



**Impact of Using Foliar Spraying of *Moringa oleifera* Leaf Extract as
Bio-stimulant of Faba Bean (*Vicia faba*) Plants Grown Under Drought Stress**

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Program: Bachelor's degree in science and education (Primary) Science (Private)

Abstract

Drought is a critical environmental stressor that significantly decreases crop productivity. Previous studies have highlighted the beneficial effects of *Moringa oleifera* leaf extract (MLAE) in mitigating abiotic stresses, including drought, in plants. The external application of MLAE is widely recognized for its role in regulating various physiological and biochemical processes in plants exposed to drought stress. This study aimed to assess the impact of foliar application of MLAE₍₂₀₎ on faba beans (*Vicia faba* L. cv. Nubaria 1), focusing on growth parameters (shoot and root length, fresh and dry weights of shoots and roots), photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, and total pigments), leaf relative water content, non-enzymatic antioxidants (ascorbic acid and anthocyanin), osmolyte compounds (proline), as well as yield quantity and quality. Special attention was given to the total carbohydrate and protein content in the harvested plants grown under both normal and drought-stressed conditions. Drought stress treatments were applied based on soil water holding capacity, with irrigation levels set at 60% (moderate drought stress) and 40% (severe drought stress) of field capacity. *Moringa oleifera* leaf extract (MLAE) was prepared at a concentration of 20 times. Water deficit (60% and 40% of field capacity) markedly reduced faba bean growth, as well as the quantity and quality of yield, by decreasing photosynthetic pigments and relative water content (RWC). This was accompanied by significant increases in certain non-enzymatic antioxidants (ascorbic acid and anthocyanin) and the osmolyte compound proline. Conversely, the application of MLAE₍₂₀₎ improved all growth parameters and enhanced yield quantity and quality (total carbohydrates and total proteins) by positively influencing these physiological and biochemical traits in faba bean plants under both normal and drought stress conditions. Overall, MLAE₍₂₀₎ proved to be

the most effective treatment in mitigating the adverse effects of drought stress on faba bean plants. In conclusion, applying MLAE₍₂₀₎ as a foliar treatment not only enhanced the growth and yield of faba bean plants under normal irrigation conditions but also mitigated the adverse effects of drought stress on the plants.

Key Words:

Drought, *Moringa*, antioxidants, Osmolytes, yield

1. Introduction:

Improving agricultural productivity and food safety is vital due to anticipated population growth and increasing living standards (Roth and Galyon, 2024). The productivity of global agroecosystems is declining due to the negative climatic impacts of modernization, industrialization, and rapid population growth, raising concerns about food security (Lee et al., 2024). Innovative agricultural technologies and practices are crucial for sustainably boosting productivity while safeguarding environmental and economic health (Roth and Galyon, 2024). Drought results from insufficient precipitation, often coupled with high temperatures that increase water loss through evapotranspiration (Raposo et al., 2023). Human activities, such as intensive agriculture and deforestation, reduce soil's capacity to retain and absorb water, worsening drought conditions (Li et al., 2024). Drought significantly restricts the transportation and availability of water-soluble nutrients such as nitrogen, phosphorus, potassium, silica, sulfate, magnesium, nitrate, and calcium, impairing nutrient assimilation (Bista et al., 2018;

Rezayian et al., 2023). This leads to decreased crop yields due to reduced photosynthesis and physiological and morphological changes (Kapoor et al., 2020; Zafar et al., 2023). Abiotic stressors like drought elevate the production of reactive oxygen species (ROS), including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), and singlet oxygen (1O_2). This imbalance between ROS production and the antioxidant defence system leads to oxidative damage in plant cells. These radicals can compromise cellular integrity, denature proteins, damage DNA and RNA, and increase levels of unsaturated fatty acids, resulting in cellular content loss (Foyer, 2018). Additionally, increased lipid damage, elevated malondialdehyde (MDA) levels due to lipid peroxidation, and membrane degradation may result from lipid peroxidase activity (Nxele et al., 2017). Plants accumulate Osmo protectants and antioxidants, including proline, phenols, soluble sugars, and scavenging radicals like glutathione, ascorbate, α -tocopherol, flavonoids, carotenoids, anthocyanins and antioxidant enzymes such as glutathione reductase (GR), superoxide dismutase (SOD), and ascorbate peroxidase (APX), which help scavenge reactive oxygen species (ROS) (El-

Beltagi & Mohamed, 2013). Therefore, researching crop management techniques to mitigate drought stress is crucial, as consumers worldwide increasingly demand high-quality, healthy foods, leading to a growing interest in eco-friendly and organic farming practices (Daryanto et al., 2020). The use of natural materials called bio-stimulants is emerging as a promising approach attracting global attention (Farraji et al., 2020). While synthetic growth regulators are effective for crop production, their widespread use has resulted in soil and water contamination, posing toxic risks to humans (Yasmeen et al., 2013, 2014). Bio stimulants, which contain biologically active substances, enhance natural processes in plants such as nutrient uptake, photosynthesis, biomass production, and flowering. They also have the potential to improve plants' tolerance to environmental stresses (Emilia et al. 2020). Biologically significant bio stimulants with hormone-like activities have been extracted from plants like *Moringa oleifera* (Yasmeen et al. 2012). Depending on the plant species, bio stimulants comprise diverse compounds, including phenols, flavonoids, minerals, and natural phytohormones (Di Mola et al. 2019). Bioactive compounds in plant extracts can act as either growth stimulators or inhibitors, leading to a recent increase in the global market for bio stimulants due to their eco-friendliness and effectiveness on plant growth (Van Oosten et al., 2017). The *Moringa oleifera* tree, known as the Miracle Tree and Mother's Best Friend, belongs to the Moringaceae family. Its

various components – root, bark, gum, leaf, flower, fruit, seed, and seed oil – offer remarkable benefits for food, medicine, and industrial applications, making it highly valuable (Adekanmi et al., 2020). *Moringa* leaf aqueous extract (MLAE) naturally enhances plant growth, is cost-effective, and improves plant tolerance to various environmental conditions, including drought. Further research is urgently needed due to its high levels of proteins, antioxidants (ascorbic acid, flavonoids, phenolics, carotenoids), essential minerals (P, Ca, Fe, K, Cr, Cu, Mg, Mn, Zn), amino acids, vitamins A, C, and B-complex, and plant hormones, particularly cytokinins like zeatin (Zahidul et al., 2021). Water stress depletes cytokinin levels in plants, but the high zeatin content in MLAE boosts its effectiveness as a natural compound that enhances plant tolerance under stressful conditions (Khan et al., 2022). Egypt has been significantly impacted by climate change in recent years, facing rising temperatures and pollution of freshwater due to human activities and global warming. The construction of the Grand Ethiopian Renaissance Dam has exacerbated the country's water scarcity for irrigation (Hegazi et al., 2018). Consequently, adopting sustainable and green technologies is essential to protect crops from water shortages (Carlo et al., 2023). We aimed to reduce the effects of water deficit stress on the growth and yield of faba bean plants (*Vicia faba*) through the application of *M. oleifera* leaf aqueous extract. Faba beans are a vital crop in Northern Africa, rich in organic

materials and minerals such as proteins, carbohydrates, thiamin, folic acid, and tocopherols, making them a key source of nutrition for both humans and animals in the food industry (Tiwari et al. 2020). Abiotic stressors like water deficiency affect faba bean plant growth and metabolism. This study aimed to evaluate the potential benefits of using aqueous extracts of *Moringa* leaves (MLAE) as a bio-organic stimulant to mitigate drought effects in faba bean (*Vicia faba*) plants.

2. PLANT MATERIALS

Healthy faba bean seeds (*Vicia faba* L. cv. Nubaria 1) were obtained from the Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt, for this study. This cultivar is noted for its early flowering, high seed yield (95 g per 100 seeds), and significant protein and carbohydrate content (24% and 58%, respectively) (Qabil et al. 2018). *M. oleifera* leaves were sourced from the botanical garden at Ain Shams University's Faculty of Education.

3. Methods of Research and the tools used

Moringa leaf aqueous extract (MLAE)
Preparation:

Fresh leaves were washed and frozen overnight at -5°C . Next, MLAE was extracted in a blender using water (1:10 wt./v) and then filtered through paper. The extract was centrifuged at $8,000 \times g$ for 15 minutes and diluted with distilled water and leaf extract to achieve a final concentration ratio

of 1:20 (MLAE₍₂₀₎) (Yasmeen et al., 2012). This diluted MLAE was used for foliar treatment on faba bean plants, while the chemical components of *Moringa* leaf extract were analysed and recorded (Table1).

The experiment took place in the winter of 2024–2025 at the Faculty of Education greenhouse, Ain Shams, Cairo, Egypt, with planting on November 6th. Faba bean seeds of uniform size and weight were sterilized in a 0.1% HgCl_2 solution for 1 minute with continuous shaking, then thoroughly rinsed in distilled water. Ten seeds were sown at a depth of 5 cm in each 25 cm diameter plastic pot containing 3.5 kg of homogeneous loamy dried soil (80% sand, 15.5% silt, 4.5% clay, 0.45% organic matter, pH 7.8, EC 0.4 dSm^{-1}). The pots were maintained under natural light and dark conditions, with temperatures ranging from 28°C during the day to 16°C at night and a relative humidity of 75%. The pots were divided into two groups based on two factors: MLAE dilution (20 times) and soil water levels (well-watered and drought stressed). Seven weeks after sowing, drought stress was applied according to soil water holding capacity, with irrigation levels maintained at 80% (well-watered), 60% (moderate drought stress), and 40% (severe drought stress) of field capacity. The plants were divided into six groups and treated as follows: The 1st group served as a control, remaining well-watered at 80%. The 2nd group was well-watered and sprayed with MLAE₍₂₀₎ extract. The 3rd group received irrigation at 40% of water-holding capacity. The 4th group was also irrigated at 40% of

water-holding capacity and sprayed with MLAE₍₂₀₎ extract. The 5th group was irrigated at 60% of water-holding capacity. The 6th group received irrigation at 60% of water-holding capacity and was sprayed with MLAE₍₂₀₎ extract. Foliar application of MLAE extract was conducted twice, at 25- and 35-days post-sowing (20 ml per plant). Plants were sampled 45 days after treatment initiation (vegetative stage), and yields were collected 64 days post-sowing. Each treatment was tested with three replications. On day 40, plant samples were collected from pot culture to assess their biochemical parameters. The measurements included shoot and root lengths, fresh and dry weights of shoots and roots, photosynthetic pigments, osmolyte compounds (proline and soluble sugars). Faba bean plants were harvested, and the leaves and roots were separated; after air drying, the samples were heated to 85 °C in an oven, followed by weighing the dried samples. The relative water content (RWC) was determined using the method of Shams et al. (2019). Fresh weight (FW) was measured immediately after cutting 10 mm diameter leaf discs. The turgor weights (TW) were obtained by floating the discs in ultrapure water at 25 °C for 24 hours. Leaf discs were dried in an oven at 70 °C for 48 hours and then weighed to determine their dry weight (DW).

RWC (%) was calculated using the following formula:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Biochemical analysis

The determination of photosynthetic pigments

As recommended by Lichtenthaler (1987), chlorophyll a, b, and carotenoids were measured using a Spekol spectrophotometer (VEB Carl Zeiss) at 663 nm and 645 nm using the recommended wavelengths. Pigment content was determined as mg g⁻¹ fresh leaf weight.

The calculation formula is as follows:

$$\text{Chl-a (mg g}^{-1}\text{)} = (12.7 \times 663 \text{ nm}) - (2.69 \times 645 \text{ nm}) \times \frac{\text{The extraction volume}}{\text{Sample weight}} \times 100$$

$$\text{Chl-b (mg g}^{-1}\text{)} = (22.91 \times 645 \text{ nm}) - (4.68 \times 663 \text{ nm}) \times \frac{\text{The extraction volume}}{\text{Sample weight}} \times 100$$

$$\text{Total Chl (mg g}^{-1}\text{)} = \text{Chl-a} + \text{Chl-b.}$$

Assay for Antioxidant compound

Determination of Ascorbic acid Contents

Fresh plant samples were crushed in 6% trichloroacetic acid (TCA), centrifuged at 12,000 g for 15 minutes, and the filtrate was measured at 530 nm using a spectrophotometer, following the method of Mukherjee and Choudhuri (1983).

Determination of Anthocyanin (ACN) Contents

Anthocyanin levels were determined following Mancinelli (1990). Fresh plant samples were homogenized in 10 mL acidified methanol (1:99 v/v HCl: methanol), centrifuged at 18,000 g for 30 min at 4 °C, and filtered through Whatman No. 1 paper. The supernatant was incubated in the dark at

5 °C for 24 hours. Anthocyanin content was measured spectrophotometrically at 550 nm and expressed as $\mu\text{mol/g}$ FW, using an extinction coefficient of $\varepsilon = 33,000/\text{mol}$.

Assay for Osmo-protectants

Determination of proline content

Proline content was measured using the Bates et al. (1973) method. Fresh bean leaves (1 g) were homogenized in 3% aqueous sulfo-salicylic acid and filtered. The extract was mixed with glacial acetic acid and ninhydrin and incubated at 100°C for 1 hour, then cooled in an ice bath and mixed with toluene. The toluene phase was separated from the aqueous phase, and proline content was determined colorimetrically at 520 nm, expressed as $\mu\text{M g}^{-1}$ fresh weight.

Determination of Total Carbohydrates:

Total carbohydrates were determined colorimetrically using a modified Dubois et al. (1956) method, following Orabi (1994). Briefly, 0.03 mg of dry powder was hydrolyzed in 10 ml of 1N H_2SO_4 at 100°C for 60 minutes. After cooling, the hydrolysate was neutralized with approximately 0.1 g barium carbonate, filtered through Whatman No. 1 paper, and diluted to 100 ml with distilled water. 1 ml filtrate aliquot was mixed with 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid. After cooling in a water bath, the solution's optical density was measured at 490 nm using a Spekol spectro-colourimeter (VEB Carl Zeiss). Total carbohydrate concentration was determined using a D-glucose standard curve.

Total carbohydrate percentage was calculated as follows:

$$\% \text{ Total Carbohydrates} = \frac{\text{Conc. (ppm)} \times 100}{\text{Wt. of sample} \times 1000} \times 100$$

Where:

Conc. ppm = reading of spectrophotometer $\times$ slope of the standard curve.

Determination of total protein

Total proteins were extracted from 0.5 g powdered seeds by grinding in 5 ml phosphate buffer (pH 6.5) and centrifuging at 6000 g for 10 min. The supernatant was collected, and the residue was washed with 2 ml distilled H_2O . The supernatant and wash were combined. Total soluble protein content was determined using the Folin-Ciocalteu method, based on Lowry et al. (1951) with Hartree (1972) modifications. 0.1 ml of protein sample was added to 5 ml alkaline copper reagent, incubated for 15 min, followed by the addition of 0.5 ml diluted Folin reagent, and further incubation for 30 min at room temperature. Optical density was then measured at 750 nm. Distilled water was used as the blank, and total protein was determined by generating a standard curve with albumin solutions.

STATICAL ANALYSIS

The experiment employed a complete randomized block design. Data analysis was conducted using one-way ANOVA, with significance determined at a 95% probability level ($p \leq 0.05$) for the LSD and Duncan's multiple range tests.

4. Results of Research

Table 1 displays the nutrient-rich, antioxidant-packed, and phytohormone-rich chemical composition of *Moringa oleifera* leaf extract (MLAE) as reported by Hanafy (2017). The results presented in Table 2 illustrate the impact of water stress, the application of *Moringa oleifera* leaf extract (MLAE₂₀), and their interactions on various plant growth parameters, including shoot and root lengths, as well as the fresh and dry weights (g) of shoots and roots in faba bean plants. The findings reveal that all growth characteristics were significantly influenced by the applied treatments during the study season. Table 2 indicates that drought stress significantly impacts growth traits. The highest mean values for shoot and root lengths (37.4 cm and 25.6 cm, respectively), as well as fresh and dry weights of shoot (6.5 g and 1.5 g) and root (2.5 g and 0.43 g), were observed in plants irrigated with water under 60% drought stress. This treatment resulted in reductions of 13.2%, 12.6%, 5.7%, 16.7%, 7.4%, and 6.5%, respectively, compared to the control group. Conversely, the lowest mean values for these traits were recorded under the 40% water holding capacity treatment. In contrast, *Moringa oleifera* leaf extract (MLAE₂₀) significantly improved growth parameters under both normal and drought conditions (Table 2). Compared to untreated controls, MLAE increased shoot and root lengths by 25.3% and 7.2%, respectively, and fresh and dry weights of shoot and root by 23.2%, 22.2%, 11.1%, and 21.7%, respectively. Notably, MLAE

application combined with drought stress resulted in significantly higher growth compared to plants subjected to drought stress alone or well-watered controls. The effects of soil drought stress, foliar application of MLAE₍₂₀₎, and their interaction on the photosynthetic pigment content (chlorophyll a, chlorophyll b, and total pigments) in faba bean leaves are presented in Table 3. The data indicate significant variation in the photosynthetic pigment percentages of faba bean plants under drought stress, particularly between treatments at 60% and 40% of water holding capacity. Irrigation deficit treatments significantly reduced the percentages of chlorophyll a, chlorophyll b, and total pigments, with average decreases of 13% and 32.7% for chlorophyll a, 15.1% and 26.3% for chlorophyll b, 9.13% and 21% for carotenoids, and 13.5% and 29.3% for total pigments, respectively. The MLAE₍₂₀₎ application demonstrated remarkable improvements in the mentioned traits, with percentage increases of 18.6%, 24.9%, 15.7%, and 22.4% for chlorophyll a, chlorophyll b, carotenoids, and total pigments, respectively. Additionally, the interaction between drought stress and MLAE₍₂₀₎ on the photosynthetic pigment percentage in Vicia faba plants are presented in the same table. The treatment of 60% + MLAE₍₂₀₎ resulted in the highest pigment percentages in faba bean plants, recorded at 15.8%, 10.2%, 1.8%, and 12.8%, respectively. In contrast, the lowest pigment percentages were observed in plants subjected to 40% drought stress without the

application of MLAE. The effects of drought on the relative water content (RWC) of faba bean leaves are summarized in Table 4. Water deficit stress significantly reduced the RWC in faba bean plants, with the most substantial decrease observed under drought conditions at 40% (29.9%), followed by 60% (19.3%) compared to their respective untreated and irrigated controls. In contrast, under non-stress conditions, the foliar application of MLAE (20) enhanced the RWC by 7.2% compared to untreated and irrigated controls. The foliar application of MLAE (20) preserved normal relative water content (RWC) levels under water deficit conditions. As shown in Table 4, the interaction between drought stress and MLAE (20) foliar spray revealed that faba bean plants treated with MLAE (20) exhibited higher RWC across all irrigation treatments (60% and 40% of water holding capacity) compared to the control group. Drought stress induces the accumulation of antioxidant compounds (ascorbic acid and anthocyanin) and osmolytes (proline), which may scavenge reactive oxygen species (ROS). Table 4 presents the effects of drought stress, foliar application of MLAE (20), and their combination of ascorbic acid, anthocyanin, and proline content in faba bean plants. The results indicate that all measured characteristics were significantly influenced by the applied treatments. Specifically, MLAE (20) application significantly increased ascorbic acid, anthocyanin, and proline levels compared to the control. Foliar application of MLAE (20) improved faba bean plant

quality under drought stress (Table 4). Plants treated with MLAE (20) exhibited enhanced quality characteristics at both 60% and 40% water holding capacity. Under severe stress (40% water holding capacity), ascorbic acid, anthocyanin, and proline levels were highest in the absence of MLAE (20) (26.4, 48.9, and 22.4%, respectively), and further increased with MLAE (20) application (31.8, 70.1, and 24.8%, respectively). Table 5 demonstrates that drought stress significantly impacted faba bean plants, with reductions observed in the number of legumes per plant, the number of seeds per plant, and the weight of seeds per plant (g) as water-holding capacity decreased to 60% and 40%, compared to the unstressed control plants. Drought stress at 40% water holding capacity significantly reduced faba bean yield, decreasing the number of legumes/plants, seeds/plant, and seed weight/plant from 14.0, 3.57, and 36.31g to 10.31, 3.50, and 25.32g, respectively, representing a decline of 26.4%, 2.0%, and 30.3% compared to the control. MLAE (20) application significantly improved yield parameters in both stressed and unstressed plants. All irrigation treatments (60% and 40% water holding capacity) resulted in a notable reduction in total carbohydrates (TC) and total protein (TP) (13.3% and 13%, 16% and 19.7%, respectively) compared to the control plants (Table 5). The application of MLAE (20) either alone or in combination, significantly enhanced the biochemical components of the harvested faba bean seeds compared to the control.

Table (1): Some chemical components of *Moringa oleifera* leaf extract (dry weight basis)

Chemical components (mg g ⁻¹ DW)	
General composition (per 100 g)	
Calcium	5.165
Magnesium	4.859
Potassium	25.57
Total amino acids	211
Ascorbic acid (vitamin C)	5.36
Tochopherol (vitamin E)	112.5
Total phenols	3.4
Total flavonoids	7.0
IAA	0.75
GA3	0.60
ABA	0.2

Table (2): Effects of foliar spraying of *Moringa* leaf aqueous extract (MLAE) extract levels on growth parameters in faba bean plants grown under drought stress conditions.

Treatments	Shoot length (cm)	Root length (cm)	Fresh wt. of shoots (g)	Dry wt. of shoots (g)	Fresh wt. of roots (g)	Dry wt. of roots (g)
Control	43.1 ^d	29.3 ^b	6.9 ^c	1.8 ^b	2.7 ^{ab}	0.46 ^b
MLAE (20)	54.0 ^a	31.4 ^a	8.5 ^a	2.2 ^a	3.0 ^a	0.56 ^a
Hold water capacity 60%	37.4 ^e	25.6 ^c	6.5 ^c	1.5 ^c	2.5 ^{bc}	0.43 ^b
Hold water capacity 60%+ MLAE (20)	50.0 ^b	31.2 ^{ab}	7.8 ^b	2.1 ^b	2.8 ^{ab}	0.52 ^a
Hold water capacity 40%	30.2 ^f	24.2 ^c	6.0 ^d	1.3 ^c	2.0 ^c	0.39 ^b
Hold water capacity 40%+ MLAE (20)	48.1 ^c	30.1 ^{ab}	7.3 ^b	1.9 ^b	2.8 ^{ab}	0.46 ^b

Table (3): Effects of foliar spraying of *Moringa* leaf aqueous extract (MLAE) extract levels on photosynthetic pigments of leaves in faba bean plants grown under drought stress conditions.

Treatments	Chl a mg g ⁻¹ FW	Chl b mg g ⁻¹ FW	Carotenoids mg g ⁻¹ FW	Total pigments mg g ⁻¹ FW
Control	9.30 ^c	4.30 ^b	3.83 ^{ab}	15.19 ^d
MLAE (20)	11.03 ^{2a}	5.37 ^a	3.23 ^{ab}	18.60 ^a
Hold water capacity 60%	8.09 ^d	3.65 ^c	3.48 ^{ab}	13.14 ^c
Hold water capacity 60%+ MLAE (20)	10.73 ^b	4.74 ^b	3.76 ^a	17.14 ^b
Hold water capacity 40%	6.26 ^e	3.17 ^d	3.04 ^b	10.74 ^f
Hold water capacity 40%+ MLAE (20)	10.14 ^b	4.54 ^b	3.95 ^a	16.16 ^c

Table (4): Effects of foliar spraying of *Moringa* leaf aqueous extract (MLAE) extract levels on RWC, antioxidant compounds (ASA, ANCs) and osmolyte compound (proline) of leaves in faba bean plants grown under drought stress conditions.

Treatments	Relative water content (RWC) %	Ascorbic acid ASA) μmol g ⁻¹ FW	Anthocyanin (ANCs) (μg g ⁻¹ FW)	Proline (μg g ⁻¹ FW)
Control	50.2 ^b	38.87 ^e	18.57 ^f	27.69 ^f
MLAE (20)	53.8 ^a	42.56 ^d	19.14 ^e	30.09 ^e
Hold water capacity 60%	40.5 ^e	46.34 ^c	22.14 ^d	31.00 ^d
Hold water capacity 60%+ MLAE (20)	49.4 ^c	47.46 ^{bc}	25.57 ^c	32.29 ^c
Hold water capacity 40%	35.2 ^f	49.15 ^{ab}	27.65 ^b	33.88 ^b
Hold water capacity 40%+ MLAE (20)	45.1 ^d	51.24 ^a	31.59 ^a	34.56 ^a

Table 5. Impact of MLE (20) on seed yield and some biochemical constituents of yield of faba bean plants grown under drought stress conditions.

Treatments	Number of legumes/ plants	Number of seeds/ plants	weight of seeds/ plant	Total carbohydrates (TC) (%)	Total protein (TP) (%)
Control	14.0 ^c	3.57 ^b	36.31 ^e	45.32 ^e	23.90 ^{ab}
MLAE (20)	17.31 ^a	3.66 ^b	49.22 ^a	51.22 ^a	29.20 ^a
Hold water capacity 60%	13.17 ^c	3.55 ^b	34.52 ^d	39.3 ^d	20.80 ^d
Hold water capacity 60%+ MLAE (20)	16.0 ^b	3.52 ^a	43.30 ^b	50.04 ^b	27.50 ^{bc}
Hold water capacity 40%	10.31 ^e	3.50 ^b	25.32 ^f	38.22 ^f	19.2 ^{cd}
Hold water capacity 40%+ MLAE (20)	14.31 ^d	3.60 ^a	37.73 ^c	49.42 ^c	25.40 ^e

5. Interpretation of Results

Water scarcity in soil directly and visibly hampers plant growth, impacting agricultural productivity and threatening food security. As a result, cultivating drought-tolerant plants and crops has become a critical focus for researchers and farmers alike. Drought significantly reduced the shoot and root lengths, as well as the fresh and dry weights of shoots and roots in stressed *Vicia faba* cv. Nubaria 1 compared to the control plants. These findings align with those of Dawood et al. (2014), who reported that both fresh and dry shoot weights in canola decreased under increasing drought stress. Such reductions are likely attributed to metabolic disturbances caused by stress and the generation of reactive oxygen species (ROS). The reduction in shoot height under drought conditions can likely be attributed to a decline in cell elongation, turgor pressure, cell volume, and overall cell growth (Alam et al., 2013). Furthermore, drought disrupts plant-water relations, decreases shoot water content, induces osmotic stress, and hampers cell expansion, cell division, and overall plant growth (Theerakulpisut and Gunnula, 2012). Assaha et al. (2016) observed that water deficit significantly inhibited shoot and root growth in huckleberry (*Solanum scabrum* Mill.) plants. This suppression of growth under drought stress may stem from stress-induced metabolic disorders, the generation of ROS that reduces cell division and elongation, meristematic activity, and cell turgor, and a decrease in photosynthetic

capacity. Moreover, obstructed translocation vessels could impede water and nutrient transport (Embiale et al., 2016). The reduction in plant height under drought conditions (Table 2) may be attributed to the adaptive response of faba bean plants to drought stress. Initially, the plants redirect assimilates away from stem growth, prioritizing enhanced root development to improve water absorption. Moringa oleifera leaf extract (MLAE), recognized as a potential source of cytokinin and zeatin, significantly enhanced faba bean growth parameters under both normal and drought stress conditions (Table 2). In drought-stressed plants, MLAE notably improved growth metrics and physio-chemical attributes, even under the challenging conditions of water deficit. The obtained readings closely align with those reported by El-Lethy et al. (2024) in their studies on wheat plants. Moringa leaf extracts are widely recognized as an affordable and highly effective source of cytokinins, such as zeatin (Yang et al., 2006). Zeatin plays a crucial role in enhancing cell division and chlorophyll biosynthesis, ultimately promoting the growth and development of wheat (Taiz and Zeiger, 2015). Yasmeen et al. (2013) reported that wheat plants exhibited enhanced growth under drought conditions when treated with MLAE, primarily by modifying their antioxidant defence pathways. Moreover, the observed increases in shoot fresh and dry weight following MLAE treatment could be attributed to seedling growth-driven dry matter accumulation in the elongating stem

and increased stem weight (Ahmed et al., 2021). This improvement may also stem from the high concentrations of vitamins, antioxidants, inorganic compounds, and minerals present in MLAE (Bakry et al., 2021). Chlorophyll content must be preserved under water stress to sustain the photosynthetic capacity of plants. In this study, the levels of chlorophyll a, chlorophyll b, and carotenoids in faba bean tissues were significantly reduced under drought stress compared to the control (Table 3). This finding aligns with the work of Jomo et al. (2016), who reported that soil water deficits significantly decreased chlorophyll content in amaranth species as drought stress intensified. The disruption of thylakoid membranes triggers photo-oxidation or chlorophyll degradation through the activation of proteolytic enzymes such as chlorophyllase, along with chloroplast deterioration and stomatal closure (Mafakheri et al., 2010). Conversely, a potential reduction in stomatal conductance may lead to chlorophyll degradation, resulting in damage to the photosynthetic apparatus in plants (Rong-Hua et al., 2006). Additionally, the decline in photosynthetic activity can be attributed to the inhibitory effects caused by reduced water content in leaves (Vurayai et al., 2011). The foliar application of MLAE₍₂₀₎ enhanced chlorophyll content in faba bean leaves. Similarly, Yasmeen et al. (2013) reported an increase in chlorophyll levels when MLAE was applied to wheat plants under drought stress. This effect may be attributed to the

ability of *Moringa* extract to stimulate the production of the phytohormone cytokinin or its association with cytokinin levels (Zeatin) present in MLAE, which helps delay leaf senescence and prevents the decline in chlorophyll content. *Moringa* leaves possess significant nutritional potential, being rich in macro- elements like magnesium, a key component of chlorophyll, which plays a crucial role in enhancing the levels of chlorophyll a and chlorophyll b in faba bean plants. Additionally, they contain notable amounts of carotenoids (α -carotene, β -carotene, xanthin, and lutein), which exhibit strong antioxidant properties (Zaki & Rady, 2015). Leaf relative water content (LRWC) is a critical physiological indicator that determines a plant's tolerance to drought stress (Sanchez-Blanco et al., 2002). Under drought conditions, the LRWC of maize plants is significantly reduced. The foliar application of MALE significantly mitigated the adverse effects of drought stress on LRWC. Notably, the positive impact of MALE on LRWC under drought conditions was more pronounced at concentrations of 25% and 12.5% (Table 4). Previous studies have documented the suppressive effects of drought stress on LRWC (Bano et al., 2012). The observed improvement in LRWC due to MALE application may be attributed to its rich phytochemical composition (Yasmeen et al., 2014). The elevated LRWC observed in MALE treatments under drought stress can likely be attributed to an increase in phenolic content within the cell wall, enhancing its hydrophobic properties. As a result, this

water-repellent apoplastic environment may restrict water movement from the metabolically active interior of the cell, hinder capillary water transport within the apoplast, and ultimately reduce cuticular transpiration (Hura et al., 2013). Research has shown that plants have evolved certain non-enzymatic antioxidant compounds with low molecular weights to mitigate the damage caused by water deficit stress. These compounds, such as ascorbic acid (AsA) and anthocyanins (ACNs), play a crucial role in directly neutralizing reactive oxygen species (ROS) during periods of stress. Drought stress in this study significantly increased ascorbic acid and anthocyanin levels in faba bean plants compared to the control group (Table 4). Similarly, Aziz et al. (2018) previously reported that endogenous AsA concentrations rose in quinoa plants subjected to water deficit stress. During drought stress, ascorbic acid (AsA) and anthocyanins function as reactive oxygen species (ROS) scavengers and cell membrane stabilizers. They also play a crucial role in regulating water retention and influencing plant growth and development (Abid et al., 2018). AsA is particularly significant in enzymatic detoxification of hydrogen peroxide, enhancing the plant's defensive mechanisms against water scarcity (Hemavathy et al., 2011). Ascorbic acid plays a pivotal role in the scavenging mechanism of reactive oxygen species (ROS) (Lushchak and Semchuk, 2012). It functions as a potent electron donor, supporting both enzymatic and non-enzymatic antioxidant systems.

This activity contributes to the regulation of cell elongation and stimulates the synthesis of defence proteins and lipids, thereby offering protection against oxidative stress (Shao et al., 2018). Previous studies have linked elevated anthocyanin levels to enhanced drought tolerance in various plant species (Gharibi et al., 2019; Cirillo et al., 2021), consistent with the current findings that demonstrate the accumulation of this phenolic compound in faba bean plants (Table 4). Despite the established role of anthocyanins in drought tolerance, their dynamic behaviour as phenolic compounds during drought hardening are not fully understood. Studies have shown that increased anthocyanin levels correlate with improved drought tolerance (Baozhu et al. 2022; Lozano et al. 2024) and enhance antioxidant activity by neutralizing ROS and mitigating oxidative stress. Regulates stomatal movement for water conservation, enhances water uptake and retention, and promotes photosynthesis. Consistent with the general effect of abiotic stress (Shafiq et al., 2021), the water deficit increased leaf proline content in faba bean under different irrigation regimes (Table 3). Osmolytes in plant cytoplasm lower osmotic potential and maintain turgor without decreasing water content (Abbas et al. 2014). Proline (Pro), a key osmolyte accumulating in plants under oxidative stress, stabilizes subcellular structures (proteins and membranes) and scavenges free radicals; however, its accumulation may also be an adaptive mechanism to mitigate water stress (EL

Moukhtari et al. 2020). There is a possibility that the increased production of proline is due to regulation of different biosynthetic and catabolic pathways. For example, Proline protects RuBisCO's carboxylase activity, regulates water absorption, ROS homeostasis, and cell signalling (Rady et al., 2013). Proline increases may enable plant cells to regulate osmotic potential, enhancing water uptake and translocation under stress conditions and protects cells and enzymes from oxidative damage by scavenging free radicals. It reduces ROS through singlet oxygen ($^1\text{O}_2$) quenching and direct chemical interaction with OH radicals (EL Moukhtari et al. 2020). Proline accumulation signals stress by acting as an osmo-protectant and stabilizing cell turgor. Additionally, reduced proline oxidase activity elevates proline levels, which are then used as C and N donors for rapid recovery and stabilization (Anaytullah et al., 2018). Conversely, MLAE mitigated water deficit stress by bolstering antioxidant defences, increasing levels of total phenols, flavonoids, anthocyanins, ascorbic acid, and proline, thereby reducing ROS, lipid peroxidation, and ion leakage (Howladar, 2014). Spraying plants with MLAE⁽²⁰⁾ showed significant increase in antioxidant and osmolytes contents of faba bean plants above that of the corresponding controls. This result is agreed with Kiran Pervez et al., (2017) on *Zea mays* plants, El-Lethy et al., (2024) on wheat plants. MLAE, rich in nutrients, amino acids, phenolics, soluble sugars, and antioxidants (e.g., proline and ascorbate), boosts ascorbic acid and

anthocyanin levels and proline levels, improving drought tolerance in faba bean plants. Drought stress reduces faba bean seed production and yield, but mitigation strategies exist. Drought stress significantly reduced the number of legumes/ plant, seeds/ plant, and seed weight (g)/plant compared to the control. Application of MLE⁽²⁰⁾ individually or under drought stress caused obvious increases of yield components of faba bean plants. These results agree with those obtained by (Begna, 2020) and Sadak and Bakry, 2020) on different crops. Drought stress reduces plant growth and yield components, ultimately impacting overall plant yield through integrated metabolic processes. Yield trait reductions may be due to growth cessation caused by plant stress. Reduced yield is associated with delayed flowering and fewer flowers and pods (Khan et al. 2016). The nutritional values of faba bean plants yielded seeds such as total carbohydrates (CHO), and protein contents and were significantly reduced by drought. Nutritional variations, such as in carbohydrate and protein content, are important due to their close link with biochemical and physiological metabolic processes (Anjum et al. 2003). Water stress reduced leaf chlorophyll content decreased photosynthetic activity and carbohydrate production. This impaired carbohydrate transport from mature leaves to developing seeds (Elewa et al. 2017). To conserve energy and protect against stress, plants often alter their metabolic systems or the seeds they produce, leading to a slowdown in seed

growth and development (Ragaey et al. 2022). The *Moringa* leaf extract, applied at a 20 times concentration, demonstrated promising results. Numerous researchers have highlighted the effectiveness of using *Moringa* extract solution as a field treatment. Its application on pepper plants significantly improved plant height, fruit quantity, foliage area, as well as fruit length and diameter, ultimately enhancing overall growth and yield performance (Hala et al., 2017). In *Triticum aestivum*, Yasmeen et al. (2012) reported that the application of *Moringa* leaf aqueous extract (MLAE) improved various growth and yield parameters, including grain yield and harvest index. This enhancement is likely due to *Moringa* extract being a natural source of cytokinin. Additionally, *Moringa* leaves are rich in ascorbates, carotenoids, potassium, and possess plant growth-promoting properties, often utilized as exogenous plant growth enhancers (Foidle et al., 2019). The effectiveness of *Moringa* can be attributed to its leaves, which are a rich source of plant growth regulators (PGR) like zeatin, as well as ascorbic acid, calcium, and potassium. *Moringa* leaves also serve as a valuable source of essential nutrients, including vitamins A and C, iron, calcium, riboflavin, beta-carotene, and phenolic acids (Nambiar et al., 2005). Therefore, foliar application of *Moringa* leaf extract (MLAE₂₀) has proven effective in enhancing the growth and yield characteristics of faba bean plants.

6. Conclusion

Drought stress adversely affects growth, biochemical composition, antioxidant levels,

and yield quality of faba bean plants compared to optimal conditions. However, the foliar application of *Moringa oleifera* leaf extract (MLAE₂₀) activates physiological compounds within the plants, mitigating oxidative damage caused by drought. This treatment enhances both physiological and biochemical parameters, promoting improved plant growth under drought conditions. *Moringa* leaf extracts can mitigate the negative impacts of water deficit stress, safeguarding plant growth and productivity under such conditions. This approach demonstrates significant promise for practical implementation in agriculture.

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