American Journal of Botany and Bioengineering
https://biojournals.us/index.php/AJBB
ISSN: 2997-9331

# Physiological of *Calendula Officinalis* L. as Affected by Foliar Application of Nano Nitrogen and Potassium Fertilizers, Under Water Stress Conditions

**Aqeel Abdul Abbas Abdul Zahra Alsudani, Qais Hussain Abbas Al Semmak**
Department of Biology, Plant physiology, College of Education for Pure Science, University of Kerbala, Iraq

**Received:** 2025 19, Jun
**Accepted:** 2025 28, Jul
**Published:** 2025 15, Aug



**Annotation:** An experiment was conducted in plastic pots at the nursery of the College of Agriculture/University of Kerbala during the agricultural season 2023-2023 to study the effect of foliar spraying with Nano nitrogen and potassium on some physiological traits of Calendula officinalis L. under water stress conditions. The experiment was implemented in a randomized complete block design with three replicates and three factors: the first, three concentrations of liquid Nano nitrogen (0, 2, and 4) ml L-1; the second, three concentrations of complex Nano potassium (0, 2, and 4) g L-1; and the third, two levels (50% and 100%) of the field capacity value, with the interaction between them. The averages were compared according to the least significant difference (L.S.D) at a probability level of 0.05.

The results showed that spraying liquid Nano nitrogen fertilizer at a concentration of 4 ml L-1 and complex Nano potassium at a concentration of 4 g L-1 at 100% of the field capacity value significantly increased the total chlorophyll content of leaves compared to the control treatment, which gave the lowest values. The results also showed that spraying liquid Nano nitrogen fertilizer at a concentration of 0 ml L-1 and complex Nano potassium at a concentration of 0 g L-1 at 50% of the field capacity value significantly increased the content of proline and abscisic acid in leaves.

**Keywords:** Nano-fertilizers, water stress, C. officinalis, Chlorophyll, Proline, Abscisic acid.

## 1. Introduction

*C. officinalis* is a popular medicinal herb from the Asteraceae family of the plant kingdom that has been used for thousands of years [1]. Water is the medium through which nutrients are transported from the soil to plant systems. Without soil water, plant growth and productivity are negatively affected. Water stress affects plant biomass production and chlorophyll content [2]. Water stress below 30% of field capacity, coupled with crop competition for soil moisture and other resources, may affect or inhibit germination, plant growth, and seed production [3]. [4] investigated the effect of water stress on plant morphological and physiological characteristics under field conditions. Water stress at 50% of field capacity resulted in decreased chlorophyll content, gas exchange, and photosynthetic efficiency, with the highest values under 100% of field capacity.

Nitrogen is an essential nutrient for plant growth and development and is the most abundant element in plant cells. It acts as a vital macronutrient that plays various essential roles in plant physiological processes. It builds essential components such as proteins and nucleic acids and also plays a role in the synthesis of some vitamins. It gives leaves their dark green color due to its presence in the chlorophyll molecule and regulates biological functions. However, human-caused changes are reducing nitrogen availability in ecosystems worldwide, impacting plant and animal populations [5].

Potassium ($K^+$) is an essential macronutrient for plants, playing a vital role in regulating various cellular processes, physiological functions, and responses to abiotic stresses such as salinity and drought. By possessing multiple transporters and channels responsible for uptake, efflux, and translocation within the cell and from the soil to different tissues as well as maintaining ionic balance under stress, it acts as a modulator of stomatal movements during water-deficient conditions [6].

[7] stated that in recent years, the global agricultural system has been negatively impacted by adverse environmental changes, affecting plant growth, physiology, and production. Abiotic stress in plants is one of the major constraints to agricultural production and global food security, and therefore there is a need to develop new methods to overcome these problems and achieve sustainability. Nanotechnology has diverse applications and has emerged as a new approach to increasing crop growth and production in agriculture through the use of Nano materials, such as Nano fertilizers. This is due to the unique properties Nano materials exhibit at the molecular level, leading to improved physical and chemical properties such as cation exchange capacity, diffusion, and ion adsorption [8].

For the above reasons, and to increase the production of this plant due to its medicinal importance, this study was designed to investigate the effect of spraying nitrogen and potassium Nano fertilizers on some physiological traits of *C. officinalis* under water stress conditions.

## 2. Materials and methods

### 2.1. Methods of planting, treatments and Collection of samples

Sandy loam soil was used in the experiment, and its physical and chemical properties are shown in Table (1). The soil was placed in plastic pots (40 cm long and 40 cm in diameter, perforated at the bottom) at a rate of (25) kg of soil per pot.

This experiment was designed with three factors and three replications in a cross-referenced design (CRD). The first factor was irrigation with river water at 50% of the soil moisture content at field capacity as the first level ($S_1$) and irrigation with river water at 100% of the soil moisture content at field capacity as the second level ($S_2$). The second factor was spraying with three different levels of Nano liquid nitrogen fertilizer (0, 2, and 4) ml. $L^{-1}$ ($N_0$, $N_1$, and $N_2$), respectively. The third spraying factor was represented by three different levels of complex Nano potassium fertilizer (0, 2, and 4) g. $L^{-1}$ ($K_0$, $K_1$, and $K_2$), respectively. The experimental units used in this study are (54) experimental units.

*C. officinalis* seeds were sown on October 1, 2023, at a rate of 10 seeds per pot. The experiment continued until the end of winter 2024, after which the number of plants was reduced to three after germination. Irrigation was based on the plant's water requirements, and the gravimetric method was used to add irrigation water according to the required level to study its content at the desired field capacity. After ensuring the emergence of 4-6 full leaves on the plant, the first foliar spray of two concentrations of Nano nitrogen fertilizer was given, and the first batch of two concentrations of Nano potassium fertilizer was added the following day as a foliar spray. Spraying at the above concentrations continued (sprayed every four weeks until 100% flowering was complete). The control treatment was sprayed with distilled water only. 50 kg $h^{-1}$ of phosphorus was added from a calcium superphosphate fertilizer source before planting. The content of chlorophyll, proline and abscisic acid in plant leaves was estimated according to the described methods [9-11], respectively.

**2.2. Statistical methods**

The data were subjected to analysis of variance using the SAS statistical package version 9.3th ed. at $P<0.05$ probability level [12].

**Table (1) Some physical and chemical characteristics of soil sample used in this study.**

| Adjective | | Value | Unit |
|---|---|---|---|
| pH | | 7.9 | - |
| Electrical conductivity ( EC ) | | 1.28 | ds.$m^{-1}$ |
| Organic matter | | 8.62 | gm. $Kg^{-1}$ |
| Soluble cations | $Ca^{2+}$ | 5.5 | C mol.$Kg^{-1}$ Soil |
| | $Mg^{2+}$ | 3.8 | C mol.$Kg^{-1}$ Soil |
| | $Na^{1+}$ | 2.7 | C mol.$Kg^{-1}$ Soil |
| | $K^{+}$ | 0.41 | C mol.$Kg^{-1}$ Soil |
| Soluble anions | $SO_4^{2-}$ | 6.6 | C mol.$Kg^{-1}$ Soil |
| | $HCO_3^{1-}$ | 2.4 | C mol.$Kg^{-1}$ Soil |
| | $CO_3^{2-}$ | Nil | C mol.$Kg^{-1}$ Soil |
| | $Cl^{-}$ | 3.3 | C mol.$Kg^{-1}$ Soil |
| Available N | | 14 | gm. $Kg^{-1}$ |
| Available P | | 13.6 | gm. $Kg^{-1}$ |
| Available K | | 85.4 | gm. $Kg^{-1}$ |
| Soil separators | Sand | 846 | gm. $Kg^{-1}$ |
| | Silt | 83 | gm. $Kg^{-1}$ |
| | Clay | 71 | gm. $Kg^{-1}$ |
| Texture Sandy loam | | | |
| Moisture at 10 kPa Tension: 9.6%<br>Moisture at 33 kPa Tension: 5.8% | | | |

*Analyzes in soil analysis laboratories at the Faculty of Agriculture - University of Baghdad

## 3. Results and discussion

### 3.1 Total chlorophyll content of fresh leaves (SPAD unit)

The results shown in Table (2) show that plants treated with irrigation at different rates of field capacity differed significantly in the trait of total chlorophyll content of their leaves, as plants that were irrigated with irrigation water at 100% of the field capacity value were significantly superior and produced leaves that were characterized by the highest total chlorophyll content, which averaged 16.45 SPAD unit, compared to plants that were treated with the highest rate of water stress in this study and at 50% of the field capacity value, as they produced leaves with the lowest average in this trait, which amounted to 14.69 SPAD unit. This shows that water stress gradually reduces the transfer of electrons to the PSII reaction center due to differences in energy absorption, retention, and electron transfer, which leads to a decrease in the efficiency of photosynthesis for PSII, as the chlorophyll content is linked to carbon metabolism, which increases the plant's yield, while the increase in chlorophyll affects the increase in the leaf area, on which the size and efficiency of the carbon metabolism process in the leaves depend according to [13] view.

The data in the same table indicate that the plants treated with a concentration of 4 ml $L^{-1}$ of Nano nitrogen were significantly superior, with an average of 17.01 SPAD unit, and an increase of 20.64% compared to the control plants, which produced the lowest total chlorophyll content in their leaves, with an average of 14.10 SPAD unit. From the effects of other individual factors, it is clear from Table (2) that the plants resulting from spraying with Nano potassium at a concentration of 4 g $L^{-1}$ were significantly superior, with an average of 16.32 SPAD unit, and an increase of 12.01% compared to the control treatment plants, whose leaves produced the lowest chlorophyll content, with an average of 14.57 SPAD unit. The difference between the plants treated with the different Nano concentrations that were sprayed on the vegetative group differs in the number of pores in their leaves, their diameters, and the extent of their compatibility with the diameter of the Nano nitrogen and potassium particles, which controls the rates of penetration through those pores, and thus is reflected in the difference in the plants' response. For Nano fertilizer additives and then their direct or indirect contribution to the estimates of total chlorophyll pigments in their leaves [14], The reason for the increase may be due to the possession of Nano fertilizers of physical and chemical activity as a result of the increase in the surface area of its particles and the presence of a large number of its atoms on the outer surface of Nano fertilizers, which is reflected in improving the vital activities and increasing the speed of enzyme activities for the process of photosynthesis and stimulating the divisions of chlorophyll, thus increasing the ability of green pigments to absorb visible light from sunlight and convert it into chemical energy stored in the form of organic materials that are transferred to the parts of the plant to benefit from them in the various functions of the plant [15, 16].

Chlorophyll content is one of the most important biochemical parameters associated with nitrogen status in plants, as most nitrogen is a structural component of this molecule [17]. [18] observed that increased chlorophyll concentrations coincide with increased nitrogen supply in plants.

The data of the two-way interaction between water stress and Nano nitrogen treatment showed a significant effect on the average of this trait, as the plants that were irrigated with 100% of the

**Table (2) The effect of spraying with Nano nitrogen and potassium on the total chlorophyll content of fresh leaves (SPAD unit) of *C. officinalis* plants growing under two different irrigation water levels.**

| Nano nitrogen spraying ($ml.L^{-1}$) N | Nano potassium spraying ($g.L^{-1}$) K | Irrigation levels (from field capacity value) S %50 $S_1$ | % 100 $S_2$ | N*K |
|---|---|---|---|---|
| 0 | 0 | 12.68 | 14.62 | 13.65 |
| | 2 | 13.56 | 14.71 | 14.14 |
| | 4 | 14.20 | 14.85 | 14.53 |
| 2 | 0 | 14.40 | 14.94 | 14.67 |
| | 2 | 14.95 | 16.41 | 15.68 |
| | 4 | 15.22 | 17.71 | 16.46 |
| 4 | 0 | 14.53 | 16.28 | 15.40 |
| | 2 | 16.13 | 19.17 | 17.65 |
| | 4 | 16.57 | 19.36 | 17.97 |
| **S** | | 14.69 | 16.45 | |
| | | | | **N** |
| **N*S** | 0 | 13.48 | 14.73 | 14.10 |
| | 2 | 14.86 | 16.35 | 15.60 |
| | 4 | 15.74 | 18.27 | 17.01 |
| | | | | **K** |
| **K*S** | 0 | 13.87 | 15.28 | 14.57 |
| | 2 | 14.88 | 16.76 | 15.82 |
| | 4 | 15.33 | 17.30 | 16.32 |

| **L.S.D 0.05** | **N** | **K** | **S** | **K * N** | **S * N** | **S * K** | *** K * N S** |
|---|---|---|---|---|---|---|---|
| | 0.3017 | 0.3017 | 0.2463 | 0.6034 | 0.548 | 0.548 | 0.8497 |

field capacity value and treated with $N_2$ at a concentration of 4 ml $L^{-1}$ achieved the highest content of total chlorophyll in their leaves with an average of 18.27 SPAD unit and an increase of 35.53% compared to the lowest total chlorophyll content with an average of 13.48 SPAD unit. The leaves of plants that were treated with the highest water stress were found to be 50% of the field capacity value with $N_0$ at a concentration of 0 ml $L^{-1}$. The interaction between water stress and Nano potassium had a significant effect on this trait, as plants irrigated with 100% of the field capacity and $K_2$ treatment at a concentration of 4 g $L^{-1}$ gave the highest average of 17.30 SPAD unit compared to the lowest total chlorophyll content recorded in plants treated with the interaction between the highest water stress at 50% of the field capacity and $K_0$ at a concentration of 0 g $L^{-1}$, with an average of 13.87 SPAD unit. The interaction between Nano nitrogen and potassium also had a significant effect on the average content of total chlorophyll pigment in *C. officinalis* leaves, as the plants treated with $N_2$ at a concentration of 4 ml $L^{-1}$ and $K_2$ at a concentration of 4 g $L^{-1}$ Nano together outperformed by giving them the highest average of 17.97 SPAD unit, with an increase of 31.65% compared to the control treatment, whose plants gave the lowest average in this trait, amounting to 13.65 SPAD unit.

The results of the three-way interaction between the studied experimental factors indicate significant differences between the treatments. Plants irrigated with 100% of the field capacity and treated with both Nano $N_2K_2$ together produced the highest total chlorophyll content in *C. officinalis* leaves, with an average of 19.36 SPAD unit, an increase of 52.68% compared to plants irrigated with 50% of the field capacity and treated with both Nano $N_0K_0$ together, with an average of 12.68 SPAD unit.

### 3.2 Proline content in leaves (mg kg$^{-1}$)

The results in Table (3) showed that the content of the proline amino acid in the leaves of *C. officinalis* plants differed significantly according to the individual effect of the three studied factors (water stress and the added concentrations of Nano nitrogen and potassium). The plants that were irrigated at 100% of the field capacity value were characterized by a clear decrease in the proline content of their leaves, with an average of only 3.73 mg kg$^{-1}$ and a decrease rate of 42.97% compared to the plants that were exposed to water stress (irrigation at 50% of the field capacity value), as they gave the highest proline content, with an average of 6.54 mg kg$^{-1}$. Plants treated with (0 and 2 ml L$^{-1}$) of Nano nitrogen significantly outperformed with an average of (6.28 and 4.88 mg kg$^{-1}$) respectively, with an increase rate of (48.11 and 15.09%) compared to the $N_2$ treatment with a concentration of (4 ml L$^{-1}$), which produced plants with the lowest proline content in their leaves, with an average of 4.24 mg kg$^{-1}$. Also, spraying plants with a Nano potassium nutrient at a concentration of (0 and 2 g L$^{-1}$) led to a significant increase in the proline content of their leaves, with an average of (5.97 and 4.99 mg kg$^{-1}$) respectively, compared to the $N_2$ treatment with a concentration of (4 g L$^{-1}$), which gave its plants the lowest average of 4.45 mg kg$^{-1}$.

The two-way interaction between water stress and Nano nitrogen treatment indicated significant differences in this trait, as plants growing under an irrigation water level of 50% of the field capacity value and treated with Nano nitrogen spraying concentration (0, 2 and 4 ml L$^{-1}$) achieved the highest proline content with an average of (8.29, 6.02 and 5.31 mg kg$^{-1}$) respectively, compared to the lowest content reached in plants growing under an irrigation water level of 100% of the field capacity value and treated with Nano nitrogen spraying concentration (0, 2 and 4 ml L$^{-1}$) with an average of (4.27, 3.75 and 3.17 mg kg$^{-1}$) respectively. The two-way interaction between water stress and Nano potassium factors was characterized by a significant effect on this trait, as the highest proline content resulted in the leaves of *C. officinalis* plants growing under an irrigation water level of 50% of the field capacity value. The Nano potassium spray treatment with concentrations of (0, 2 and 4 g L$^{-1}$) and an average of (7.80, 6.17 and 5.66 mg kg$^{-1}$) respectively, compared to the lowest content of this amino acid recorded in plants growing under an irrigation water level of 100% of the field capacity value, and the Nano potassium spray treatment with concentrations of (0, 2 and 4 g L$^{-1}$) and an average of (4.13, 3.81 and 3.24 mg kg$^{-1}$) respectively. The binary interaction between Nano nitrogen and potassium was characterized by a significant effect on the proline content of plant leaves, as the data in the same table show the superiority of treating plants with $N_0$ $K_0$ that were treated by spraying with

**Table (3) The effect of spraying with Nano nitrogen and potassium on the percentage of proline (mg kg$^{-1}$) in the leaves of the *C. officinalis* plant growing under two different irrigation water levels.**

| Nano nitrogen spraying (ml.L$^{-1}$) N | Nano potassium spraying (g.L$^{-1}$) K | Irrigation levels (from field capacity value) S %50 $S_1$ % 100 $S_2$ | | N*K |
|---|---|---|---|---|
| 0 | 0 | 10.81 | 4.57 | 7.69 |
| | 2 | 7.28 | 4.27 | 5.77 |
| | 4 | 6.79 | 3.97 | 5.38 |
| 2 | 0 | 6.63 | 3.94 | 5.28 |
| | 2 | 5.92 | 3.78 | 4.85 |
| | 4 | 5.51 | 3.53 | 4.52 |
| 4 | 0 | 5.95 | 3.90 | 4.92 |
| | 2 | 5.32 | 3.40 | 4.36 |
| | 4 | 4.67 | 2.22 | 3.45 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **S** | | 6.54 | | | 3.73 | | | |
| | | | | | | | | **N** |
| **N*S** | 0 | 8.29 | | | 4.27 | | | 6.28 |
| | 2 | 6.02 | | | 3.75 | | | 4.88 |
| | 4 | 5.31 | | | 3.17 | | | 4.24 |
| | | | | | | | | **K** |
| **K*S** | 0 | 7.80 | | | 4.13 | | | 5.97 |
| | 2 | 6.17 | | | 3.81 | | | 4.99 |
| | 4 | 5.66 | | | 3.24 | | | 4.45 |
| **L.S.D 0.05** | | **N** | **K** | **S** | **K * N** | **S * N** | **S * K** | *** K * N S** |
| | | 0.058 | 0.058 | 0.0474 | 0.116 | 0.1054 | 0.1054 | 0.1634 |

distilled water by giving them plants characterized by the highest proline content, which reached an average of 7.69 mg $kg^{-1}$ and an increase rate of 122.90% compared to treating plants with $N_2$ $K_2$ The concentration of each of them was 4 (ml $L^{-1}$, g $L^{-1}$) which gave its plants the lowest content of it, which amounted to 3.45 mg $kg^{-1}$.

Through the results of the three-way interaction between the study factors shown in the same table, it became clear that they had a significant effect on the proline content of plant leaves, as the treatment of plants with $N_2K_2S_2$ produced the lowest proline content with an average of 2.22 mg $kg^{-1}$, while the $N_0K_0S_1$ treatment produced plants whose leaves were characterized by a high proline content with an average of 10.81 mg $kg^{-1}$, with a significant increase rate of 386.94% compared to the first treatment mentioned.

[19] demonstrated that plant osmotic pressure maintains turgor pressure at low water potential. Proline accumulation contributes to maintaining turgor pressure in the cell by concentrating various solutes, which reduces the osmotic potential of the cytosol, thus maintaining plant growth under drought conditions through the osmoprotective and growth-promoting functions of amino acids [20]. [21] indicated that the relationship between proline accumulation and increased or decreased stress tolerance remains controversial in scientific studies, as high proline concentrations are associated with increased or decreased stress tolerance. This amino acid is involved in the scavenging of reactive oxygen species (ROS), maintaining protein stability and pH balance in the cytoplasm [22].

[23] demonstrated that foliar application of Nano nitrogen and potassium increases proline content and antioxidant enzyme activity, which is critical for stress tolerance, reduces signs of oxidative stress, and improves photosynthetic efficiency in stressed plants.

### 3.3 Abscisic acid content of leaves (µg $g^{-1}$ dry weight)

The results of the study presented in Table (4) showed the significant effect of all the individual factors studied, as well as their double and triple interactions on the average concentration of the plant hormone ABA in the leaves of the *C. officinalis* plant. When the concentration of Nano nitrogen increased from 0 to 4 ml $L^{-1}$, the average concentration of the acid decreased from 28.13 µg $g^{-1}$ dry weight to 24.43 µg $g^{-1}$ dry weight, with a decrease rate of 13.15%, while when spraying the nutrient element Nano potassium, it did not differ significantly from its predecessor in this trait, i.e., the average concentration of abscisic acid decreased from 27.45 µg $g^{-1}$ dry weight to 24.91 µg $g^{-1}$ dry weight when the concentration of Nano potassium increased from 0 to 4 g $L^{-1}$. As for the single factor (S), the plants growing under an irrigation water level of 50% of the field capacity value increased significantly in the average of this trait, which amounted to Its value was 29.14 µg $g^{-1}$ dry weight compared to plants growing under 100% irrigation water level of the field capacity value, which achieved an average abscisic acid content of 22.74 µg $g^{-1}$ dry weight.

The results shown in Table (4) confirmed the presence of a significant interaction effect between the effect of spraying with the nutrient element represented by Nano nitrogen and the effect of water stress on the average ABA concentration, as the highest value of ABA concentration reached 32.28 micrograms $g^{-1}$ dry weight in the $N_0S_1$ treatment, while the lowest value reached 21.79 micrograms $g^{-1}$ dry weight in the $N_2S_2$ treatment. The same table also showed a significant interaction in the average ABA content for both experimental factors represented by Nano potassium and water stress. The $K_0S_1$ treatment gave the highest average of 31.47 micrograms $g^{-1}$ dry weight, while the lowest average reached 22.20 micrograms $g^{-1}$ dry weight in the $K_2S_2$ treatment. In addition, there was a significant decrease in the average ABA concentration with an increase in the concentration of Nano nitrogen and potassium, as the $N_2K_2$ treatment recorded a low average of this trait, reaching 23.18 micrograms $g^{-1}$ dry weight, with a decrease of 19.23% compared to the $N_0K_0$ treatment, which gave an increase in the level of ABA concentration with an average of 28.70 micrograms $g^{-1}$ dry weight.

Regarding the triple interactions, there was a clear significant difference in this trait, as the treatments $N_0K_0S_1$ and $N_0K_1S_1$ achieved the highest value of ABA concentration in the leaves of the plant under study, reaching 32.97 and 32.63 micrograms $g^{-1}$ dry weight, respectively, while the lowest value for this trait reached 21.06 micrograms $g^{-1}$ dry weight in the treatment $N_2K_2S_2$.

**Table (4) The effect of spraying with Nano nitrogen and potassium on the leaf content of abscisic acid (micrograms $gm^{-1}$ dry weight) of *C. officinalis* plants growing under two different irrigation water levels.**

| Nano nitrogen spraying ($ml.L^{-1}$) N | Nano potassium spraying ($g.L^{-1}$) K | Irrigation levels (from field capacity value) S %50 $S_1$ % 100 $S_2$ | | N*K |
|---|---|---|---|---|
| 0 | 0 | 32.97 | 24.42 | 28.70 |
| | 2 | 32.63 | 23.78 | 28.21 |
| | 4 | 31.22 | 23.74 | 27.48 |
| 2 | 0 | 31.14 | 23.12 | 27.13 |
| | 2 | 26.77 | 22.42 | 24.59 |
| | 4 | 26.33 | 21.81 | 24.07 |
| 4 | 0 | 30.32 | 22.75 | 26.53 |
| | 2 | 25.59 | 21.57 | 23.58 |
| | 4 | 25.31 | 21.06 | 23.18 |
| **S** | | 29.14 | 22.74 | |
| | | | | **N** |
| **N*S** | 0 | 32.28 | 23.98 | 28.13 |
| | 2 | 28.08 | 22.45 | 25.26 |
| | 4 | 27.07 | 21.79 | 24.43 |
| | | | | **K** |
| **K*S** | 0 | 31.47 | 23.43 | 27.45 |
| | 2 | 28.33 | 22.59 | 25.46 |
| | 4 | 27.62 | 22.20 | 24.91 |

| **L.S.D 0.05** | **N** | **K** | **S** | *** N K** | **S * N** | **S * K** | **K * N S *** |
|---|---|---|---|---|---|---|---|
| | 0.0755 | 0.0755 | 0.0616 | 0.151 | 0.1371 | 0.1371 | 0.2265 |

[24] indicated that plants produce molecules that act as negative regulators of various plant responses, including abscisic acid (ABA), which is an inhibitory hormone that helps plants adapt to stress, maintains water balance, prevents seed embryos from germinating, and stimulates seed

and bud dormancy.

In plants, abscisic acid regulates many plant processes, including plant growth and development, stress physiology (response to biotic and abiotic stresses), antioxidant activities, and important roles in the cell such as plant adaptation to environmental influences, seed development, dormancy, budding, and fruit ripening. It is critical for enhancing stress tolerance. This includes ABA-mediated control of gene expression to increase antioxidant activities to remove reactive oxygen species [25].

Abscisic acid plays an important vital role in reducing a variety of stress reactions in plants, including salinity, low temperature and drought stress. Drought is an abiotic stress and is defined as a prolonged period of limited water supply. It regulates processes involved in plant growth such as flower induction, seed dormancy, embryo maturation, seed germination, root and fruit development and ripening by regulating growth, senescence and interactions with other hormones such as ethylene and defoliation. This was reported by [26] when applying abscisic acid to selected medicinal plant species to analyze drought tolerance and leaf yellowing.

Abscisic acid (ABA), present in the root hairs of *C. officinalis*, enhances the biosynthesis of terpenoids such as the triterpenoid oleanolic acid and the release of their glycosides (saponins), acting as a potent catalyst. Therefore, phytohormones (plant growth regulators) can be used as effective stimulants to enhance plant productivity due to their importance in regulating plant metabolism and their strong influence on plant defense responses. In addition, the application of ABA to *C. officinalis* under water-deficit conditions resulted in a decrease in stomatal conductivity and net photosynthesis, particularly affecting gas exchange parameters while restricting flavonoid biosynthesis without altering the total flavonoid content in the inflorescences. These results highlight the multifaceted effect of ABA on *C. officinalis*, affecting both physiological parameters and the production of secondary metabolites in response to environmental stresses [27].

**References**

1. Shahane, K.; Kshirsagar, M.; Tambe, S.; Jain, D.; Rout, S.; Karolina, M.; Ferreira, M.; Mali, S.; Amin, P.; Prakash, P.; Srivastav, J. C. and Lima, R. (2023). An Updated Review on the Multifaceted Therapeutic Potential of *Calendula officinalis* L.Pharmaceuticals. 16(4):1-21.

2. Adebanjo, O.; Setordjie, E. A. and Li, R. W.(2022). Water Stress Effect on the Growth of Lettuce (*Lactuca sativa*) and Moth Plant (*Araujia sericifera*). Polytechnic University of Valencia, Valencia, Spain. PP:1-16.

3. Singh, M.; Thapa, R.; Kukal, M. S.; Irmak, S.; Mirsky, S.and Jhala, A. J. (2022). Effect of water stress on weed germination, growth characteristics and seed production. a global meta-analysis. Weed Sci. (70): 621-640.

4. Laskari, M.; Menexes ,G.; Kalfas, I.; Gatzolis, I. and Dordas, C. (2022).Water Stress Effects on the Morphological, Physiological Characteristics of Maize (*Zea mays* L.), and on Environmental Cost .Agronomy, 12(10): 2386.

5. Mason, R.(2023). Nitrogen is becoming less available in ecosystems around the world. The Science Breaker. 9 (3):722.

6. Ankit,A. and Singh, A.(2022). Cation Transporters in Plants.Potassium ($K^+$) transporters in plants: regulation and functional role in K+ uptake and homeostasis. National Institute of Plant Genome Research, New Delhi, India. Pp: 29-47.

7. Al-Khayri, J.M.; Rashmi, R.;Surya Ulhas, R.; Sudheer, W.N.; Banadka, A.;Nagella, P.; Aldaej, M.I.; Rezk, A.A.S.; Shehata, W.F. and Almaghasla, M.I. (2023).The Role of Nanoparticles in Response of Plants to Abiotic Stress at Physiological, Biochemical, and Molecular Levels. Plants, 12: 292.

8. Napagoda, M.; Jayathunga, D. and Witharana, S. (2023). Introduction to Nanotechnology. In: Witharana, S. and Napagoda, M.T. (eds) Nanotechnology in Modern Medicine. PP: 1-17.

9. Reynolds, M. P.; Singh, P. R; Ibrahim, A.; Ageeb, O. A.; saavedra, A. and Quik, J. S. (1998). Evaluating physiological traits to complement empirical selection of wheat in warm environments. H. J. Braum et al. ( Eds ). Wheat prospects for Global improvement.Pp: 143 . -152

10. Bates, L.S.; Waldren, R.P.and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and soil. Aug .39(1): 7-205.

11. Nuray, E. and Topcuoglu, S. F. (2002). Auxin (indole-3-acetic acid), gibberellic acid (GA3), abscisic acid (ABA) and cytokinin (zeatin) production by some species of mosses and lichens. Turk J Bot. 26(1): 13-18.

12. Cary, N. (2018).Statistical analysis system, User's guide. Statistical. Version 9, SAS. Inst. Inc. USA .

13. Khatri, K. and Rathore, M.S. (2019). Photosystem photochemistry, prompt and delayed fluorescence, photosynthetic responses and electron flow in tobacco under drought and salt stress. Photosynthetica. 57:61-74.

14. Nair, R.; Varghese, S.H.; Nair, B.G.; Maekawa, T.; Yoshida, Y. and Kumar, D.S. (2010). Nanoparticulate Material Delivery to Plants. Plant Sci. 179 (3): 154-163.

15. Al-Mutairi, T. (2012). A Recent Working Paper - Uses in Disaster Response. Naif Arab University for Security Sciences, pp: 1-29.

16. Siddiqui, M.H.; Al-Whaibi, M.H.; Faisal, M. and Al-Sahli, A.A. (2014). Nanosilicon dioxide mitigates the adverse effects of salt stress on *Cucurbita pepo* L. Environ. Toxicol. Chem. 33 (11): 2429-2437.

17. Bassi, D.; Menossi, M.and Mattiello, L. (2018). Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. Sci. Rep. 8: 2327.

18. Deng,B.; Li, Y.; Xu, D.; Ye, Q.and Liu, G.(2019). Nitrogen availability alters flavonoid accumulation in Cyclocarya paliurus via the effects on the internal carbon/nitrogen balance. Sci. Rep. 9: 2370.

19. Bhutto, L.A.; Osborne, C.P.and Quick, W.P. (2023). Osmotic adjustment and metabolic changes under drought stress conditions in wheat (*Triticum aestivum* L.) genotypes. Pakistan Journal of Botany .55:915-923.

20. Batista-Silva, W.; Heinemann, B.; Rugen, N.; Nunes-Nesi, A.; Araújo, W.L.; Braun, H.P.and Hildebrandt, T.M. (2019). The role of amino acid me tabolism during abiotic stress release. Plant, Cell and Environment. 42:1630-1644.

21. Arteaga, S.; Yabor, L.; Díez, M.J.; Prohens, J.; Boscaiu, M.and Vicente, O. (2020). The use of proline in screening for tolerance to drought and salin ity in common bean (*Phaseolus vulgaris* L.) genotypes. Agronomy. 10:817.

22. Bandurska, H.; Niedziela, J.; Pietrowska-Borek, M.; Nuc, K.; Chadzinikolau, T.and Radzikowska, D. (2017). Regulation of proline biosynthesis and resistance to drought stress in two barley (*Hordeum vulgare* L.) genotypes of different origin. Plant Physiology and Biochemistry. 118:427-437.

23. Rady, M. M. ; Mossa , A. H.; Youssof, A. M.A. ; Osman, A. S.; Ahmed, S. and Mohamed, I. A. A. (2023). Exploring the reinforcing effect of nano-potassium on the antioxidant defense system reflecting the increased yield and quality of salt-stressed squash plants. Scientia Horticulturae. 308: 1-11.

24. Bhatla, S.C.and Lal, M.A. (2023). Abscisic Acid. In: Plant Physiology, Development and Metabolism. Pp: 443-451.

25. Utama, N. A. and Dewi, S. S.(2023). Effects of Abscisic Acid on Bioactive Compounds and Postharvest Quality of Climacteric and Non-Climacteric Fruit. Planta Tropika: Jurnal Agrosains.11(1) : 41- 49.

26. Latha, A. S. and Sree, K. (2023). Effect of Abscisic Acid on Drought Tolerance of Selected Plant Species. Ecology, Environment and Conservation. 29: 313-318.

27. Markowski, M.; Alsoufi, A.S.M.; Szakiel, A. and Długosz, M.( 2022).Effect of Ethylene and Abscisic Acid on Steroid and Triterpenoid Synthesis in *Calendula officinalis* Hairy Roots and Saponin Release to the Culture Medium. Plants. 11(3):303.