International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Comparing Effects of Priming Chili Pepper Seed with Different Plant Biostimulants, with Balancing Effects on Vegetative and Root Growths and Seedling Quality

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ARTICLE INFO

Article history:

Received: 1 June 2024,
Received in revised form: 3 December 2024,
Accepted: 14 December 2024

Article type:

Research paper

Keywords:

Biostimulants
Chili
Establishment
Quality
Seedling

ABSTRACT

Plant extracts, derived from natural sources, are increasingly recognized as cost-effective biostimulants for enhancing plant growth. In this study, aqueous and ethanolic extracts from various parts of *Moringa oleifera* (leaves, flowers, and seeds), along with aqueous extracts from prickly pear (*Opuntia ficus-indica*), red beetroot (*Beta vulgaris*), and carrot root (*Daucus carota* L.), were used to prime seeds of three Yemeni hot pepper cultivars (Zaaitri, Haimi, and Dhamari) at two concentrations. The results demonstrated significant differences among the cultivars. Water extracts from moringa flowers, moringa leaves, and prickly pear significantly enhanced root growth across all cultivars. Furthermore, beetroot extract, a combination of moringa flower and seed extracts, and prickly pear extract markedly improved vegetative growth traits. For biomass-related parameters (DM%, MM%, and OC%), moringa seed extract performed best on the Haimi cultivar, while the combination of moringa seed and flower extracts yielded the most favorable results in the Dhamari cultivar, and beetroot extract was most effective in the Zaaitri cultivar. These findings offer valuable insights into optimizing seedling production practices and highlight the potential of affordable, plant-based biostimulants for agricultural applications.

Introduction

Hot peppers (*Capsicum* spp.) are an essential vegetable crop with a significant role in global culinary practices. Their importance is underscored by their rich nutritional profile, particularly their high vitamin C content and capsaicin, a compound widely recognized for its medicinal properties. Hot peppers are utilized in various forms, including fresh, dried, as a condiment, and as a key ingredient in chili-based products, enhancing the flavor profiles of international cuisines. According to the United Nations Food and Agriculture Organization (FAO, 2022), hot pepper production has shown a steady linear increase over the past five decades. In 2022, global pepper production reached 788,032.04 t from an estimated cultivated area of 689,336 ha, with Yemen contributing 18,223.37 t from 3,240 ha, accounting for approximately 2.3% of global production. Pepper cultivation generally relies on seedlings and exhibits a longer germination period compared to other vegetable crops.

Seedling quality is crucial, as it significantly impacts plant productivity. The growing

emphasis on organic agriculture has highlighted the need for natural alternatives to chemical inputs, which can pose residual toxicological risks. Plant biostimulants, first described in the literature in 1951 (Yakhin et al., 2017), represent a promising natural solution. These organic compounds, distinct from pesticides and fertilizers, include amino acids, sugars, vitamins, and humic substances. They also contain hormonal compounds that activate endogenous plant hormones, thereby promoting growth, development, and overall vitality. When applied to soil or plants, either alone or with fertilizers and pesticides, biostimulants enhance plant resilience to environmental stressors.

The prickly pear (*Opuntia ficus-indica*), a member of the Cactaceae family, is rich in mineral elements, carbohydrates, vitamins, amino acids, organic acids, antioxidative compounds, and plant pigments (Rodriguez-Amaya, 2015; Albuquerque et al., 2020; Shoukat et al., 2023). Its fruits and seeds are particularly abundant in protein, beta-carotene, amino acids, phenolic compounds, and vitamin C (AbdelFattah et al., 2020). These properties make it an effective biostimulant, promoting plant growth under adverse conditions such as salinity, drought, and wastewater stress, primarily due to its antimicrobial attributes.

Moringa oleifera, belonging to the moringaceae family, thrives in tropical and subtropical climates with temperatures ranging from 25 to 40 °C (Zulfiqar et al., 2020). Extracts from various parts of moringa serve as biostimulants due to their nutrient-dense composition, including minerals, alkaloids, sugars, and vitamins, which enhance plant growth and development (Arif et al., 2023). Moringa seeds are particularly rich in unsaturated fatty acids, while its foliage contains high concentrations of free cytokinin, especially zeatin (Fuglie, 2000; Davies, 2004; Basra et al., 2011; Davies, 2012; Abdalla, 2013). The plant has demonstrated efficacy in supporting growth under saline and drought stress (Gharsallah et al., 2021), facilitating osmotic regulation, hormone synthesis, and cellular resilience to environmental stress, while mitigating heavy metal toxicity (Arif et al., 2023). Additionally, moringa extract stimulates chlorophyll synthesis in saline environments, aiding mineral balance and stabilizing photosynthetic enzyme activity. Carrots (*Daucus carota* L.), members of the Apiaceae family, are rich in nutrients such as beta-carotene, protein, carbohydrates, fats, and a variety of vitamins (A, B1, B2, B6, C, D, and E), which function as antioxidants protecting against cellular damage (Baranska et al., 2005; Kasim et al., 2017). These nutrients, especially trace vitamins, act as biological stimulants by modulating physiological processes such as enzyme synthesis (Youssef and Talaat, 2003). Beta-carotene, a prominent carotenoid, not only imparts carrots with their distinctive orange hue but also plays a critical role in photosynthesis and protects photosystems from reactive oxygen species (Ramel et al., 2012; Kasim et al., 2017). Carrot extract, containing compounds such as indole-3-acetic acid (IAA) and beta-carotene, is a cost-effective biostimulant that mitigates drought stress (Alasalvar et al., 2001; Puchooa and Ramburn, 2004; Abbas and Akladious, 2013). Additionally, carrot roots are abundant in anthocyanins, amino acids, proline, sugars, flavonoids, protein, fiber, and lycopene, making carrot extract a robust biostimulant and plant growth regulator (Jiménez et al., 2005; Anwar et al., 2011; Boadi et al., 2021). Red beetroot (*Beta vulgaris*), commonly consumed in juices, salads, and various culinary preparations, is rich in vitamins, minerals, carotenoids, glucose, fructose, organic acids (e.g., lactic acid), and betalains, which possess potent antioxidant properties that counteract reactive oxygen species (Madadi et al., 2020; Moreno-Ley et al., 2021; Wijesinghe and Choo, 2022). Betalains, in particular, exhibit exceptional antioxidant activity (Georgiev et al., 2010; Vieira Teixeira da Silva et al., 2019). While much of the literature focuses on the nutritional and health benefits of red beetroot, its extract also contains compounds that promote plant growth and development. This study investigates the effects of extracts from moringa, prickly pear, red beetroot, and carrot root, all recognized as effective natural biostimulants, on the quality of hot chili seedlings.

Material and methods

Experimental lay out

The extraction process was carried out at the horticulture laboratory of Sana'a University, while the seedlings were cultivated in a controlled greenhouse environment at Al Nahda Vegetable Nursery during the summer of 2023.

Plant materials

Chili peppers from three Yemeni cultivars (*Zaaitri*, *Haimi*, and *Dhamari*) were collected. Their seeds extracted and stored in labeled glass jars for future use in the experiment. Moringa leaves, flowers, and seeds were collected from large trees.

Plant biostimulants extract

Plant materials were extracted using two methods: water for fresh samples and 80%

ethanol for dry samples. The dried samples were extracted using a Soxhlet apparatus. A blender was used to extract the prickly pear fruits, beetroots, and carrot roots. Two concentrations were prepared for each extract, including double and triple combinations of ethanolic extracts. The highest concentration for moringa extracts was 20%, while the highest concentration for the other extracts was 25%. The lowest concentration for all extracts was 10%. Table 1 presents the extract ratios (w:v) and the characteristics of each concentration.

Table 1. Plant biostimulants stock preparing used in the experiment, and the characterizes of each concentration of the extract.

Extract	Plant part	T.E	Stock reparation		Concentration								code
			W(g)	V(ml)	Lower (A)				Higher (B)				
					pH	Ec	TSS	Ψs	pH	Ec	TSS	Ψs	
Control	-	D.W	-	-	7	0	0	0	7	0	0	0	N
Prickly pear	Fruit	W	300	-	6.78	0.37	3.6	-13.32	6.75	0.83	5.4	-29.88	O (W)
Beatroot	Root	W	100	150	6.93	0.68	3.2	-24.48	6.95	1.42	3.2	-51.12	B (W)
Carrot	Root	W	100	150	6.84	0.39	3.2	-14.04	6.81	0.88	3.4	-31.68	C (W)
Moringa	Leaf	W	100	30	6.43	1.53	3.8	-55.08	6.33	2.32	4.2	-83.52	ML (W)
Moringa	Flower	W	82.5	30	6.16	0.56	4.2	-20.16	6.12	1.25	6.2	-45.00	MF (W)
Moringa	Leaf	E	3	50	6.01	1.14	8	-41.04	6.09	2.52	10	-90.72	ML (E)
Moringa	Flower	E	3	50	5.53	1.78	8	-64.08	5.55	1.86	8.4	-66.96	MF (E)
Moringa	Seed	E	3	50	5.96	0.72	5.6	-25.92	6.09	1.31	5	-47.16	MS (E)
Moringa	Leaf + Flower	E			5.61	2.88	5.4	-103.68	5.76	4.2	7.4	-151.20	ML+MF (E)
Moringa	Leaf + Seed	E			5.91	2.95	5.2	-106.20	6.01	4.35	7.4	-156.60	ML+MS (E)
Moringa	Seed + Flower	E			5.63	2.84	8	-102.24	5.48	3.77	8.4	-135.72	MS+MF (E)
Moringa	Leaf + Seed + Flower	E			5.75	2.04	7.2	-73.44	5.52	3.59	7.8	-129.24	ALL (E)

T.E. = Type of extract, where "W" denotes water extract and "E" denotes ethanolic extract. The pH was measured using a pH meter, electrical conductivity (EC, ms cm^{-1}) was determined with an EC meter, and total soluble solids (TSS) were measured using a hand refractometer. Osmotic potential (Ψs) was calculated using the formula: Ψs = -0.36 × EC.

Treatment of seeds in extracts

The seeds were sterilized using a 10% chlorine solution mixed with 90% distilled water and a drop of Tween 20 for 5 min. After sterilization, the seeds were rinsed several times with running water and distilled water before being air-dried. The seeds were then soaked for 18 h in varying concentrations of each extract, with distilled water used as the control. After treatment, the seeds were planted in 216-hole dispersion plates, with each plate divided into two treatments and 36 seedlings per replicate. A factorial experiment was conducted using a completely randomized design (CRD) with three replications.

Parameters of study

After 60 d of planting, 18 seedlings per treatment, including six seedlings from each replicate, were measured for vegetative growth, root development, dry matter, and mineral content.

Chlorophyll levels

Chlorophyll levels were measured using the SPAD 502 chlorophyll meter.

Vegetative and root growth measurements

The methodology outlined by Al-Madhagi and Al-Sharaqi (2019) was followed closely. Six seedlings were harvested from each replicate, and soil adhering to the roots was carefully removed. The seedlings were then separated into leaves, roots, and stems. Fresh weights were recorded for each plant part, and each part was placed on a black paper plate with a calibration mark for each plant. Photographs were taken using a mobile phone camera.

Photo data processing

Photographs were transferred to a laptop in JPEG format for further processing. The images were initially processed using *IrfanView* or *Fiji* software and saved at a resolution of 200 after size correction with a calibration ruler. Vegetative trait data, including the number of leaves, leaf area (cm^2), stem length (cm), and stem diameter (mm), were analyzed using the *Tomato Analyzer* 3.0 program (available at http://oardc.osu.edu/vanderknaap/tomato_analyzer.htm). Root trait data were analyzed using *Giaroot* software, provided by the Georgia Tech Research Corporation and Duke University, USA. The method is illustrated in Figure 1.

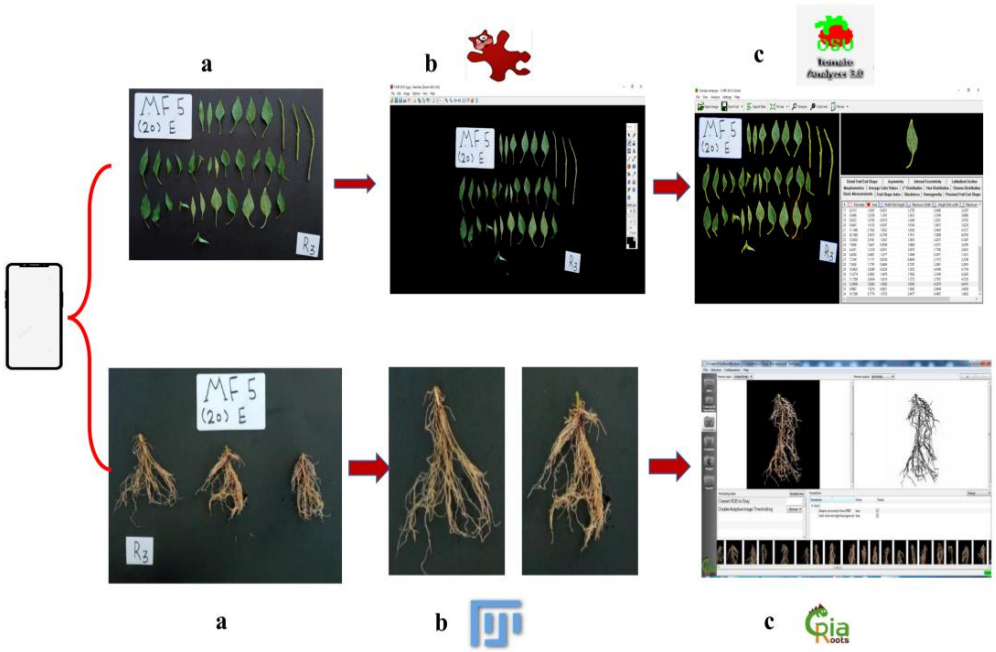

Fig. 1. Steps for measuring vegetative and root growth, (a) capturing images using a mobile phone camera, (b) enhancing and resizing the images using *IrfanView* software for vegetative parameters and by *Fiji* software for root parameters, and (c) measuring vegetative parameters with Tomato Analyzer software and root parameter with *Giaroot* software.

Dry matter of vegetative and root

The different plant parts of the seedlings (vegetative and root), along with their fresh weights recorded during vegetative and root growth measurements, were placed in paper bags of known empty weight. The samples were then air-dried at room temperature until they reached a stable weight. The dry weights were recorded, and the percentage of dry matter was calculated using the equation provided by Al-Madhagi et al. (2011).

$$\%DM = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100 \quad (1)$$

Organic matter (OC%) and mineral matter (MM%) evaluation

After grinding the dried samples using a specialized electric grinder, a known weight of the ground material was taken and incinerated at 550 °C for 8 h to estimate the mineral matter (MM%) and organic carbon (OC%) content. These parameters were calculated using the following equations, as described by Armecin and Gabon (2008).

$$\%OC = \frac{\%OM}{1.724} \quad (2)$$

$$\%MM = \frac{AW}{DW} \times 100 \quad (3)$$

Data analyses

The data were analyzed using the statistical analysis program *GenStat* 12, and then the means of single factors (cultivars, priming extract and concentration) were compared using the least significant difference (LSD 0.50) test. The values of the means of the interactions (cultivars × priming extract × concentration) were displayed according to the efficiency of the extract, as they were calculated by converting the data as a percentage to express the amount of increase or decrease over the control according to the recipe as the equation described by Jang and Kuk (2019).

$$\text{Extract} = 100 \text{ x } \left[\frac{\text{Treated}}{\text{Control}} - 1\right] \quad (4)$$

Results

Leaf count seedling^{-1} (LC)

All factors, except for extract concentration and its interaction with cultivars, significantly influenced leaf count seedling^{-1} ($P < 0.05$, ANOVA). After 60 d, the *Dhamari* cultivar achieved the highest average leaf count, with 8.92 leaves seedling^{-1}. In contrast, the *Zaaitri* and *Haimi* cultivars showed no statistically significant differences in leaf count. Among the extracts, beetroot extract [B (W)] resulted in the highest average leaf count (8.8 leaves seedling^{-1}), while combined moringa flower and seed extracts [MF+MS (E)] produced the lowest count (6.73 leaves seedling^{-1}). The concentration of plant extracts did not significantly affect the results (Table 2).

Table 2. *F probability* values and means for the individual factors (genotypes, extract, and concentration) affecting vegetative growth, root development, and dry matter of 60-day-old chili seedlings.

Source of variation		d.f.	Leaf number	Total leaf area (cm^2)	Seedling height (cm)	Seedling diameter (mm)	Chlorophyll (SPAD)	Root Number	Root Specific Length (cm/cm^3)	Root Dry matter%	Vegetative dry matter %	Soot root $^{-1}$ratio
			<.001	**0.041**	**<.001**	**<.001**	**<.001**	**0.001**	**<.001**	**<.001**	**0.149**	**<.001**
Genotype (G)	**Zaaitri**	**2**	7.26^{b}	17 ^{b}	8.4^{b}	0.27^{a}	43.43^{a}	21.84 ^{b}	223 ^{a}	15.08^{b}	23.22^{a}	1.58^{b}
	Haimi		7.46^{b}	17.05^{b}	8.19^{b}	0.21^{b}	41.45^{b}	23.6 ^{a}	210.9 ^{b}	16.31^{a}	23.41^{a}	1.53^{b}
	Dhamari		8.92^{a}	18.34^{a}	9.48^{a}	0.28^{a}	42.86^{a}	20.78 ^{c}	174.2 ^{c}	13.53^{c}	22.62^{b}	1.71^{a}
Lsd$^{0.05}$			**0.351**	**0.779**	**0.253**	**0.015**	**0.576**	**4.936**	**0.051**	**0.55**	**0.503**	**0.959**
			<.001	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.007**	**0.004**
	ALL(E)		8.02^{bcde}	15.89^{fg}	7.81 ^{g}	0.24^{bcd}	42.00^{de}	23.03 ^{b}	211.1 ^{bc}	14.29^{cd}	23.93^{ab}	1.71^{bc}
	B(W)		8.78 ^{a}	21.83^{a}	9.06^{abcd}	0.27^{b}	43.39^{abc}	20.78 ^{c}	199.2 ^{d}	12.95 ^{e}	23.44^{abc}	1.824^{a}
	C(W)		8.5^{ab}	18.47^{bcd}	8.15^{fg}	0.26^{bc}	43.88^{ab}	21.01 ^{c}	199.9 ^{d}	14.55^{cd}	22.90 ^{bc}	1.615^{cd}
	MF(E)		8.43^{abc}	19.81^{b}	9.52^{a}	0.33^{a}	40.91^{ef}	25.81 ^{a}	225.0 ^{a}	14.82^{bc}	21.80^{d}	1.547^{de}
	MF(W)		7.44^{efg}	17.6^{cde}	9.36^{ab}	0.25^{cd}	44.19 ^{ab}	23.07 ^{b}	204.4 ^{cd}	16.06^{a}	24.13 ^{a}	1.52 ^{de}
	MF+MS(E)		6.73^{g}	13.65 ^{h}	8.64^{def}	0.24^{bcd}	43.53^{abc}	20.34^{cd}	194.4 ^{d}	16.10 ^{a}	23.33^{abc}	1.56^{de}
Extract (E)	**ML(E)**	**12**	7.01^{fg}	16.28^{efg}	8.52^{ef}	0.24^{bcd}	43.00^{bcd}	20.52^{cd}	198.0 ^{d}	14.27^{cd}	21.52^{d}	1.52^{de}
	ML(W)		7.64 ^{def}	16.26^{efg}	9.25^{abc}	0.25^{bc}	42.58^{cd}	18.55 ^{d}	171.9 ^{e}	16.35^{a}	24.27^{a}	1.521^{de}
	ML+MF(E)		8.24^{abcd}	17.18^{def}	8.16^{fg}	0.24^{cd}	44.37^{a}	25.31 ^{a}	219.4 ^{ab}	13.44^{de}	21.69^{d}	1.682^{bc}
	ML+MS(E)		7.72^{cdef}	15.31^{g}	8.13^{fg}	0.25^{bc}	43.06^{bcd}	23.06 ^{b}	200.0 ^{d}	14.54^{cd}	23.63^{ab}	1.735^{ab}
	MS(E)		7.62^{def}	17.26^{def}	8.73^{cde}	0.21^{d}	42.16^{d}	21.40 ^{bc}	211.8 ^{bc}	16.86^{a}	23.01 ^{bc}	1.491 ^{e}
	N		8.33^{abcd}	18.42^{bcd}	8.77^{cde}	0.24^{cd}	40.03^{f}	20.83 ^{c}	199.4 ^{d}	14.61 ^{c}	22.48^{cd}	1.566^{de}
	O(W)		7.85^{bcde}	19.04^{bc}	8.84^{bcde}	0.25^{bc}	40.41 ^{f}	23.22 ^{b}	200.6 ^{d}	15.81^{ab}	23.94^{ab}	1.565^{de}
Lsd$^{0.05}$			**0.73**	**1.622**	**0.526**	**0.032**	**1.199**	**10.276**	**0.105**	**1.146**	**1.047**	**1.997**
			0.369	**0.487**	**<.001**	**0.026**	**0.006**	**0.019**	**0.713**	**0.016**	**0.156**	**0.003**
Concentration (C)	**A**	**1**	7.96	17.63	8.95^{a}	0.24^{b}	43.15^{a}	22.81^{a}	203.32 ^{a}	15.25^{a}	22.93	1.57^{b}
	B		7.79	17.29	8.43^{b}	0.26^{a}	42.01^{b}	21.33^{b}	202.1 ^{a}	14.7^{b}	23.23	1.64^{a}
G × E		**24**	**<.001**	**<.001**	**<.001**	**0.017**	**0.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
G × C		**2**	**0.302**	**0.604**	**0.698**	**0.898**	**0.016**	**0.78**	**0.092**	**0.002**	**0.433**	**0.011**
E × C		**12**	**0.697**	**0.062**	**0.002**	**0.011**	**0.053**	**<.001**	**<.001**	**<.001**	**0.048**	**<.001**
G ×E× C		**24**	**0.011**	**<.001**	**0.042**	**0.200**	**0.011**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**

(E) refers to ethanolic extract, (W) to water extract; "A" indicates the lower concentration, and "B" denotes the higher concentration. Means of individual factors sharing the same lowercase Latin letters are not significantly different at the 0.05 level, as determined by the Least Significant Difference (LSD 0.05) test.

The rate of change in leaf count compared to the control (untreated group) varied across treatments. The *Dhamari* cultivar showed the largest increase (47.3%) in response to ethanolic extracts from all parts of the moringa plant [ALL (E)], while the most significant decrease (-34.2%) was observed in the *Haimi* cultivar in response to a 20% concentration of prickly pear extract [O (W)] (Fig. 2).

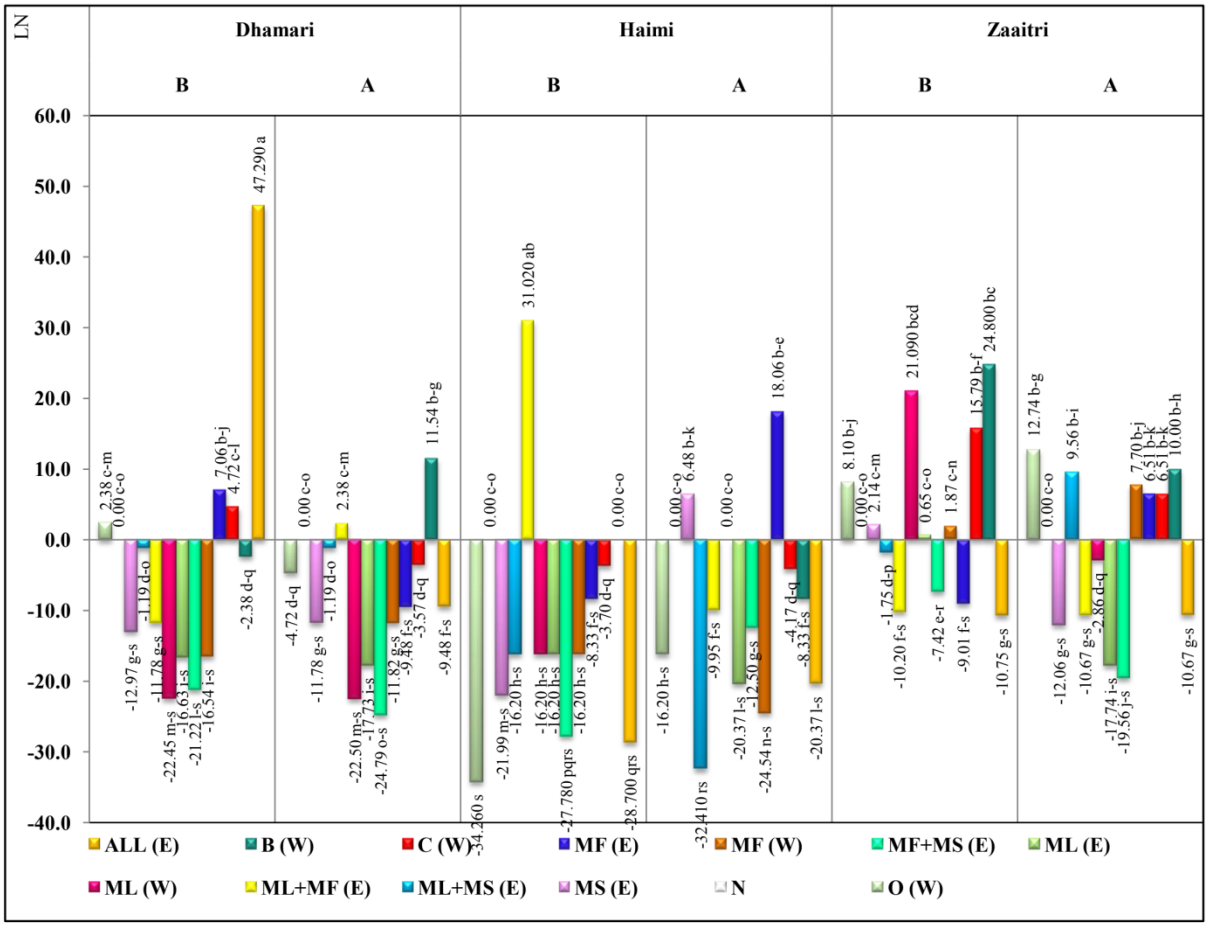

Fig. 2. The rate of change (%) in leaf count (LC) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

For the Zaaitri cultivar, beetroot extract [B (W)] at a 25% concentration increased leaf count by 24.8%. In the Haimi cultivar, a 20% concentration of combined moringa leaf and flower extracts [(ML+MF) (E)] resulted in a 31% increase. For the Dhamari cultivar, seeds primed with ethanolic extracts from all parts of the moringa plant [(ALL) (E)] showed a 47% increase compared to the control. Aqueous moringa leaf extract [ML (W)] at 10% and 20% concentrations reduced leaf count in all cultivars except Zaaitri, where a 20% concentration positively affected leaf count. The carrot extract [C (W)] at a 10% concentration increased leaf count but reduced it at a 25% concentration in Zaaitri and Haimi, whereas the Dhamari cultivar exhibited the opposite trend. Ethanolic moringa leaf extract [ML (E)] negatively impacted the Haimi and Dhamari cultivars but elicited a positive response in Zaaitri at a 20% concentration. Similarly, ethanolic moringa flower extract [MF (E)] increased leaf count at a 10% concentration but decreased it at 20% in both Zaaitri and Haimi cultivars, while Dhamari displayed the opposite trend. Prickly pear extract [O (W)] produced cultivar-specific effects: it increased leaf count in Zaaitri, decreased it in Haimi, and enhanced it in Dhamari when applied at a 25% concentration (Fig. 2).

Total leaf area (TLA) (cm² seedling⁻¹)

The total leaf area (cm² seedling^{-1}) was significantly influenced by both cultivars and extract types, along with their interaction effects. However, varying concentrations of plant

extracts did not have a significant impact, as indicated by the ANOVA results ($P < 0.05$, Table 2). At 60 d, the cultivars showed notable differences in total leaf area. The *Dhamari* cultivar exhibited the highest average leaf area (18.34 cm^2 $seedling^{-1}$), with no significant difference between the *Zaaitri* and *Haimi* cultivars. The extract type also significantly affected total leaf area, with beetroot extract [B (W)] producing the highest average value (21.83 cm^2 $seedling^{-1}$) and the mixture of ethanolic moringa flower and seed extracts [MF+MS (E)] resulting in the lowest value (13.65 cm^2 $seedling^{-1}$). The plant extract concentrations, however, had no discernible impact (Table 2). Figure 3 illustrates the percentage of changes in total leaf area compared to the control (untreated seeds) following seed priming with plant extracts. *Zaaitri* seedlings treated with a 25% concentration of beetroot extract [B W)] showed a significant increase of 60.9%, while *Haimi* seedlings treated with a 10% concentration of [MF+MS(E)] recorded a pronounced decrease of -75.6%.

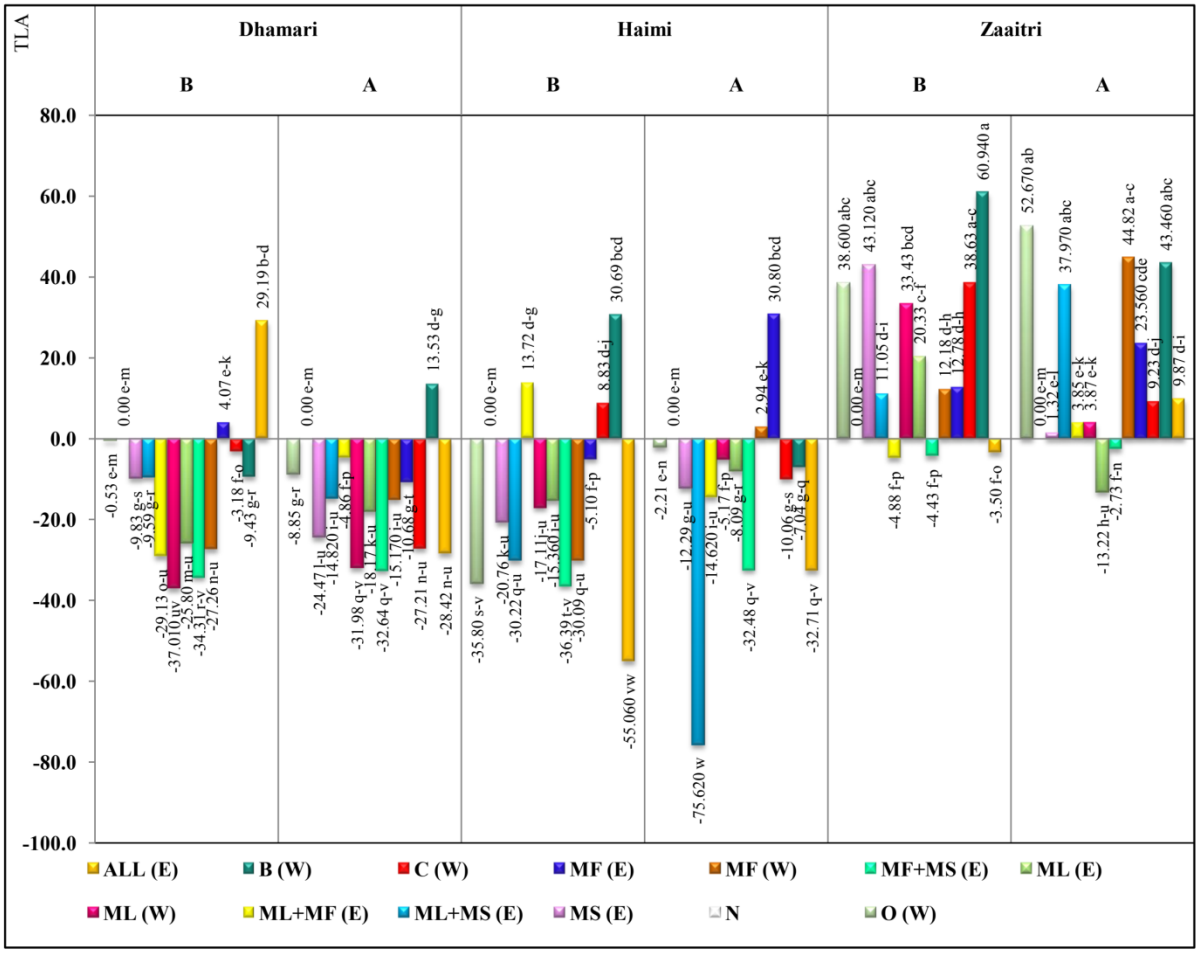

Fig. 3. The rate of change (%) in leaf area $plant^{-1}$ (TLA) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Zaaitri cultivar, most priming treatments had positive effects on leaf area, with the exception of ethanolic moringa leaf extract [ML (E)] at 10%, ethanolic moringa flower and seed extract mixture [MF+MS (E)], and ethanolic moringa leaf and flower extract mixture [ML+MF (E)] at 20%, all of which reduced leaf area compared to the control (Fig. 3). Conversely, seedlings of the Haimi cultivar generally exhibited negative responses to most priming treatments. Notable exceptions included a 30.8% increase in leaf area with 10% ethanolic moringa flower extract [MF (E)], as well as positive effects from treatments with 10% aqueous moringa flower extract [MF (W)], carrot extract [C (W)], 25% beetroot extract [B (W)], and the 20% ethanolic mixture of moringa leaf and flower extracts [ML+MF (E)] (Fig. 3). For the Dhamari

cultivar, most treatments reduced total leaf area compared to the control. However, a 10% beetroot extract [B (W)] and a 20% ethanolic mixture of all moringa parts [ALL (E)] resulted in a 29.19% increase in leaf area. Additionally, treatment with 20% ethanolic moringa flower extract [MF (E)] produced positive effects, exceeding the control values (Fig. 3).

Seedling height (SH) (cm)

Seedling height (cm) was significantly influenced by all individual factors in the study (cultivars, plant extracts, and concentrations) and their interactions, except for the two-way interaction between cultivars and concentrations (G × C), where the F-probability values exceeded 0.05 (Table 2). Among the chili cultivars, Dhamari exhibited the longest average seedling height (9.48 cm), while Haimi had the shortest (8.19 cm). However, the difference between Haimi and Zaaitri was not statistically significant (Table 2). Significant variation in seedling height was observed across the plant extracts used for seed priming. Seedlings treated with ethanolic moringa flower extract [MF (E)] attained the greatest height (9.52 cm), whereas the shortest height (7.81 cm) was observed in seedlings treated with the ethanolic mixture of all moringa parts [ALL (E)]. Additionally, lower concentrations of plant extracts generally resulted in significantly taller seedlings compared to higher concentrations (Table 2). The rates of change in seedling height relative to the control varied, influenced by extract type, concentration, and cultivar (Fig. 4). The percentage change ranged from a maximum increase of 45.6% in Zaaitri seedlings to a minimum decrease of -29.8% in Haimi seedlings.

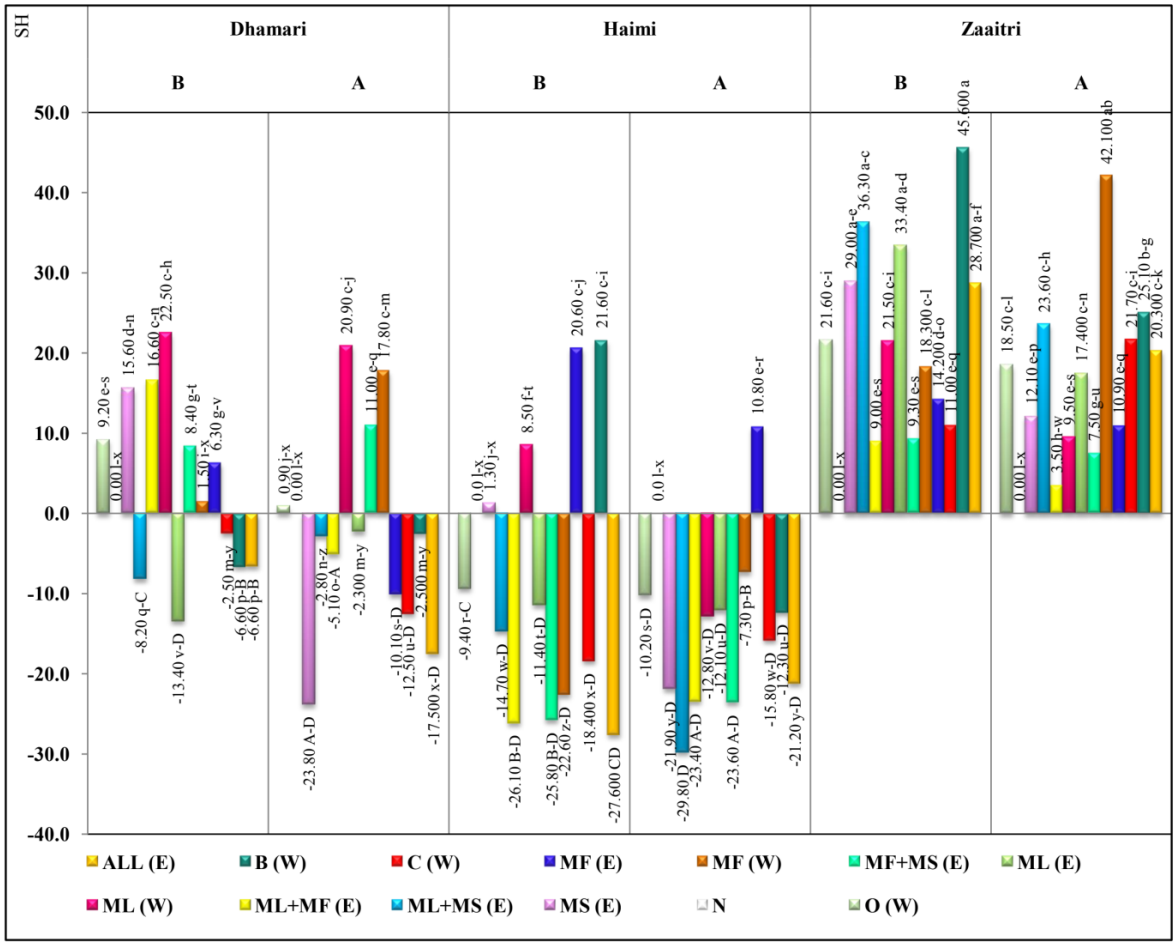

Fig. 4. The rate of change % in shoot height (SH) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Zaaitri cultivar, all seed priming treatments enhanced seedling height. The greatest increase (45.6%) was observed in seedlings primed with red beetroot extract [B (W)] at the highest concentration (25%), followed by moringa flower extract [MF (W)] at a lower concentration (10%), which resulted in a 42.1% increase. However, increases below 20% were not statistically significant when compared to the control (Fig. 4). In the Haimi cultivar, seedling height increased with priming treatments involving ethanolic moringa flower extract [MF (E)] at both low and high concentrations (10% and 20%), as well as with beetroot extract [B (W)], aqueous moringa leaf extract [ML (W)], and ethanolic moringa seed extract [MS (E)]. The highest increase (21.6%) was recorded in seedlings treated with beetroot extract [B (W)] at a 25% concentration (Fig. 4). For the Dhamari cultivar, aqueous moringa leaf extract [ML (W)] boosted seedling height by 22.5% and 20.9% at its highest and lowest concentrations, respectively. At high concentrations (20%), ethanolic moringa seed extract [MS (E)] and a mixture of moringa leaf and flower extracts [ML+MF (E)] increased seedling height. In contrast, these extracts caused decreases in seedling height at lower concentrations compared to the control (Fig. 4).

Seedling diameter (SD) (mm)

Various factors, including the three-way interaction, significantly influenced seedling diameter ($P < 0.05$). Seedling diameter varied among the chili cultivars, with the Haimi cultivar having the smallest average diameter (0.21 mm) and the Zaaitri cultivar the largest (0.28 mm) (Table 2). The type of plant extract had a significant impact on stem diameter. Seedlings treated with ethanolic moringa flower extract [MF (E)] exhibited the largest diameter (0.33 mm), significantly exceeding those treated with other extracts. In contrast, seedlings treated with ethanolic moringa seed extract [MS (E)] displayed the smallest diameter (0.21 mm). Additionally, higher concentrations of plant extracts consistently resulted in greater stem diameters compared to lower concentrations (Table 2). As illustrated in Figure 5, priming chili seeds with plant extracts before planting led to variable changes in stem diameter across treatments relative to the control. The percentage change ranged from a maximum increase of 76.3% to a minimum decrease of -30.5%.

Higher concentrations of plant extracts generally resulted in significant increases in seedling diameter. For instance, seedlings primed with the lowest concentration (10%) of ethanolic moringa flower extract [MF (E)] demonstrated notable increases in stem diameter, reaching 61.9%, 76.3%, and 40.3% in the Zaaitri, Haimi, and Dhamari cultivars, respectively (Fig. 5). In the Zaaitri cultivar, most plant extracts positively influenced stem diameter. However, treatments with ethanolic moringa leaf extract [ML (E)] or a mixture of moringa flower and seed extracts [MF+MS (E)] at the highest concentration (20%) resulted in reductions in stem diameter, though these decreases were not statistically significant compared to the control (Fig. 5). In the Haimi cultivar, higher concentrations of carrot extract [C (W)] and prickly pear extract [O (W)] negatively impacted stem diameter, while ethanolic moringa flower extract [MF (E)] exhibited a positive effect. Conversely, treatments with aqueous moringa flower extract [MF (W)] and the mixture of ethanolic moringa leaf and flower extracts [ML+MF (E)] resulted in reductions in stem diameter at both low (10%) and high (20%) concentrations relative to the control (Fig. 5). The Dhamari cultivar generally exhibited decreases in stem diameter following treatment with most plant extracts. However, positive effects were observed in seedlings treated with beetroot extract [B (W)], carrot extract [C (W)], ethanolic moringa flower extract [MF (E)], and the mixture of all moringa parts [ALL (E)] at the lower concentration (10%) when compared to the control (Fig. 5).

Chlorophyll (SPAD) (Chl)

The average leaf chlorophyll content (SPAD Chlorophyll) was significantly affected by all individual factors and their interactions (F-probability < 0.05) (Table 2). Among the chili cultivars, Zaaitri exhibited the highest chlorophyll content (43.43), which was not significantly different from that of Dhamari. Conversely, the Haimi cultivar recorded the lowest SPAD chlorophyll content (41.54). Lower concentrations of plant extracts generally led to significantly higher chlorophyll values compared to higher concentrations (Table 2). The chlorophyll content varied significantly depending on the type of plant extract used for seed priming. The mixture of moringa leaf and flower extracts [ML+MF (E)] resulted in the highest average chlorophyll content (44.37), while the control treatment exhibited the lowest value (40.03). Most plant extracts significantly enhanced chlorophyll content compared to the control, with the exceptions being prickly pear fruit extract [O (W)] and ethanolic moringa flower extract [MF (E)], which did not demonstrate significant improvements. Notably,

lower concentrations of extracts consistently produced higher chlorophyll content than higher concentrations (Table 2). As shown in Figure 6, the effect of seed priming with plant extracts on chlorophyll content varied across cultivars when compared to the control. The percentage change ranged from a maximum increase of 24.3% to a minimum decrease of -9.2%, highlighting the differential impact of treatments across cultivars.

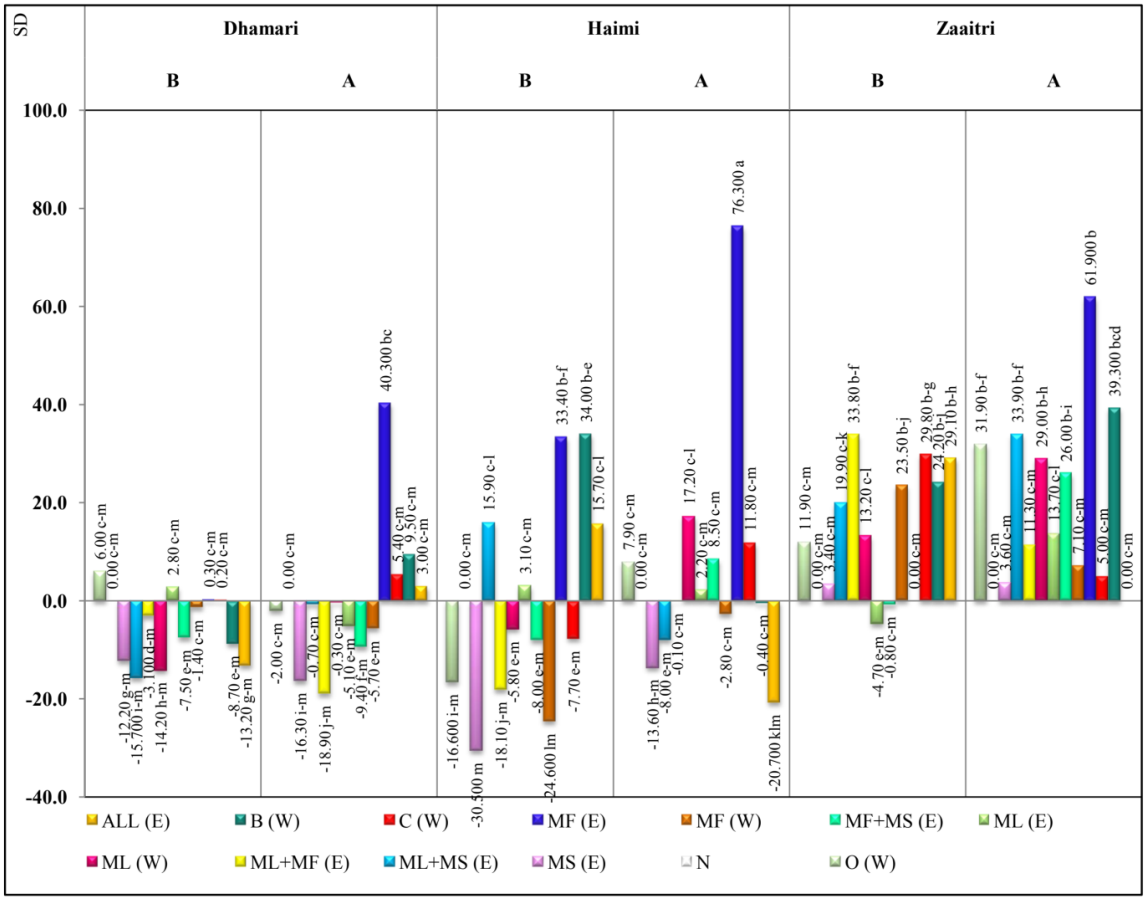

Fig. 5. The rate of change (%) in shoot diameter (SD) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Zaaitri cultivar, the chlorophyll content exhibited the highest increase (16.5%) in seedlings treated with a 20% mixture of moringa leaf and flower extracts [ML+MF (E)]. Conversely, a 3.5% decrease in chlorophyll content was observed at the lower concentration (10%), although this reduction was not statistically significant compared to the control (Fig. 6). In the Haimi cultivar, plant extract treatments generally resulted in positive changes, with a few exceptions. Seedlings treated with 10% ethanolic moringa flower extract [MF (E)] and those treated with higher concentrations (20% and 25%) of moringa leaf extract [ML (W)] or beetroot extract [B (W)] showed negative effects. The greatest increase in chlorophyll content (24.3%) was observed with the mixture of moringa flower and seed extracts [MF+MS (E)]. However, treatments that increased chlorophyll content by less than 13% did not differ significantly from the control (Fig. 6). For the Dhamari cultivar, most priming treatments positively affected chlorophyll content. Negative effects were recorded for seedlings treated with prickly pear fruit extract [O (W)] at both 10% and 25%, as well as for those treated with a 10% mixture of all moringa parts [ALL (E)]. The highest increase in chlorophyll content (21.6%) was achieved with the moringa leaf and seed extract mixture [ML+MS (E)] and beetroot extract [B (W)] at 25%. Similar to the other cultivars, treatments that resulted in chlorophyll increases of less than 12% did not show statistically significant differences from the control (Fig. 6).

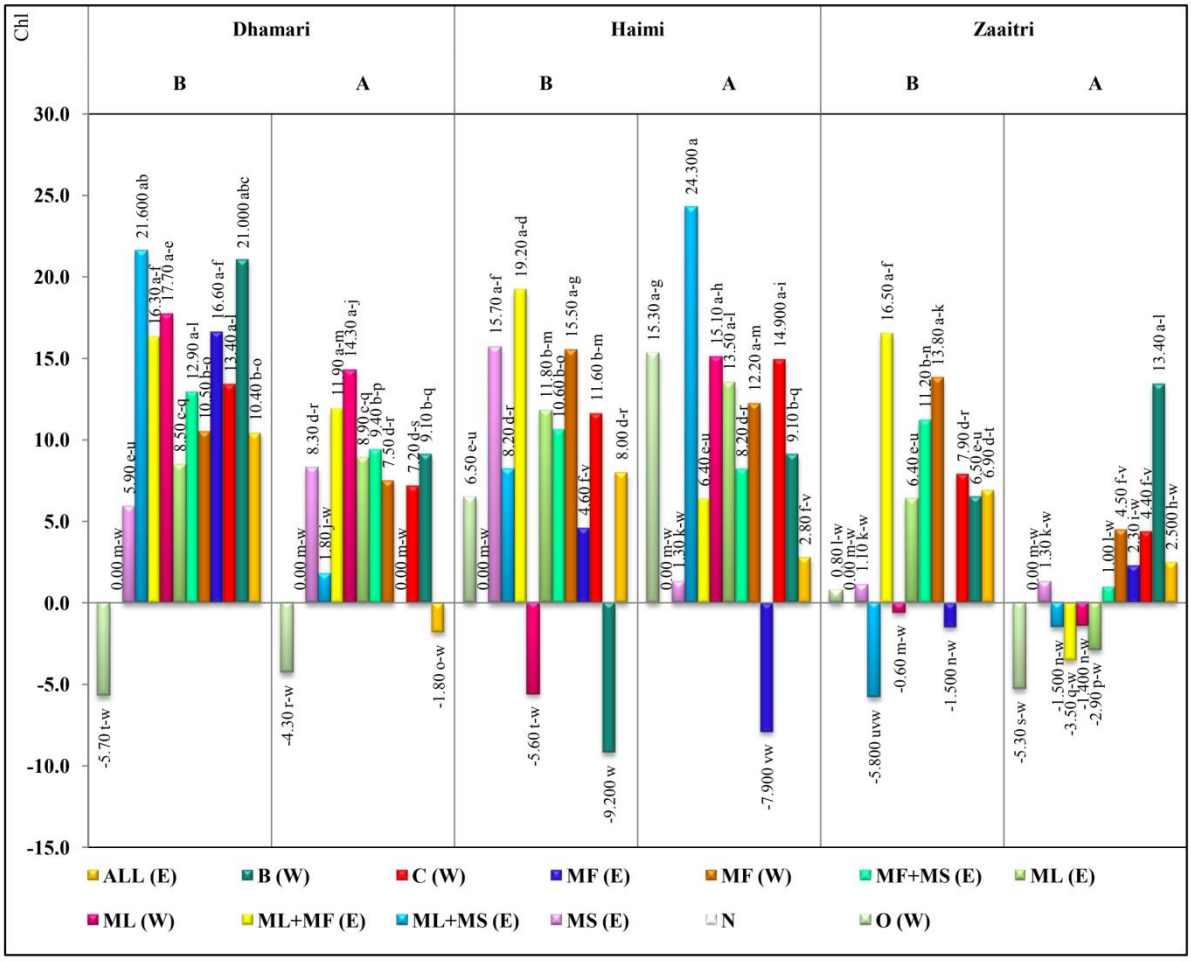

Fig. 6. The rate of change (%) in chlorophyll (SPAD) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract. "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

Root count (RC)

The ANOVA results revealed that cultivars, priming treatments, concentrations, and their interactions significantly influenced the mean root count of chili seedlings, as indicated by F-probabilities below 0.05 (Table 2). Among the cultivars, Haimi exhibited the highest root count seedling^{-1} (23.6), while Dhamari recorded the lowest (20.78). Priming with plant extracts had a significant impact on root count across treatments. Seedlings treated with ethanolic moringa flower extract [MF (E)] displayed the highest root count (25.81), which was statistically comparable to those treated with a mixture of moringa leaf and flower extracts [ML+MF (E)]. In contrast, the lowest root count (18.55) was observed in seedlings treated with aqueous moringa leaf extract [ML (W)]. In general, lower concentrations of plant extracts resulted in significantly higher root counts compared to higher concentrations (Table 2). The percentage of change in root count seedling^{-1} varied widely, ranging from an increase of 136.5% to a decrease of -81.7%, depending on the priming treatment, concentration, and cultivar (Fig. 7).

In the Zaaitri cultivar, most extracts at the lower concentration (10%) resulted in negative effects on root count. However, aqueous and ethanolic moringa flower extracts [MF (W) and MF (E)] produced positive changes, with the highest increase reaching 18.3% above the control. Additionally, beetroot extract [B (W)] at a 25% concentration significantly enhanced root count, achieving a 30.5% increase compared to the control (Fig. 7). For the Haimi cultivar, the combination of ethanolic moringa leaf and seed extracts [ML+MS (E)] at the lower concentration (10%) induced a remarkable 136.5% increase in root count over the control. In contrast, aqueous

moringa leaf extract [ML (W)] consistently showed negative effects. Positive changes were observed with prickly pear fruit extract [O (W)], the mixture of moringa leaf and flower extracts [ML+MF (E)], and aqueous moringa flower extract [MF (W)] at both concentrations (Fig. 7). In the Dhamari cultivar, the most substantial increase in root count (43.2%) occurred in response to a 10% concentration of aqueous moringa leaf extract [ML (W)] compared to the control. A similar increase was observed at the 20% concentration of the same extract. Extract concentration significantly influenced root count variations. Negative changes were observed with the mixture of moringa flower and seed extracts [MF+MS (E)] at both 10% and 20% concentrations, aqueous moringa leaf extract [ML (E)] at 10%, and lower concentrations of prickly pear fruit extract [O (W)] compared to the control (Fig. 7).

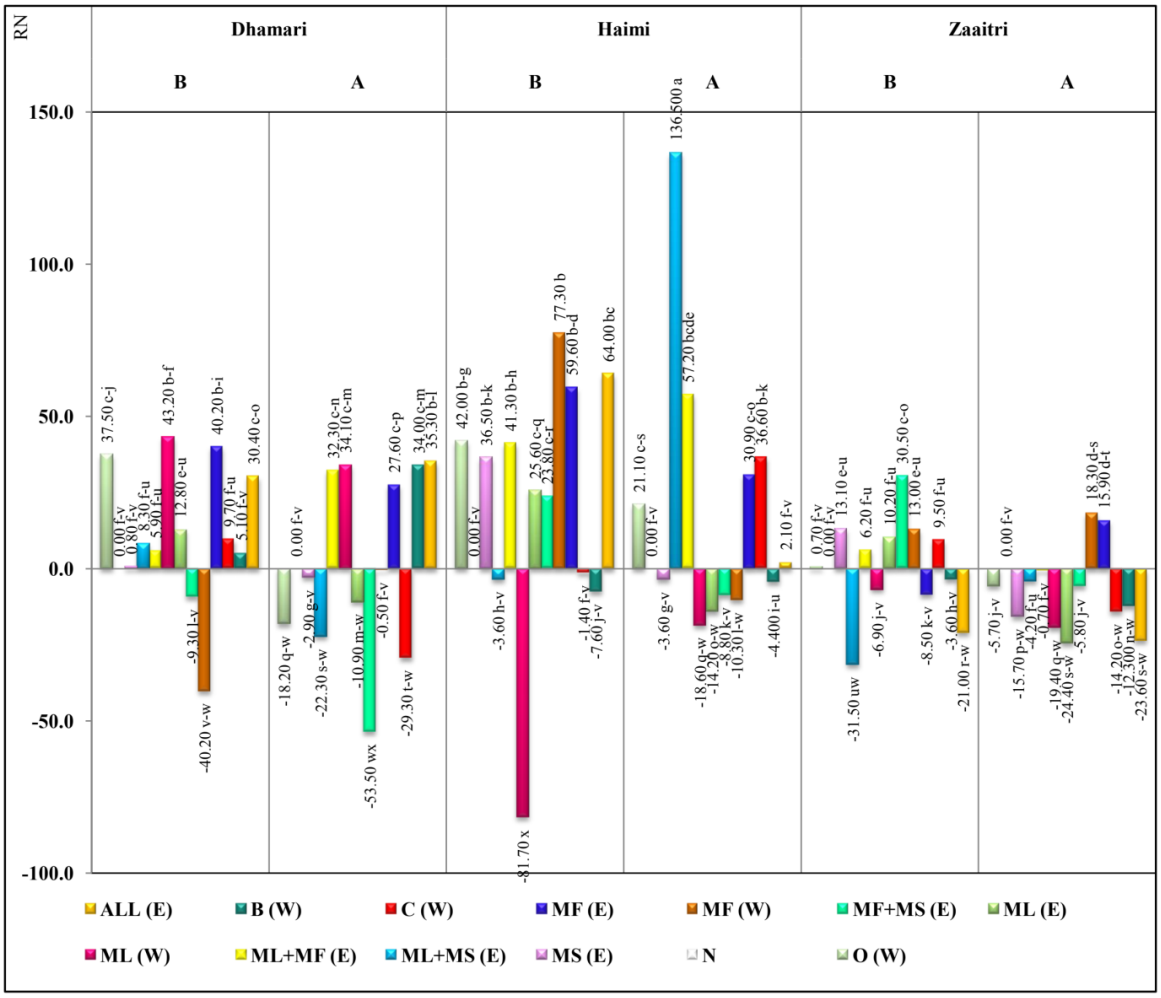

Fig. 7. The rate of change (%) in root count (RC) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and B the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

Specific root length (RSL cm cm^{-3})

The statistical analysis revealed that neither concentration nor the interaction between cultivars and concentrations had a statistically significant effect on specific root length (RSL, cm cm^{-3}) in chili seedlings ($P > 0.05$). However, other factors significantly influenced RSL (Table 2). Among the cultivars, *Zaaitri* exhibited the highest average RSL (223 cm cm^{-3}), while Dhamari recording the lowest (174.2 cm cm^{-3}). Priming extracts significantly affected RSL. Seedlings treated with ethanolic moringa flower extract [MF (E)] achieved the highest RSL (255 cm cm^{-3}), whereas those treated with aqueous moringa leaf extract [ML (W)] showed the lowest (171.9 cm cm^{-3}). The concentration of extracts did not significantly alter RSL (Table 2). Figure 8

illustrates the changes in RSL compared to the control, reflecting the influence of cultivar, priming type, and concentration. In Zaaitri, most extracts decreased RSL compared to the control, except for the 10% ethanolic mixture of moringa flowers and seeds [MF+MS (E)], which caused a minor increase of 0.4%. The 20% moringa leaf and seed extract [ML+MS (E)] produced the most substantial reduction (-23.3%).

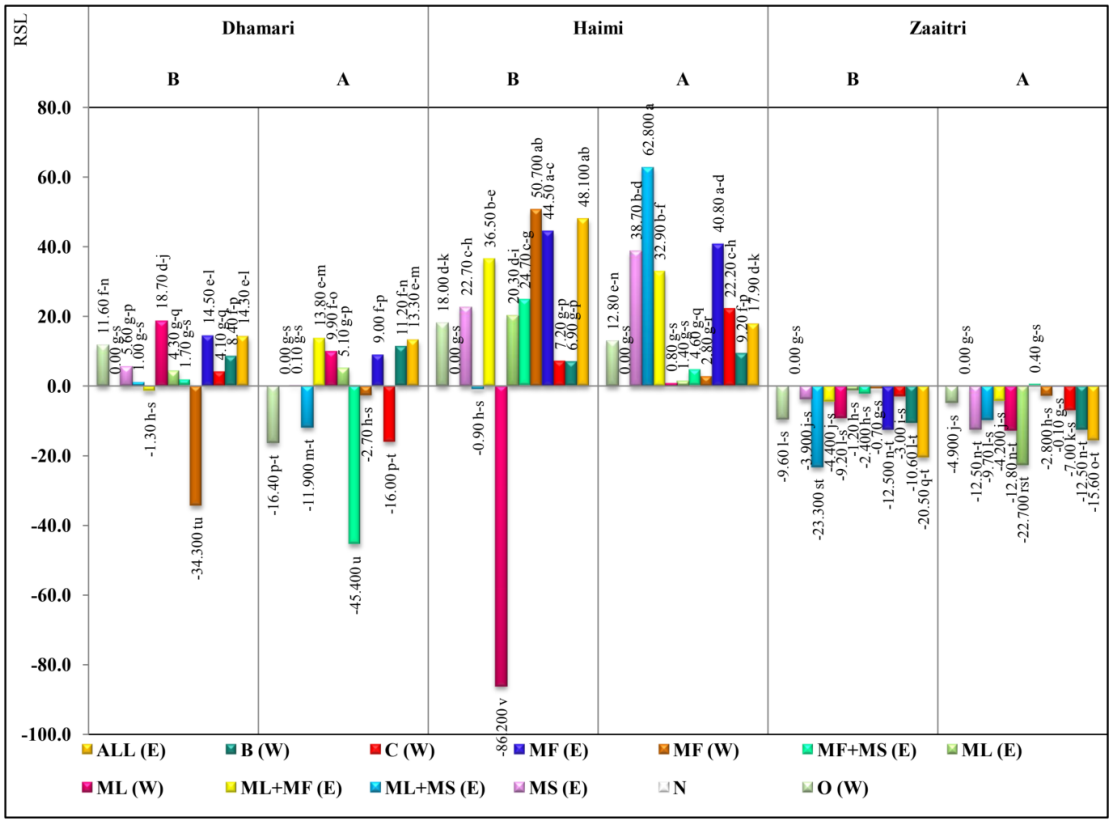

Fig. 8. The rate of change (%) over the control in Specific Root Length (RSL) as a result of seeds priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and B the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the *Haimi* cultivar, most extracts increased the RSL compared to the control. However, aqueous moringa leaf extract [ML (W)] caused the largest decline, reducing RSL by 86.2%. In contrast, the 10% aqueous mixture of moringa leaves and seeds [ML+MS (E)] produced the highest RSL increase at 62.8%. For the Dhamari cultivar, the 20% aqueous moringa leaves extract [ML (W)] increased the RSL by 18.7% compared to the control. Some extracts, including the 10% moringa flower and seed extract mixture [MF+MS (E)], 20% aqueous moringa flower extract [MF (W)], 10% prickly pear fruit extract [O (W)], and 10% carrot extract [C (W)], reduced the RSL compared to the control (Fig. 8).

Vegetative dry matter (VD%)

The percentage of dry matter in chili seedlings varied significantly among cultivars, priming extracts, and their interactions, as indicated by F-probability values below 0.05 (Table 2). The Haimi cultivar exhibited the highest dry matter percentage in its vegetative parts (23.41%), while the Dhamari cultivar had the lowest (22.26%) (Table 2). The shoot dry matter percentage of seedlings was significantly affected by the type of plant extract used for priming. The highest dry matter percentage (24.27%) was observed in seedlings treated with aqueous moringa leaf extract [ML (W)]. This value was statistically comparable to those treated with aqueous moringa flower extract [MF (W)], beetroot extract [B (W)], prickly pear fruit extract [O (W)], and the mixture of all ethanolic moringa extracts [ALL (E)], which included all moringa parts. In contrast, the lowest dry matter percentage (21.6%) was recorded in seedlings primed with a combination of ethanolic moringa leaf and flower extracts [ML+MF (E)]. Extract concentration levels did not significantly influence the dry

matter percentage (Table 2). However, the percentage of change in dry matter content in the vegetative parts of seedlings differed significantly across treatments, ranging from a maximum increase of 72.95% to a minimum decrease of -83.12% (Fig. 9).

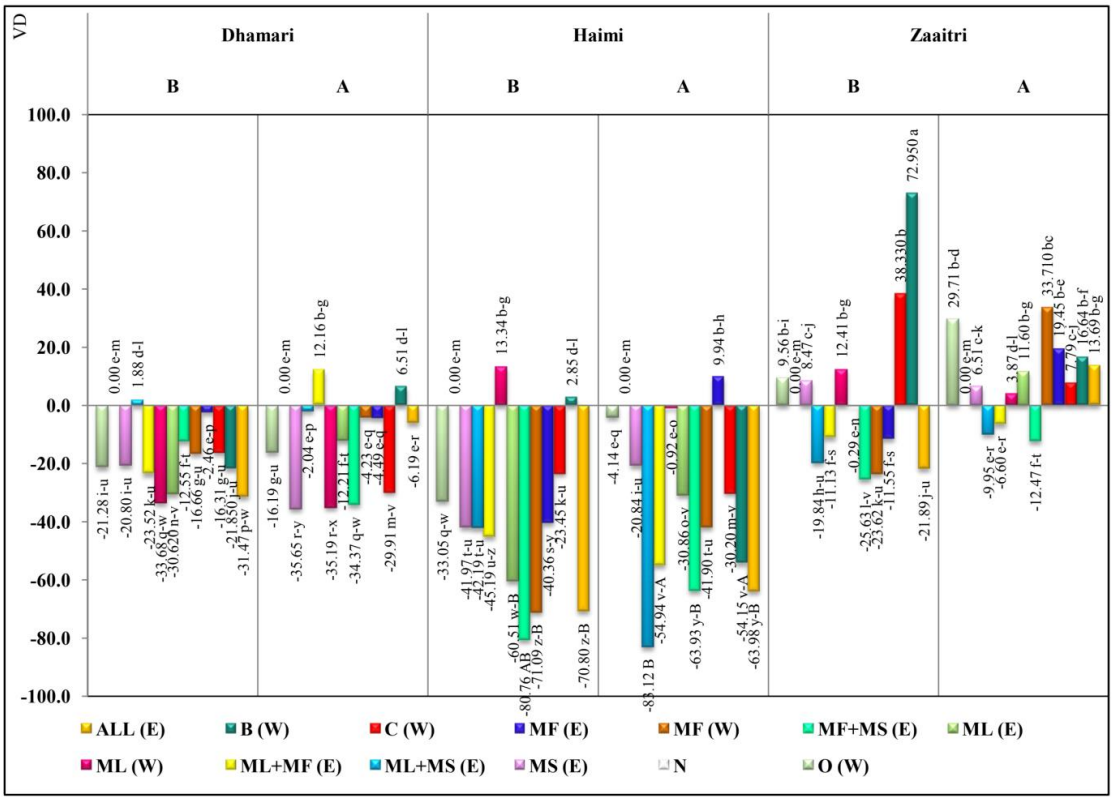

Fig. 9. The rate of change (%) on shoot dry mater (VD) compared to the control due to seed priming with plant Biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Zaaitri cultivar, seedlings primed with red beetroot extract [B (W)] at its highest concentration (25%) exhibited a substantial 72.95% increase in dry matter content compared to the control. Priming treatments that resulted in negative changes did not differ significantly from the control (Fig. 9). The Haimi cultivar showed distinct responses to priming, with most plant extracts causing reductions in dry matter content. However, positive effects were observed in seedlings primed with 10% ethanolic moringa flower extract [MF (E)], 20% aqueous moringa leaf extract [ML (W)], or red beetroot extract [B (W)]. Among these, the highest increase (13.34%) was recorded in seedlings treated with 20% aqueous moringa leaf extract (Fig. 9). In the Dhamari cultivar, the response pattern was similar to that of the Haimi cultivar. Most extracts resulted in reductions in dry matter content. Exceptions included seedlings primed with 10% red beetroot extract [B (W)] or 10% of the ethanolic moringa leaf and flower mixture [ML+MF (E)], which achieved a maximum positive increase of 12.16%. Treatments involving individual moringa extracts led to significantly lower dry matter content compared to the control (Fig. 9).

Root dry matter content (RD%)

According to the analysis of variance in Table 2, all individual factors (cultivars, plant extracts, and extract concentrations) and their interactions significantly influenced the root dry matter percentage in chili seedlings, as evidenced by F values below 0.05. The Haimi cultivar demonstrated the highest root dry matter percentage (16.31%), while the Dhamari cultivar exhibited the lowest (13.53%) (Table 2). Priming with different extracts significantly affected root dry matter percentages. Ethanolic moringa seed extract [MS (E)] resulted in the highest root dry matter percentage (16.86%), which was statistically comparable to treatments with prickly pear extract [O (W)], aqueous moringa leaf extract [ML (W)], aqueous moringa flower

extract [MF (W)], the mixture of moringa flowers and seeds [MF+MS (E)], and ethanolic moringa flower extract [MF (W)]. Red beetroot extract [B (W)] produced the lowest root dry matter percentage (12.95%), which was significantly lower than most other extracts, except for the moringa leaf and flower mixture [ML+MF (E)]. Lower extract concentrations consistently yielded higher root dry matter percentages than higher concentrations (Table 2). The percentage of change in root dry matter ranged from an increase of +89% to a decrease of -37.7%, depending on the priming type, concentration level, and cultivar (Fig. 10). In the Zaaitri cultivar, several treatments showed positive effects, including 10% and 20% aqueous moringa flower extract [MF (W)], 10% aqueous moringa leaf extract [ML (W)], 25% prickly pear extract [O (W)], and 20% ethanolic moringa leaf and flower mixture [ML+MF (E)]. The greatest increase (15.7%) was observed in seedlings pre-treated with 10% aqueous moringa leaf extract.

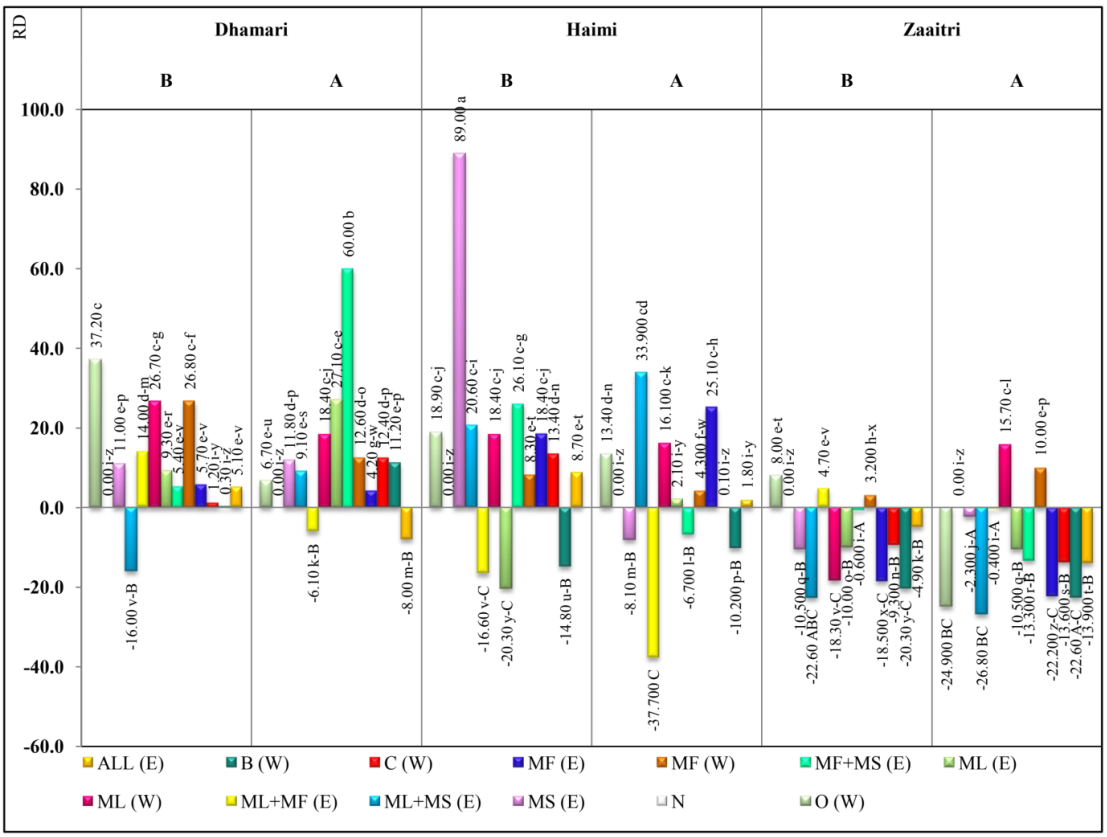

Fig. 10. The rate of change (%) on the percentage of root dry matter (RD) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Haimi cultivar, root dry matter percentage increased by 89% compared to the control when seeds were primed with 20% moringa seed extract [MS (E)]. The priming extract significantly influenced the rate of change, with treatments such as red beetroot extract [B (W)] and the mixture of moringa leaves and flowers [ML+MF (E)] at both 10% and 20% concentrations resulting in negative changes. Additionally, increasing the concentration of moringa leaf extract [ML (E)] reduced root dry matter content in Haimi seedlings compared to the control (Fig. 10). In the Dhamari cultivar, most ethanolic extracts had positive effects, with notable exceptions. Seedlings pre-treated with 20% moringa leaf and seed mixture [ML+MS (E)], 10% moringa leaf and flower mixture [ML+MF (E)], and 10% of the mixture of all ethanol extracts [ALL (E)] exhibited negative outcomes. However, pre-treatment with 10% moringa flower and seed ethanolic extract resulted in a 60% increase in root dry matter content in Dhamari seedlings (Fig. 10).

Shoot root ratio-1 (S:R)

The analysis of variance revealed that all experimental factors—cultivars, priming extracts, and extract concentrations—

significantly influenced the shoot-to-root dry matter ratio in chili seedlings, with F-values below 0.05 (Table 2). The Haimi cultivar exhibited a significantly lower shoot-to-root ratio (1.53) compared to the other cultivars, which had an average ratio of 1.71. The dry matter ratios of seedlings varied notably based on the type of priming extract used, ranging from 1.5 for seedlings primed with moringa seed extract [MS (E)] to 1.82 for those treated with red beetroot extract [B (W)] or the ethanolic mixture of moringa leaf and seed extract [ML+MS (E)]. Higher extract concentrations led to significantly increased shoot-to-root ratios (Table 2). The changes in the shoot-to-root dry matter ratio relative to the control ranged from a 42.9% increase to a 43.9% decrease (Fig. 11).

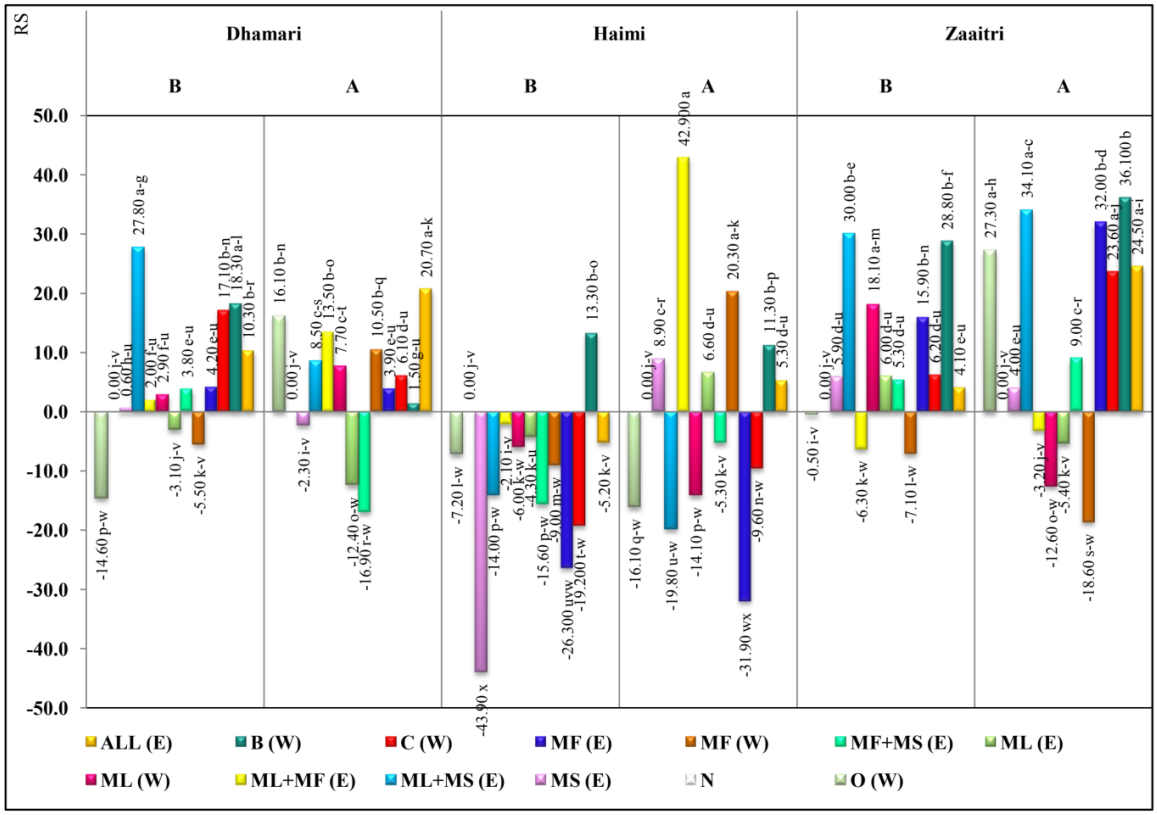

Fig. 11. The rate of change (%) in shoot to root ratio (S/R) compared to the control due to seed priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Zaaitri cultivar, negative changes in the shoot-to-root ratio were observed with moringa flower extract [MF (W)] at both high (20%) and low (10%) concentrations, moringa leaf extract [ML (E)] at 10%, and the moringa leaf and flower mixture [ML+MF (E)] at both 20% and 10% concentrations. In contrast, all other extracts resulted in positive changes, with the highest increase (36.1%) recorded in seedlings treated with beetroot extract [B (W)] at 10% concentration (Fig. 11). For the Haimi cultivar, the ethanolic mixture of moringa leaves and flowers [ML+MF (E)] at 10% concentration produced a 42.9% increase in the shoot-to-root ratio compared to the control. However, higher concentrations of all extracts, except beetroot extract [B (W)], resulted in negative values. Additionally, moringa flower and seed extract [MF+MS (E)] and moringa leaf extract [ML (W)] at 10% concentration also caused negative changes relative to the control (Fig. 11). In the Dhamari cultivar, most extracts led to positive changes, except for the ethanolic mixture of moringa flower and seed extracts [MF+MS (E)] at 10%, moringa leaf extract [ML (E)] at both concentrations, moringa seed extract [MS (E)] at 10% and 25%, and prickly pear fruit extract [O (W)] at the high concentration. Despite these exceptions, the Dhamari cultivar seedlings exhibited a 27.8% higher shoot-to-root ratio when primed with the 10% ethanolic mixture of moringa leaf and seed extracts [ML+MS (E)] compared to the control (Fig. 11).

Organic carbon percentage (OC%)

The percentage of organic matter was significantly influenced by cultivars, extracts, plant portions, and, to a lesser extent, concentrations (Table 3). According to the ANOVA results ($P < 0.05$), significant differences were observed for plant parts, extracts, and cultivars, while concentrations had no significant effect. The Haimi cultivar exhibited the highest organic carbon content (52.75%), whereas the Zaaitri cultivar recorded the lowest (45.63%). Among the extracts, carrot extract [C (W)] showed the highest organic carbon percentage (50.53%), which was not significantly different from the control. In contrast, the lowest value (45.97%) was observed in the ethanolic mixture of moringa leaf and seed extracts [ML+MS (E)], which also did not significantly differ from the control. Roots contained significantly higher organic carbon levels than shoot parts, as shown in Table 3.

Table 3. *F* probability values and means for the individual factors (cultivar, extract, concentration, and plant part) affecting mineral content and organic carbon parameters in the shoot and root of 60-day old chili seedlings.

Source of variation	OC	MM
Cultivar (G)	**<.001**	**<.001**
Zaaitri	45.63^{c}	21.34^{a}
Haimi	52.75^{a}	9.06^{c}
Dhamari	47.46^{b}	18.19^{b}
Lsd 0.05	1.245	2.146
Extract (E)	**<.001**	**<.001**
ALL (E)	48.14^{abc}	17^{a-c}
B (w)	50.33^{a}	13.24^{c}
C (w)	50.53^{a}	12.89^{c}
MF (E)	50.26^{a}	13.35^{c}
MF (w)	48.92^{a}	15.67^{bc}
MF+MS (E)	46.48^{a}	19.86^{ab}
ML (E)	50.03^{a}	13.74^{c}
ML (w)	48.16^{a-c}	16.98^{a-c}
ML+MF (E)	46.6^{bc}	19.65^{ab}
ML+MS (E)	45.97^{c}	20.75^{a}
MS (E)	47.97^{a-c}	17.3^{a-c}
N	48.18^{a-c}	16.94^{a-c}
O (w)	50.36^{a}	13.18^{c}
Lsd 0.05	**2.592**	**4.468**
Concentration (C)	**0.064**	**0.064**
A	49.09^{a}	15.37^{a}
B	48.13^{a}	17.02^{a}
Lsd 0.05	**1.016**	**1.752**
Plant part (P)	<.001	<.001
Root	53.05^{a}	8.55^{b}
Shoot	44.17^{b}	23.84^{a}
Lsd 0.05	**1.016**	**1.752**
G × E	0.004	0.004
G × C	0.003	0.003
E × C	0.301	0.301
G × P	<.001	<.001
E × P	0.003	0.003
C × P	0.019	0.019
G × E × C	0.113	0.113
G × E × P	0.012	0.012
G × C × P	0.001	0.001
E × C × P	0.214	0.214
G × E × C × P	0.141	0.141

(E) refers to ethanolic extract, (W) to water extract; "A" indicates the lower concentration, and "B" denotes the higher concentration. Means of individual factors sharing the same lowercase Latin letters are not significantly different at the 0.05 level, as determined by the Least Significant Difference (LSD 0.05) test.

Table 3 indicates that the interaction between cultivar, extract, concentration, and plant part were not significant. However, the three-way interaction (cultivar × extract × plant part) was significant ($P < 0.05$), with an F value of 0.012. Figure 12 demonstrates the effects of this three-way interaction on organic carbon content.

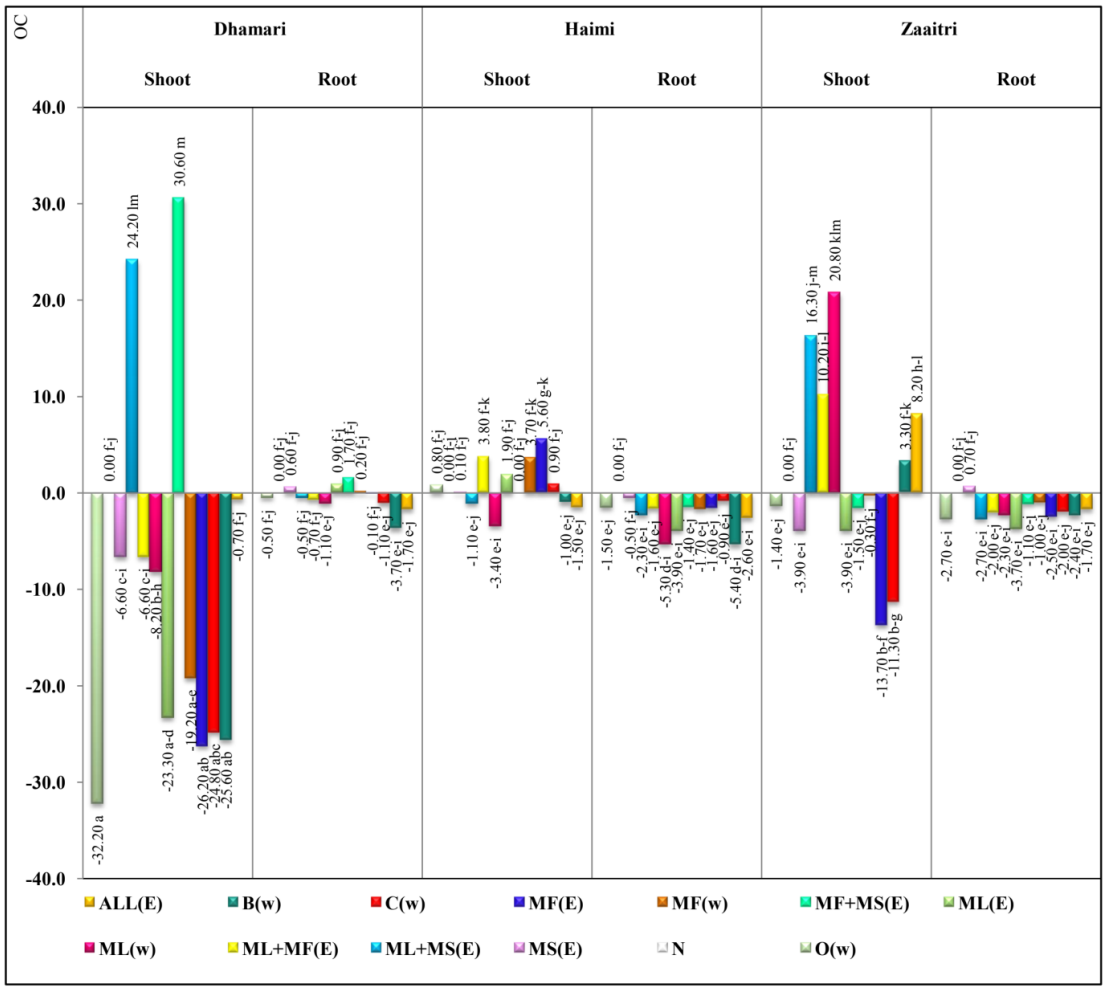

Fig. 12. The rate of change (%) over the control in organic carbon (OC) as a result of seeds priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Dhamari cultivar, the mixtures of moringa flower and seed extracts [MF+MS (E)] and moringa leaf and seed extracts [ML+MS (E)] resulted in the highest increases in organic carbon content in the vegetative parts, with increases of 30.6% and 24.2%, respectively, compared to the control. Conversely, prickly pear fruit extract [O (W)] caused the largest reduction, decreasing organic carbon content by 32.2%. In the roots, no significant differences were observed between the control and the extracts, although carrot extract [C (W)] exhibited the largest decrease (Fig. 12). In the Haimi cultivar, organic carbon content in the shoots showed no significant differences between the control and the extracts. In the roots, all extracts displayed a negative trend in carbon content, but none of these reductions were statistically significant compared to the control (Fig. 12). In the Zaaitri cultivar, moringa leaf extract [ML (W)] increased organic carbon content in the shoots by 20.8% compared to the control. The other extracts exhibited negative effects on the shoot carbon content, though none of these effects were significantly different from the control. In the roots, all extracts, except for moringa seed extract [MS (E)], showed negative values, but none differed significantly from the control (Fig. 12).

Percentage of mineral matter (MM%)

The mineral composition in both shoot and root growth showed notable variations depending on the cultivar, type of extract, and plant part, although differences due to extract concentrations were not statistically significant, as indicated by the ANOVA ($P < 0.05$) (Table 3). The Zaaitri cultivar recorded the highest mineral content (21.34%), while the Haimi cultivar exhibited the lowest (9.06%). The effects of plant extracts on mineral content ranged from 12.89%

for carrot extract [C (W)] to 20.75% for the combination of moringa leaf and seed extracts [ML+MS (E)]. Notably, the mineral content in shoots was significantly higher than that in roots (Table 3). While the overall interaction of all study factors (cultivars × extracts × concentrations × plant part) was not significant (Table 3), the three-way interaction of cultivars, extracts, and plant parts was significant, as illustrated in Figure 13.

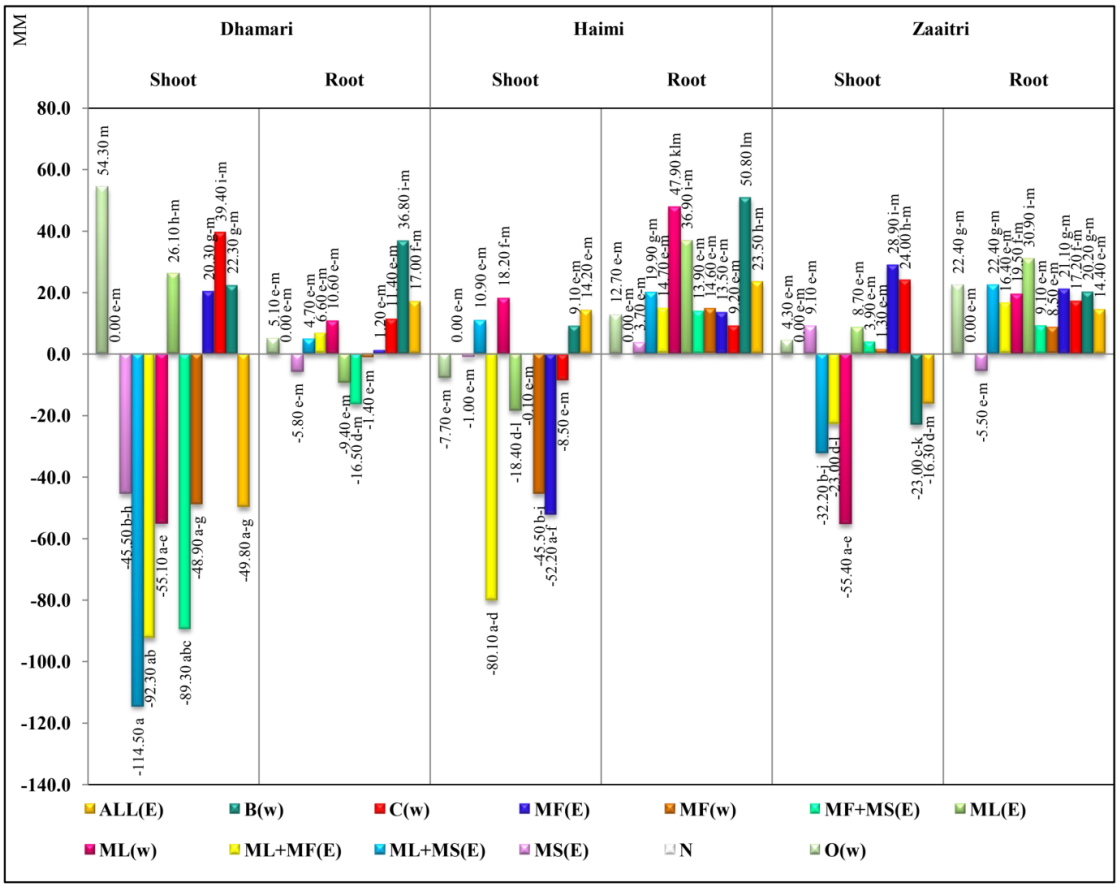

Fig. 13. The rate of change (%) in the control in mineral matter (MM) as a result of seeds priming with plant biostimulants. (E) refers to ethanolic extract, (W) to water extract, "A" indicates the lower concentration, and "B" the higher concentration. Values sharing the same uppercase or lowercase Latin letters are not significantly different at a significance level of 0.05 according to Duncan's test.

In the Dhamari cultivar, prickly pear fruit extract [O (W)] increased shoot mineral content by 54.3% compared to the control. In contrast, moringa seed extracts [MS (E)], both individually and in combination with moringa leaf or flower extracts, significantly reduced mineral content. Other extracts caused insignificant changes, either positive or negative. In the root, no significant differences were observed between the extracts and the control, though carrot extract resulted in the highest increase (Fig. 13). In the Haimi cultivar, the maximum shoot mineral content (18.20%) was achieved with an aqueous extract of moringa leaves [ML (W)], though this did not differ significantly from the control. Conversely, the mixture of moringa leaf and flower extracts [ML+MF (E)] significantly reduced shoot mineral content by 80%. In the root, all extracts showed positive effects, with beetroot extract [B (W)] producing a 50.8% increase, although this change was not significantly different from the control (Fig. 13). For the Zaaitri cultivar, the aqueous moringa leaf extract [ML (W)] led to the lowest shoot mineral content, reducing it by 55.4% compared to the control. However, no significant differences were found between the positive and negative changes caused by different extracts. In the root, all extracts except moringa seed extract [MS (E)] increased mineral content, but none of these increases were significantly different from the control (Fig. 13).

Correlation between parameters

Figure 14 illustrates several significant correlations between growth and physiological

traits. Specific root length exhibited a strong positive correlation with the number of roots ($r = 0.907$, $P < 0.01$). For the total leaf area (TLA) trait, the strongest positive correlation was observed with leaf count (LC) ($r = 0.786$, $P < 0.01$). Chlorophyll content showed positive correlations with the percentage of dry matter in the shoot (SD) and roots (RD), with correlation coefficients of 0.372 and 0.284, respectively ($P < 0.01$). However, chlorophyll content was moderately negatively correlated with vegetative growth characteristics. Shoot mineral matter (MM) was positively correlated with stem diameter ($r = 0.224$, $P < 0.01$) but negatively correlated with organic carbon content. Similarly, shoot height (SH) demonstrated a positive correlation with stem diameter (SD) ($r = 0.442$, $P < 0.01$).

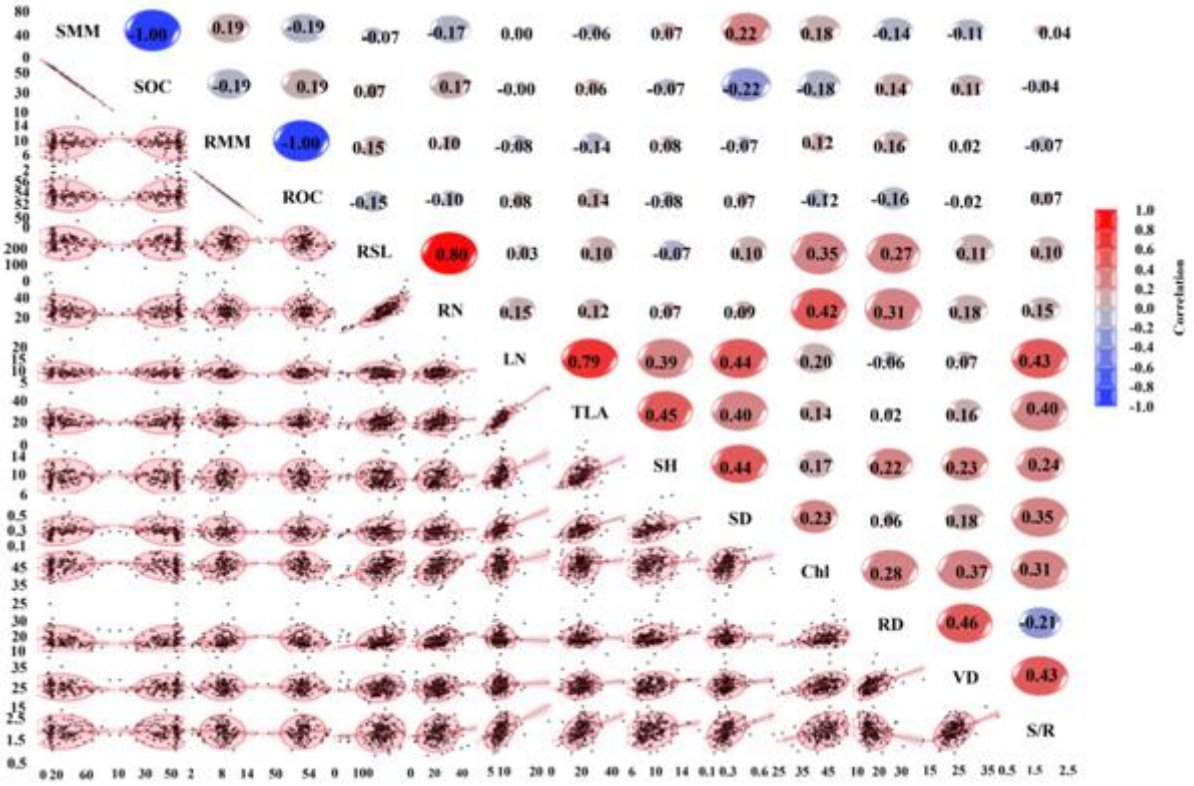

Fig. 14. A correlation matrix (Pearson's correlation) displaying the relationships among parameters investigated in the current study. Significant correlations are detailed below the diagonal, whereas above the diagonal, there are correlations between parameters under various treatments. The degree of correlations between these parameters under treatment is shown by varying sizes of circles and shades of color that correspond with different correlation values.

Discussion

Plant extracts have gained increasing popularity due to their numerous advantages, including enhancing seedling quality, promoting growth, and supporting the development of root and shoot systems. However, a key challenge remains: the prolonged germination time of pepper seeds, which delays their readiness for transplanting into greenhouses or fields. The chili cultivars examined in this study exhibited notable variations in various traits, largely influenced by their genetic backgrounds. While *Capsicum frutescens* is associated with the Zaaitri and Haimi cultivars, *C. annuum* is linked to the Dhamari cultivar. Despite Zaaitri and Haimi both belonging to *C. frutescens*, their measurable differences highlight the unique reactions across these cultivars. The high genotypic diversity of Yemeni chili peppers has previously been documented by Aldobai and Al-shabi (2010) and Colonna et al. (2019). Beetroot extract [B (W)] significantly increased the dry matter percentage in seedling shoots but had a comparatively lower effect on root dry matter. This suggests that beetroot extract primarily enhances vegetative growth rather than root development. The increased chlorophyll content facilitated by the extract allows the plant to absorb more sunlight and conduct photosynthesis more efficiently, resulting in greater carbohydrate accumulation,

the development of new plant structures, and overall improved plant health. While beetroot extract promoted root mineral accumulation—particularly in the Haimi and Dhamari cultivars—it did not significantly influence vegetative growth compared to the control. The active compound betaine, a key component of beetroot, along with betalains, has been shown to mitigate salinity stress in *Carthamus tinctorius* L. (Kim et al., 2021). Moringa flowers, leaves, and seeds contain distinct active compounds, leading to varying effects on root and vegetative development. Among these, the flowers contain higher concentrations of water-insoluble aromatic chemicals compared to other plant parts. When combined into an ethanolic extract, moringa flowers [MF (E)] demonstrated notable improvements in several aspects of root and vegetative growth, particularly seedling height and diameter.

Moringa leaves are rich in active compounds, particularly zeatin, with concentrations reaching up to 200 μg g^{-1} (Fuglie, 2000; Davies, 2004; Basra et al., 2011; Davies, 2012; Abdalla, 2013). This high zeatin content likely explains why seedlings treated with fresh aqueous moringa leaf extract [ML (W)] outperformed those treated with dried ethanolic moringa leaf extract [ML (E)]. Plant hormones, including cytokinins like zeatin, tend to degrade during drying, reducing their efficacy. Cytokinins are known to promote cell division and overall plant development (Alqadasi et al., 2022). When applied externally, cytokinins also enhance auxin activity, creating a hormonal balance favorable for exceptional seedling development (Al-Madhagi, 2012). In this study, the application of fresh moringa leaf extract resulted in significant elongation, outperforming the control in seedling length and specific root length. This aligns with previous research demonstrating that fresh moringa leaf extract enhances plant height across various species, such as sunflower (Farhat et al., 2023), pepper (Matthew, 2016), and cowpea (Maishanu et al., 2017). Additionally, fresh moringa leaves accelerate chlorophyll production, enhancing photosynthesis and carbohydrate storage. This is evident in the increased dry matter content observed in both shoots and roots of treated seedlings. Noreen et al. (2024) found that soaking pea seeds in moringa extract before planting or applying the extract post-planting increased total carbohydrates, photosynthetic pigments (chlorophyll and carotenoids), and the fresh and dry weight of shoots and roots (Hassan et al., 2021). Moreover, the ability of moringa to boost chlorophyll in salinity-prone environments suggests a stabilization of photosynthetic enzyme activity and mineral equilibrium (Noreen et al., 2024). The ethanolic extract of dried moringa leaves [ML (E)] specifically increased chlorophyll content but had minimal effects on other vegetative or root traits. This enhancement is not solely due to hormonal activity; bioactive compounds in dried moringa leaves also contribute. For instance, fresh leaves contain higher levels of vitamin C but lower amounts of protein, calcium, potassium, and vitamin E compared to dried leaves (Devkota and Bhusal, 2020). Conversely, dried leaves are richer in protein (Islam et al., 2021). The amino acids and ascorbic acid in ethanolic extracts of dried leaves likely play critical roles in photosynthesis (Dolatabadian et al., 2009), with ascorbic acid acting as a cofactor for enzymes essential to the photosynthetic process (Xu et al., 2015).

Moringa leaves tend to outperform seeds, likely due to their higher mineral and vitamin content, with the notable exception of vitamin E, which is more abundant in the seeds (Islam et al., 2021). In this experiment, the application of moringa flower extract alone had more pronounced effects than when flowers were combined with seeds. The lack of improvement in the combined extract could stem from chemical antagonism between these parts, as seeds contain various fatty acids that may interfere with the beneficial effects of the flowers. On the other hand, combining moringa leaves and seeds enhanced shoot dry matter and increased root numbers, surpassing the results from using ethanolic extracts of either seeds or leaves alone. Prickly pear extract significantly enhanced root development and increased dry matter content in both shoots and roots. However, it had a detrimental effect on leaf count and chlorophyll levels, particularly at higher concentrations (Fig. 6). Its beneficial effects on dry matter accumulation and root growth are likely due to improved soil nutrient uptake and direct stimulation of photosynthesis. Dry matter formation occurs through the absorption of organic and mineral materials from the soil, coupled with carbon assimilation via photosynthesis, which may explain the higher dry matter and chlorophyll levels observed in seedlings treated with prickly pear extract compared to the control. Notably, the Zaaitri cultivar showed a greater response to the extract than the others. Priming seeds with carrot extract also significantly influenced the length of Zaaitri seedlings and the chlorophyll content in the Haimi and Dhamari cultivars. Previous research has shown that carrot extract enhances chlorophyll a and b content in sunflowers under both normal and drought-stressed conditions (Dawood et al., 2019). In another study, carrot extract showed benefits only in drought-stressed plants, such as pea (Arafa et al., 2021) and faba bean plants (Kasim et al., 2019), where it increased proline content and decreased reactive oxygen species (ROS). Carrot roots are nutritionally rich and packed with bioactive

components, antioxidants like phenolics and carotenoids, and vitamins A and C, as well as essential minerals like calcium, potassium, phosphorus, and magnesium. These nutrients contribute to enhanced chlorophyll formation (Waraich et al., 2011; Dawood et al., 2019; Arafa et al., 2021). Additionally, carrot extract plays a significant role in the production of indole acetic acid (IAA), which further supports plant growth (Dawood et al., 2019).

Conclusions

Using plant extracts significantly improved the growth of hot chili seedlings from Yemeni cultivars. Although the responses varied across cultivars, water extracts of moringa flowers [MF (W)], moringa leaves [ML (W)], and prickly pear fruits [O (W)] notably stimulated root development. Meanwhile, beetroot extract [B (W)], a combination of moringa flower and seed extracts [MF+MS (E)], and prickly pear extract [O (W)] were particularly effective in enhancing vegetative growth parameters. In terms of biomass-related traits, such as dry matter (DM%), mineral content (MM%), and organic content (OC%), the most promising results were observed with moringa seed extract, the moringa seed and flower extract combination, and beetroot extract. Since plant extracts contain a wide range of bioactive compounds, it is difficult to determine the exact mechanisms by which these extracts promote seedling growth without a more detailed investigation. However, it is likely that amino acids, macro- and micronutrients, and growth regulators like hormones contribute to their biostimulatory effects. Therefore, further research is needed to better understand the specific mechanisms through which plant extracts enhance chili seedling growth.

Acknowledgements

Special thanks and appreciation are extended to Engineer Hamir Heisam, Director of Al Nahda Nurseries, for his invaluable support and provision of the necessary resources to conduct this study.

Author Contributions

The authors of this article collaborated to conduct this study. EA, the first author of the manuscript, contributed to the experimental application, methodology, data recording, and the preparation of the original draft. IA contributed to the methodology, software, formal analysis, investigation, supervision, review, editing, and writing. All authors have read and agreed to the final version of the manuscript.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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