{-1} significantly increased leaf number to 621.0 leaves plant^{-1}, whereas the non-application treatment recorded the lowest value of 490.8 leaves plant^{-1}

The interaction between the Armuti cultivar and foliar application of Brassinolide (2 mg L^{-1}**)** significantly increased leaf number to 675.0 leaves plant^{-1}, whereas the lowest value (473.0 leaves plant^{-1}) was recorded for the interaction between spray number and the Bembawi cultivar. Similarly, the interaction between the Armuti cultivar and soil application of Manvert Soil Best (3.5 mL L^{-1}) produced 620.0 leaves plant^{-1}, while the Bembawi cultivar without addition recorded the lowest mean (445.8 leaves plant^{-1}).The combined treatment of Brassinolide (2 mg L^{-1}) and Manvert Soil Best (3.5 mL L^{-1}) gave the highest leaf number (710.0 leaves plant^{-1}) compared with the control (412.3 leaves plant^{-1}).

Table 1. Effect of Brassinolide Spray and Manvert Soil Best on Leaf Number in Three Ziziphus Cultivars

Cultivars x Manvert Soil Best	Brassinolide		Manvert Soil Best	Cultivars
	2 mg L^{-1}	0 mg L^{-1}		
493.6 bc	531.3 b	456.0 c	0 mL L^{-1}	Tufahe
618.1 a	713.3 a	523.0 b	3.5 mL L^{-1}	
445.8 c	533.3 b	358.3 d	0 mL L^{-1}	Bembawi
615.0 a	706.6 a	523.3 b	3.5 mL L^{-1}	
533.0 b	643.3 a	422.6 cd	0 mL L^{-1}	Armuti
620.0 a	710.0 a	530.0 b	3.5 mL L^{-1}	
Cultivars				
558.4 ab	622.3 b	494.5 c	Tufahe	Cultivars X Brassinolide
536.8 b	600.6 c	473.0 c	Bembawi	
574.0 a	675.0 a	499.1 d	Armuti	
Manvert Soil Best				
490.8 b	569.3 b	412.3 d	0 mL L^{-1}	Manvert Soil Best x Brassinolide
621.0 a	710.0 a	532.1 c	3.5 mL L^{-1}	
	639.6a	468.8 b	Brassinolide	

Means with the same letter are not significantly different at P ≤ 0.05 according to Duncan's Multiple Range Test

- **Rate of increase in number of main branches (branch plant^{-1})**

Table 2 shows that the Armuti cultivar recorded the highest number of main branches (49.99 branches plant^{-1}), whereas the

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Bembawi cultivar produced the lowest value (34.33 branches plant^{-1}). Foliar application of branches to 50.33 branches plant^{-1}, compared with 29.77 branches plant^{-1} in the control.. Application of Manvert Soil Best at 3.5 ml L^{-1} resulted in a significant increase in the number of branches (45.49 branches plant^{-1}), compared with the untreated control, which recorded the lowest value (34.61 branches plant^{-1}).

Interaction between cultivar and Brassinolide showed the highest number of branches in Tufahe (59.33 branches plant^{-1}) and the lowest in Bembawi (23.50 branches plant^{-1}).Interaction between cultivar and Manvert Soil Best showed the highest number of branches in Tufahe (53.83 branches plant^{-1}) and the lowest in Bembawi (27.49 branches Brassinolide at 2 mg L^{-1} significantly increased the number of plant^{-1}),while the interaction between Brassinolide and Manvert Soil Best showed the highest number of branches with Brassinolide at 2 mg L^{-1} combined with Manvert Soil Best at 3.5 ml L^{-1} (57.55 branches plant^{-1}) and the lowest with no spray and no addition (26.11 branches plant^{-1}).The three-way interaction showed the highest number of branches in Tufahe sprayed with Brassinolide at 2 mg L^{-1} and supplemented with Manvert Soil Best at 3.5 ml L^{-1} (63.66 branches plant^{-1}), while the lowest was recorded in Bembawi sprayed with distilled water and without soil addition (20.33 branches plant^{-1}).

Table 2 Effect of Brassinolide Spray and Manvert Soil Best on Branch Number in Three Ziziphus Cultivars

Cultivars X Manvert Soil Best	Brassinolide		Manvert Soil Best	Cultivars
	2 mg L^{-1}	0 mg L^{-1}		
30.16 c	39.66 d	20.66 f	0 mL L^{-1}	Tufahe
43.50 b	59.00 ab	28.00 ef	3.5 mL L^{-1}	
27.49 c	34.66 de	20.33 f	0 mL L^{-1}	Bembawi
39.16 b	50.00 bc	28.33 ef	3.5 mL L^{-1}	
46.16 b	55.00 ab	37.33 de	0 mL L^{-1}	Armuti
53.83 a	63.66 a	44.00 cd	3.5 mL L^{-1}	
Cultivars				
35.49 b	46.83 b	24.16 c	Tufahe	Cultivars X Brassinolide
34.33 b	44.83 b	23.50 c	Bembawi	
49.99 a	59.33 a	40.66 b	Armuti	
Manvert Soil Best				
34.61 b	43.11 b	26.11 d	0 mL L^{-1}	Manvert Soil Best X Brassinolide
45.49 a	57.55 a	33.44 c	3.5 mL L^{-1}	
	50.33 a	29.77 b	Brassinolide	

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- **Leaf phosphorus concentration (%)**

Table 2 shows that Armuti cultivar of Ziziphus showed the highest leaf phosphorus content (0.322%), while Bembawi had the lowest (0.289%). Brassinolide spray at 2 mg L^{-1} resulted in the highest phosphorus content (0.358%), and no spray gave the lowest (0.254%). Manvert Soil Best at 3.5 ml L^{-1} led to the highest leaf phosphorus (0.335%), while no addition resulted in the lowest (0.277%).

Armuti treated with 2 mg L^{-1} Brassinolide recorded the highest leaf phosphorus content (0.378%), while control with Bembawi cultivar showed the lowest value (0.243%). The highest leaf phosphorus content (0.350%) was recorded in Armuti treated with 3.5 ml L^{-1} Manvert Soil Best, while the control treatment of Bembawi showed the lowest value (0.260%). The interaction between foliar application of Brassinolide at 2 mg L^{-1} and Manvert Soil Best at 3.5 ml L^{-1} showed significant differences, recording the highest leaf phosphorus content (0.391%) compared with the control treatment (0.230%). Armuti treated with 2 mg L^{-1} Brassinolide and 3.5 ml L^{-1} Manvert Soil Best had the highest leaf phosphorus (0.413%), while Bembawi sprayed with distilled water had the lowest (0.218%).

Table 4. Effect of Brassinolide Spray and Manvert Soil Best on Leaf Phosphorus Content (%) in three Ziziphus Cultivars

Cultivars X Manvert Soil Best	Brassinolide		Manvert Soil Best	Cultivars
	2 mg L^{-1}	0 mg L^{-1}		
0.278 de	0.328 cde	0.228 hi	0 mL L^{-1}	Tufahe
0.338 ab	0.393 ab	0.283 fg	3.5 mL L^{-1}	
0.260 e	0.303 def	0.218 i	0 mL L^{-1}	Bembawi
0.318 bc	0.368 bc	0.268 fgh	3.5 mL L^{-1}	
0.293 cd	0.343 cd	0.243 ghi	0 mL L^{-1}	Armuti
0.350 a	0.413 a	0.288 ef	3.5 mL L^{-1}	
Cultivars				
0.308 b	0.360 ab	0.255 c	Tufahe	Cultivars X Brassinolide
0.289 c	0.335 b	0.243 c	Bembawi	
0.322 a	0.378 a	0.265 c	Armuti	
Manvert Soil Best				
0.277 b	0.325 b	0.230 d	0 mL L^{-1}	Manvert Soil Best x Brassinolide
0.335 a	0.391 a	0.280 c	3.5 mL L^{-1}	
	0.358 a	0.254 b	Brassinolide	

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- **Leaf magnesium concentration (%)**

Table 5 shows that Armuti had the highest leaf magnesium (185.4%), Bembawi the lowest (174.6%), while Tufahe and Armuti showed no significant difference. Manvert Soil Best at 3.5 ml L^{-1} increased magnesium content to 183.5% compared with 178.5% in the control. Table 6 shows that foliar application of Brassinolide at 2 mg L^{-1} gave the highest leaf magnesium content (188.2%), while the control treatment recorded the lowest value (173.7%).

Interaction between Armuti and Manvert Soil Best had the highest leaf magnesium (189.3%), while Bembawi without addition had the lowest (170.5%). The interaction between cultivar and Brassinolide spray showed significant differences, with Armuti sprayed with 2 mg L^{-1} Brassinolide recording the highest leaf magnesium (193.8%), while Bembawi sprayed with distilled water had the lowest (168.5%). Brassinolide spray (2 mg L^{-1}) with Manvert Soil Best (3.5 ml L^{-1}) gave the highest leaf magnesium (189.5%), while the control had the lowest (170.1%).The three-way interaction showed that Armuti sprayed with 2 mg L^{-1} Brassinolide and treated with 3.5 ml L^{-1} Manvert Soil Best had the highest leaf magnesium (198.0%), while Bembawi sprayed with distilled water without Manvert Soil Best had the lowest (164.0%).

Table 5. Effect of Brassinolide Spray and Manvert Soil Best on Leaf magnesium Content (%) in three Ziziphus Cultivars

Cultivars X Manvert Soil Best	Brassinolide		Manvert Soil Best	Cultivars
	2 mg L^{-1}	0 mg L^{-1}		
179.8 cd	186.6 cd	173.0 g	0 mL L^{-1}	Tufahe
186.1 ab	193.6 ab	178.6 efg	3.5 mL L^{-1}	
170.5 d	177.0 cde	164.0 h	0 mL L^{-1}	Bembawi
178.8 d	184.6 fg	173.0 g	3.5 mL L^{-1}	
181.5 bc	189.6 bc	173.3 g	0 mL L^{-1}	Armuti
189.3 a	198.0 a	180.6 def	3.5 mL L^{-1}	
Cultivars				
183.0 a	190.1 c	175.8 cb	Tufahe	Cultivars X Brassinolide
174.6 b	180.8 b	168.5 e	Bembawi	
185.4 a	193.8 a	177.0 d	Armuti	
Manvert Soil Best				
178.5 b	187.0 a	170.1 c	0 mL L^{-1}	Manvert Soil Best X Brassinolide
183.5 a	189.5 a	177.4 b	3.5 mL L^{-1}	
	188.2 a	173.7 b	Brassinolide	

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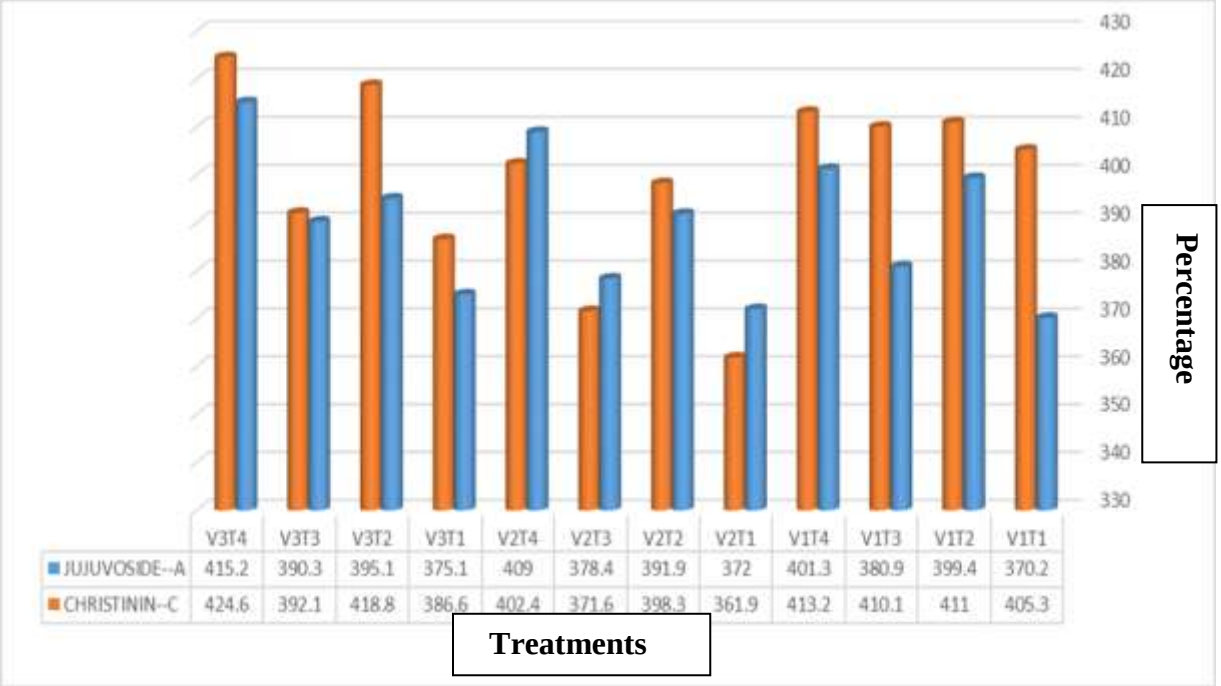
- **Leaf saponin content (Jujuvoside-A and Christinin-C)**

Figure 1 shows that the interaction of Armuti with 2 mg L^{-1} Brassinolide and 3.5 ml L^{-1} Manvert Soil Best outperformed Tufahe and Bembawi. The highest values were 415.2 mg L^{-1} for Jujuvoside-A and 424.6 mg L^{-1} for Christinin-C, while the lowest values were 370.2 mg L^{-1} (Tufahe) and 361.9 mg L^{-1} (Bembawi), respectively.



	V3T4	V3T3	V3T2	V3T1	V2T4	V2T3	V2T2	V2T1	V1T4	V1T3	V1T2	V1T1
JUJUVOSIDE--A	415.2	390.3	395.1	375.1	409	378.4	391.9	372	401.3	380.9	399.4	370.2
CHRISTININ--C	424.6	392.1	418.8	386.6	402.4	371.6	398.3	361.9	413.2	410.1	411	405.3

Figure 1. Effect of Brassinolide Spray and Manvert Soil Best on Jujuboside-A and Christinin-C Content (%) in Three Ziziphus Cultivars

The results presented in Tables 1–5 and Figure 1 indicate variability in plant growth among the three cultivars, which can be attributed to genetic factors that determine the degree of plant development and their interaction with environmental conditions (14). Notably, the Armuti cultivar outperformed the other cultivars (Tufahe and Bembawi) in total leaf number, which enhanced photosynthesis through increased sunlight absorption, thereby boosting energy production and cell division and root elongation in both longitudinal and radial axes, thereby improving photosynthesis and increasing water and nutrient uptake, even when improving transpiration and respiration. These physiological processes ultimately led to significant increases in growth, branch number, and leaf area. This finding is consistent with (15), who reported that Ziziphus cultivars are strongly influenced by soil factors and climatic conditions, which vary across regions, corroborating the observations of (16).

Foliar application of Brassinolide at 2 mg L^{-1} enhanced most vegetative growth traits by stimulating meristematic applied only to leaves, as it functions as a general growth regulator (17) Similar growth-promoting effects were reported in

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Ziziphus (18) and in bitter orange seedlings (19).

The tables indicate that the application of Manvert Soil Best at 3.5 mL L^{-1} enhanced overall vegetative growth by improving soil structure and permeability, alleviating salinity stress, and enhancing magnesium uptake due to its high cation exchange capacity. This promoted strong root development, increased water and nutrient absorption, and stimulated metabolic activity (20). These results are consistent with (21), who reported improved vegetative growth in citrus orchards following magnesium addition, and with (22), who observed similar effects in apple trees.

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The tables show that the interaction between cultivar, foliar application of Brassinolide at 2 mg L^{-1}, and soil application of Manvert Soil Best at 3.5 mL L^{-1} resulted in significant improvements. This effect can be attributed to the synergy between the genetic traits of each cultivar, foliar nutrition enhancing direct nutrient uptake, and soil amendments providing a sustained nutrient supply that supports root growth. Consequently, photosynthesis and tissue development were enhanced more effectively than with individual treatments (23), consistent with (24), who reported similar effects in foliar and soil fertilization of two orange cultivars

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