



Effect of Conventional and Nano Potassium on the Chemical Characteristics of Alfalfa (*Medicago sativa* L.) under Different Levels of Water Stress

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Abstract: This study was conducted to evaluate the effect of conventional potassium fertilization and nano-potassium application on some chemical characteristics of alfalfa (*Medicago sativa* L.) under different levels of water stress. The experiment was carried out in a randomized complete block design (RCBD) with three replicates, using a factorial arrangement. The treatments included three concentrations of nano-potassium (1, 2, and 3 g L⁻¹), three levels of conventional potassium added to soil (4, 6, and 8 g kg⁻¹ soil), and a control treatment without potassium fertilization. Four irrigation levels representing water stress were applied: 100%, 75%, 50%, and 25% of field capacity. Several chemical characteristics were measured, including chlorophyll content, proline concentration, mineral nutrient content (N, P, and K), protein percentage, and total carbohydrates in the leaves. The results indicated that nano-potassium application significantly increased chlorophyll content, protein percentage, and total carbohydrates compared with the control treatment. Conventional potassium fertilization significantly improved mineral nutrient concentrations, particularly potassium in plant tissues. Water stress levels significantly affected most of the studied traits, with moderate levels enhancing some physiological responses. The interaction between potassium treatments and irrigation levels showed significant effects on several chemical characteristics of alfalfa plants. Overall, potassium nutrition, especially in nano form, improved chemical characteristics and enhanced alfalfa plants' tolerance to water stress. These findings highlight the potential role of nano-potassium fertilization in improving plant performance and forage quality under limited water availability.

Keywords: Alfalfa; water stress; conventional potassium fertilizer; nano-potassium fertilizer

1. Introduction

Alfalfa (*Medicago sativa* L.) is considered one of the most important perennial forage crops worldwide. This importance stems from its numerous agricultural and nutritional characteristics. It has a high ability to adapt to a wide range of environmental conditions, in addition to its high nutritional value due to its elevated protein content, high biomass productivity, and high digestibility for ruminant animals. Moreover, alfalfa can fix atmospheric nitrogen through its symbiotic relationship with root-nodule bacteria, thereby improving soil fertility and reducing the need for nitrogen fertilizers [1, 2]. Furthermore, producing this crop is relatively less expensive than that of many other forage crops, thereby

enhancing its economic importance in agricultural production systems [3, 4]. Alfalfa belongs to the family Fabaceae, which is considered one of the largest plant families in the plant kingdom [5].

This crop is particularly well-suited to arid and semi-arid environments, where it is widely used in livestock feeding due to its high protein content, which may reach about 20%. In addition, it contains carotenoids, vitamins, and minerals that improve forage quality and enhance animal production efficiency [6, 7]. Alfalfa has also been used in folk medicine due to its bioactive compounds, which possess antioxidant and anti-inflammatory properties. It has been traditionally used to treat conditions such as arthritis, digestive disorders, and metabolic problems. Recent studies have also shown its effectiveness in supporting the central nervous system and helping in the treatment of obesity [8, 9]. Despite the agricultural and economic importance of this crop, its productivity is greatly affected by environmental factors, particularly abiotic stresses. These stresses influence plant growth, physiological processes, biomass accumulation, and forage quality [2]. Water stress resulting from drought is considered one of the most important limiting factors affecting agricultural production in many regions of the world [10]. This problem has intensified in recent decades due to declining water resources, reduced and irregular rainfall, and the increasing impacts of climate change. These factors have negatively affected agricultural production and reduced cultivated areas, especially amid the increasing demand for food driven by rapid population growth.

Water deficiency causes several morphological and physiological changes in plants [11]. It disrupts cellular water balance, leads to stomatal closure, and reduces photosynthesis by decreasing chlorophyll content. Water stress also disrupts the balance between the production of Reactive Oxygen Species (ROS) and antioxidant defense systems, leading to the accumulation of ROS and oxidative stress, which damages proteins, membrane lipids, and various cellular components [12]. Consequently, plant productivity and crop quality decline under drought conditions [13, 14]. Many studies indicate that plant mineral nutritional status plays a fundamental role in enhancing their ability to tolerate environmental stresses [15]. Potassium is considered an essential macronutrient for plant growth and development, as it plays a crucial role in regulating many physiological processes [16]. In alfalfa in particular, the demand for potassium increases to ensure continued growth and maintain forage productivity [17]. Potassium contributes to the activation of many enzymes involved in biosynthetic processes, such as protein and starch synthesis, and the production of the energy molecule adenosine triphosphate (ATP) [18]. It also plays an important role in regulating stomatal movement and maintaining ionic balance within plant cells, in addition to its roles in carbohydrate transport through the phloem and in regulating plant water balance [19]. Moreover, potassium enhances plant tolerance to various biotic and abiotic stresses [20, 21]. Several studies have demonstrated that the use of conventional potassium fertilizers, such as potassium sulfate, can improve the growth and productivity of alfalfa, especially under water-stress conditions [17].

In recent decades, agricultural technologies aimed at managing plant stresses have witnessed significant development, among which nanotechnology applications in agriculture have emerged as one of the most prominent innovations. This technology represents a modern approach that aims to improve nutrient-use efficiency, increase crop productivity, and reduce the negative environmental impacts of conventional agricultural inputs [22]. Nanoparticles have demonstrated a high capacity to penetrate plant cellular barriers, thereby enhancing the efficiency of nutrient absorption and their translocation within plant tissues due to their small size, large surface area, and high solubility [23]. The high efficiency and concentration of these fertilizers enhance the ability of nutrient-loaded nanoparticles to penetrate plant surfaces, thereby increasing the interaction area between nanoparticles and plant tissues. This, in turn, enhances physiological processes, particularly photosynthesis. As a result, plant dry matter content increases, confirming the high efficiency of nano-fertilizers used in small quantities, while achieving high productivity in both quantity and quality [24]. Recent studies also indicate that integrating nanoparticles into agricultural practices can improve both quantitative and qualitative crop traits, particularly under exposure to different environmental stresses. This is attributed to nanoparticles' ability to influence molecular signaling pathways in plants, activate antioxidant defense systems, regulate plant hormones, and enhance nutrient absorption efficiency and distribution within plant tissues [25, 26]. Therefore, this technology represents a promising approach for developing more efficient and environmentally sustainable agricultural production systems.

2. Materials and Methods

2.1 Study Site

The experiment was conducted in a nursery belonging to the Directorate of Agriculture of Al-Muthanna, in the center of Al-Samawah city, on 4 October 2025, to study the effects of conventional and nano forms of potassium on reducing the impact of water stress on alfalfa (*Medicago sativa* L.) growth. Local alfalfa seeds were obtained from the local market and manually cleaned to remove impurities before sowing. The seeds were planted in plastic pots with a capacity of 20 kg, filled with 11 kg of a growth medium consisting of loamy soil mixed with peat moss at a 1:3 ratio (soil:peat moss). Before planting, a representative soil sample was collected to conduct some physical and chemical analyses in the U-Science Laboratory in Al-Diwaniyah city, as shown in Table 1 according to the methods described by John [27] and Ryan et al. [28].

Table 1. Physical and chemical properties of the experimental soil before planting

Property	Value	Unit
pH	7.30	–
EC	2.06	ms/cm
Organic matter (OM)	1.12	%
CaCO ₃	36	%
CaSO ₃	1.15	%
Bulk density	1.13	g·cm ⁻³
N available	2.8	mg/kg
K available	108.65	mg/kg
P available	7.48	mg/kg
K soluble	51.43	mg/kg
Na	273.6	mg/kg
Ca	216	mg/kg
Cl	441	mg/kg
Mg	200.08	mg/kg
HCO ₃	280	mg/kg
SO ₄	878.42	mg/kg
Sand	68.4	%
Silt	20.5	%
Clay	11.1	%
Soil texture	Sandy loam	–

2.2 Experimental Design

The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates. The first factor was the source of potassium, comprising seven treatments: conventional potassium fertilizer applied at three levels (4, 6, and 8 g kg⁻¹ soil), where 6 g kg⁻¹ represents the standard recommended rate; nano-potassium fertilizer applied at three concentrations (1, 2, and 3 g L⁻¹), where 2 g L⁻¹ represents the concentration recommended by the producing company; and an unfertilized control. The second factor consisted of four water-stress levels applied as percentages of field capacity: 100% (well-watered control), 75%, 50%, and 25%. Accordingly, the experiment comprised 84 experimental units resulting from the full factorial combination of seven potassium treatments and four irrigation levels with three replicates (7 × 4 × 3).

2.3 Experimental Treatments

Conventional potassium sulfate fertilizer (K₂SO₄; K₂O content: 50%; purity ≥98%) obtained from a local agricultural office was applied at three concentrations (4, 6, and 8 g kg⁻¹ soil), equivalent to 44, 66, and 88 g pot⁻¹, based on the soil weight per pot (11 kg). Fertilizer was applied to the soil after alfalfa plant germination in two doses. Nano potassium fertilizer produced by KHAZRA Company (Iran) was used. According to the manufacturer's technical data sheet, the product contains 10% K₂O (as K) with a nominal particle size ≤50 nm. It is acknowledged that independent physicochemical verification (e.g., TEM, DLS, XRD) of particle

characteristics was not conducted, which represents a limitation of the present study. Three concentrations (1, 2, and 3 g L⁻¹) were prepared by dissolving them in distilled water. The treatments were applied as foliar sprays in two applications, following the protocols reported for other crops [29]. Four irrigation levels were applied according to field capacity: 100% (well-watered control), 75%, 50%, and 25%.

2.4 Determination of Soil Field Capacity

The field capacity of the soil was determined gravimetrically following the method of Klute [30], using three pots each filled with 11 kg of air-dried soil. The soil was irrigated to complete saturation, then covered with plastic bags to minimize evaporation losses and left to drain for 48 hours until gravitational water drainage from the bottom holes ceased, after which the pots were reweighed. Field capacity was calculated from the difference between wet and dry soil weight, yielding an average gravimetric field capacity of 25%, based on which the four irrigation treatments were set at 25%, 50%, 75%, and 100% of field capacity, corresponding to approximate water additions of 0.7, 1.4, 2.0, and 2.7 L per pot, respectively.

2.5 Studied Chemical Traits and Measurement Methods

Total chlorophyll content in leaves was determined according to the method described by Goodwin [31]. A 0.5 g leaf sample of alfalfa was ground with 10 ml of 80% acetone. The extract was kept in darkness in a refrigerator for at least 30 minutes to ensure efficient extraction. The extract was then filtered or centrifuged to obtain a clear solution. Absorbance was measured at 663, 645, and 470 nm using a spectrophotometer, with 80% acetone as the blank. Chlorophyll concentration was calculated according to the following equations:

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times 2$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times 2$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Plant samples were digested according to the method of Cresser and Parsons [32] after drying the shoot samples in an oven at 70°C for 48 hours until constant weight. A 0.2 g sample of dry powder was mixed with 10 ml concentrated sulfuric acid (H₂SO₄) and 2 ml perchloric acid (HClO₄), then heated on a hot plate until a clear digest was obtained. After cooling, the volume was completed to 100 ml with distilled water. Total nitrogen was determined by the Kjeldahl method [33] according to the AOAC International standard procedures [34]. Phosphorus was determined using the Ammonium Molybdate–Ascorbic Acid method, and absorbance was measured at 882 nm using a UV-Visible spectrophotometer, as described by John [35]. Potassium was determined using a Flame Emission Photometer at 766.5 nm, according to the AOAC method [36]. Proline content was determined according to the method described by Bates et al. [37]. A 0.1 g dry sample was ground with 10 ml of 3% sulfosalicylic acid. The extract was centrifuged for 10 minutes and filtered. Then 1.5 ml of filtrate was mixed with 1.5 ml acetic acid and 1.5 ml acid ninhydrin solution, and the tubes were heated in a boiling water bath for 30 minutes. The reaction was stopped by placing the tubes in an ice bath. Afterward, 4 mL toluene was added and mixed using a vortex mixer. The upper toluene layer was separated, and its absorbance was measured at 520 nm using a UV-Visible spectrophotometer. Proline concentration was determined from a standard curve prepared with varying proline concentrations. Protein percentage in plant samples was calculated by multiplying the nitrogen percentage by the conversion factor of 6.25, as described by Kalra [38] and Vopyan [39].

$$\text{Protein (\%)} = \text{N\%} \times 6.25$$

Total carbohydrates were determined according to the method described by Joslyn [40]. A 0.29 g dry sample was mixed with 5 ml of perchloric acid and heated in a water bath at 60°C for one hour, with the process repeated three times. The supernatant was separated by centrifugation (15 min, 3000 rpm). One ml of the extract was diluted to 100 ml with distilled water, and 1 ml of the diluted solution was mixed with 1 ml of 5% phenol and 5 ml concentrated sulfuric acid, then left to cool. Absorbance was measured at 490 nm using a spectrophotometer, and readings were recorded in mg L⁻¹. Carbohydrate percentage was calculated using the following equation:

Carbohydrates (%) = (Instrument reading × (extract volume / sample weight) × dilution factor) / 10000

2.6 Statistical analysis

All experimental data were analyzed using the Statistical Analysis System (SAS) program according to a factorial experiment within a Randomized Complete Block Design (RCBD). Significant differences among treatment means were determined using Duncan's Multiple Range Test at a probability level of 0.05.

3. Results and Discussion

3.1 Effect of conventional potassium fertilization and foliar application of nano-potassium under different levels of water stress on some chemical traits of alfalfa

3.1.1 Total chlorophyll concentration in leaves (mg 100 g⁻¹)

The results of the statistical analysis presented in Table 2 showed that foliar application of nano-potassium significantly increased chlorophyll concentration in alfalfa leaves. The spraying treatments (1, 2, and 3 g L⁻¹) significantly increased chlorophyll content compared with the control treatment, which recorded 100.59 mg 100 g⁻¹, while the highest mean value was observed in the 1 g L⁻¹ treatment, reaching 135.01 mg 100 g⁻¹. The results also indicated that soil-applied potassium fertilizer significantly increased chlorophyll concentration. The treatment 4 g kg⁻¹ soil recorded the highest mean value of 128.76 mg 100 g⁻¹ compared with the control treatment. Water stress levels also significantly affected chlorophyll concentration. Chlorophyll content increased with increasing water stress levels, where the highest mean value (120.04 mg 100 g⁻¹) was recorded at 50% irrigation level, with no significant difference compared with 75% irrigation level, while the lowest mean (105.79 mg 100 g⁻¹) was recorded at 100% irrigation level. Regarding the interaction between experimental factors, the combination of nano-potassium at 2 g L⁻¹ and a 50% irrigation level yielded the highest mean chlorophyll concentration (154.69 mg 100 g⁻¹), with no significant difference from the 3 g L⁻¹ nano-potassium at the same irrigation level. In contrast, the lowest mean value (70.53 mg 100 g⁻¹) was recorded for the interaction between soil potassium treatment at 8 g kg⁻¹ and irrigation level of 50%.

Table 2. Effect of conventional and nano potassium fertilization on the mean total chlorophyll concentration (mg 100 g⁻¹) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	86.43 q	102.38 m	108.39 k	105.17 l	100.59 d
Nano K (2 g L ⁻¹)	102.38 m	145.50 c	154.69 a	127.74 h	132.58 b
Nano K (3 g L ⁻¹)	102.90 m	150.21 b	153.55 a	121.88 i	132.14 b
Nano K (1 g L ⁻¹)	133.45 f	142.64	141.19 d	122.77 i	135.01 a
K (4 g kg ⁻¹)	131.60 g	127.13 h	139.39 e	116.90 j	128.76 c
K (6 g kg ⁻¹)	99.65 n	82.76 r	72.51 s	90.54 p	86.37 e
K (8 g kg ⁻¹)	84.10 r	86.09 q	70.53 t	92.88 o	83.40 f
Mean	105.79 c	119.53 a	120.04 a	111.13 b	

*Means with the same letter are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

3.1.2 Proline concentration (mg 100 g⁻¹)

The results of the statistical analysis presented in Table 3 indicate that foliar application of nano-potassium had a significant effect on proline concentration in alfalfa leaves. The spraying treatments (1, 2, and 3 g L⁻¹) reduced proline concentration compared with the control treatment, which recorded 81.25 mg 100 g⁻¹; the lowest mean value was observed at 1 g L⁻¹ (79.13 mg 100 g⁻¹). In contrast, soil application of conventional potassium fertilizer showed no significant differences in proline concentration compared with the control treatment. The lowest mean value was 80.92 mg 100 g⁻¹ in the treatment 4 g kg⁻¹, while the highest mean value was 81.98 mg 100 g⁻¹ in the treatment 6 g kg⁻¹, without a significant difference compared with the treatment 8 g kg⁻¹. Water stress levels also significantly affected proline concentration. The highest mean value (81.00 mg 100 g⁻¹) was recorded at the 100% irrigation level, while the lowest mean value (80.22 mg 100 g⁻¹) was observed at the 50% irrigation level. Regarding the interaction between the experimental factors, the interaction between the control treatment and the 25% irrigation level recorded the highest mean proline concentration in leaves

(86.66 mg 100 g⁻¹). In contrast, the lowest mean value (73.63 mg 100 g⁻¹) was recorded for the interaction between the control treatment and irrigation level of 100%.

Table 3. Effect of conventional and nano potassium fertilization on the mean proline concentration (mg 100 g⁻¹) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	73.63 j	81.77 cd	82.95 bc	86.66 a	81.25 ab
Nano K (2 g L ⁻¹)	81.94 cd	80.33 def	81.10 cde	78.08 ghi	80.36 c
Nano K (3 g L ⁻¹)	84.13 b	78.95 fgh	79.30 e-h	78.10 ghi	80.12 c
Nano K (1 g L ⁻¹)	84.12 b	78.16 ghi	76.72 i	77.52 hi	79.13 d
K (4 g kg ⁻¹)	82.00 cd	80.33 def	81.03 de	80.31 def	80.92 bc
K (6 g kg ⁻¹)	80.94 de	81.43 cd	81.06 de	84.50 b	81.98 a
K (8 g kg ⁻¹)	80.21 def	84.50 b	79.39 efg	81.11 cde	81.30 ab
Mean	81.00 a	80.78 ab	80.22 b	80.90 b	

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

3.1.3 Mineral content

3.1.3.1 Nitrogen concentration (%)

The results of the statistical analysis presented in Table 4 indicate that foliar application of nano-potassium had no significant effect on nitrogen concentration in alfalfa leaves compared with the control treatment, which recorded 3.30%. However, the highest mean value (4.10%) was observed in the 2 g L⁻¹ treatment, with no significant difference compared with the 1 and 3 g L⁻¹ treatments. Similarly, soil application of conventional potassium fertilizer showed no significant differences in nitrogen concentration compared with the control treatment, although the highest mean value (3.70%) was recorded in the 4 g kg⁻¹ treatment. In contrast, water stress levels significantly affected nitrogen concentration. Nitrogen values decreased with increasing water stress levels. The highest mean value (3.94%) was recorded at the 100% irrigation level, with no significant difference compared with 75% and 25% irrigation levels, while the lowest mean value (2.30%) was observed at the 50% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the nano-potassium at 2 g L⁻¹ and 100% irrigation level recorded the highest leaf nitrogen concentration (4.80%), with no significant difference from other interactions. In contrast, the lowest mean value (2.30%) was recorded for the interaction between conventional potassium treatment at 8 g kg⁻¹ and an irrigation level of 50%.

Table 4. Effect of conventional and nano potassium fertilization on the mean nitrogen concentration (%) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	3.60 abc	3.40 abc	3.20 abc	3.00 abc	3.30 abc
Nano K (2 g L ⁻¹)	4.80 a	4.40 ab	3.80 abc	3.40 abc	4.10 a
Nano K (3 g L ⁻¹)	4.40 ab	3.60 abc	3.60 abc	3.60 abc	3.80 ab
Nano K (1 g L ⁻¹)	4.40 abc	4.00 abc	3.40 abc	4.20 abc	4.00 ab
K (4 g kg ⁻¹)	4.00 abc	3.40 abc	3.60 abc	3.80 abc	3.70 abc
K (6 g kg ⁻¹)	2.80 abc	2.60 bc	2.60 bc	3.40 abc	2.85 c
K (8 g kg ⁻¹)	3.60 abc	3.00 abc	2.30 c	3.60 abc	3.13 bc
Mean	3.94 a	3.49 ab	3.21 b	3.57 ab	

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

3.1.3.2 Phosphorus concentration (%)

The results of the statistical analysis presented in Table 5 indicate that foliar application of nano-potassium had a significant effect on phosphorus concentration in alfalfa leaves. The spraying treatments (1, 2, and 3 g L⁻¹) significantly increased phosphorus concentration compared with the control treatment, which recorded 0.580%, while the highest mean value (0.724%) was observed in treatment 1 g L⁻¹. Similarly, soil

application of conventional potassium fertilizer showed a significant increase in phosphorus concentration compared with the control treatment. The 6 g kg⁻¹ treatment recorded the highest mean value (0.652%), with no significant difference from the 4 g kg⁻¹ treatment. Water stress levels also significantly affected phosphorus concentration. Phosphorus content increased with increasing water stress levels. The highest mean value (0.750%) was recorded at the 50% irrigation level, whereas the lowest mean value (0.473%) was observed at the 100% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the nano-potassium treatments (2, 1, and 3 g L⁻¹) and the 50% irrigation level recorded the highest mean phosphorus concentrations, without significant differences among them. The highest value (0.893%) was recorded in treatment 2 g L⁻¹ at 50% irrigation level. In comparison, the lowest mean value (0.243%) was observed in the interaction between the control treatment and irrigation level of 100%, with no significant difference from the interaction between nano-potassium treatment (2 g L⁻¹) at the same irrigation level.

Table 5. Effect of conventional and nano potassium fertilization on the mean phosphorus concentration (%) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	0.243 i	0.609 c-f	0.710 b-e	0.660 b-f	0.556 c
Nano K (2 g L ⁻¹)	0.340 hi	0.715 b-e	0.893 a	0.748 bc	0.674 ab
Nano K (3 g L ⁻¹)	0.444 gh	0.670 b-f	0.875 a	0.659 b-f	0.662 ab
Nano K (1 g L ⁻¹)	0.533 fg	0.700 b-e	0.880 a	0.782 ab	0.724 a
K (4 g kg ⁻¹)	0.573 efg	0.659 b-f	0.659 b-f	0.700 b-e	0.648 b
K (6 g kg ⁻¹)	0.650 b-f	0.659 b-f	0.651 b-f	0.649 b-f	0.652 b
K (8 g kg ⁻¹)	0.526 fg	0.723 bcd	0.585 def	0.659 b-f	0.623 b
Mean	0.473 c	0.676 b	0.750 a	0.694 b	

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

3.1.3.3 Potassium concentration (%)

The results of the statistical analysis presented in Table 6 indicate that foliar application of nano-potassium had a significant effect on potassium concentration in alfalfa leaves. The 3 g L⁻¹ treatment showed a significant increase compared with the control, which recorded 1.53%, achieving the highest mean value of 2.05%. Similarly, soil-applied conventional potassium fertilizer resulted in a significant increase in leaf potassium concentration. The treatment 8 g kg⁻¹ recorded the highest mean value of 3.79% compared with the control treatment. Water stress also significantly affected potassium concentration. Potassium concentration decreased with increasing water stress levels. The highest mean value (3.25%) was recorded at the 100% irrigation level, while the lowest mean value (2.01%) was observed at the 25% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the interaction between conventional potassium at 8 g kg⁻¹ and irrigation level of 100% recorded the highest mean potassium concentration in leaves (4.83%), with no significant difference compared with the interaction of the same treatment at 75% irrigation level, as well as the interaction between 6 g kg⁻¹ potassium and 100% irrigation level. In contrast, the lowest mean value (0.88%) was recorded for the interaction between the control treatment and the 50% irrigation level.

3.1.4 Total protein concentration (%)

The results of the statistical analysis presented in Table 7 indicate that foliar application of nano-potassium had a significant effect on protein concentration in alfalfa leaves. The treatments significantly increased protein concentration compared with the control treatment, which recorded 21.25%, where the 2 g L⁻¹ treatment recorded the highest mean value (25.63%), with no significant difference compared with the 1 g L⁻¹ treatment. Similarly, soil application of conventional potassium fertilizer significantly increased protein concentration. The treatment 4 g kg⁻¹ recorded the highest mean value (22.50%) compared with the control treatment. Water stress levels also significantly affected protein content. Protein concentration decreased with increasing water stress levels. The highest mean value (24.64%) was recorded at the 100% irrigation level, while the lowest mean value (20.12%) was recorded at the 50% irrigation level. Regarding the interaction

between potassium fertilization treatments and water stress levels, the nano-potassium treatment (2 g L^{-1}) at 100% irrigation level achieved the highest mean leaf protein concentration (30.00%). In contrast, the lowest mean value (14.58%) was recorded for the interaction between conventional potassium treatment (8 g kg^{-1}) and irrigation level of 50%.

Table 6. Effect of conventional and nano potassium fertilization on the mean potassium concentration (%) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	2.20 f–k	1.75 h–m	0.88 m	1.28 klm	1.53 d
Nano K (2 g L^{-1})	2.43 f–j	2.31 f–k	1.51 i–m	1.39 j–m	1.91 cd
Nano K (3 g L^{-1})	2.91 c–g	1.93 g–l	1.85 h–m	1.51 i–m	2.05 c
Nano K (1 g L^{-1})	2.52 e–i	1.87 h–m	1.15 lm	1.78 h–m	1.83 cd
K (4 g kg^{-1})	3.53 bcd	2.91 c–g	3.63 bc	2.03 f–l	3.03 b
K (6 g kg^{-1})	4.35 ab	3.49 b–e	3.00 c–f	2.39 f–j	3.31 b
K (8 g kg^{-1})	4.83 a	4.02 ab	2.61 d–g	3.69 bc	3.79 a
Mean	3.25 a	2.61 b	2.09 c	2.01 c	

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

Table 7. Effect of conventional and nano potassium fertilization on the mean total protein concentration (%) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	22.50 ef	21.25 fg	22.50 ef	18.75 hi	21.25 d
Nano K (2 g L^{-1})	30.00 a	27.50 b	23.75 de	21.25 fg	25.63 a
Nano K (3 g L^{-1})	27.50 b	22.50 ef	22.50 ef	22.50 ef	23.75 b
Nano K (1 g L^{-1})	27.50 b	25.00 cd	21.25 fg	26.25 bc	25.00 a
K (4 g kg^{-1})	25.00 cd	21.25 fg	20.00 gh	23.75 de	22.50 c
K (6 g kg^{-1})	17.50 ij	16.25 jk	16.25 jk	21.25 fg	17.81 f
K (8 g kg^{-1})	22.50 ef	18.75 hi	14.58 k	22.50 ef	19.58 e
Mean	24.64 a	21.79 b	20.12 c	22.32 b	

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

3.1.5 Total carbohydrates concentration (%)

The results of the statistical analysis presented in Table 8 indicate that foliar application of nano-potassium had a significant effect on carbohydrate concentration in alfalfa leaves. The treatments significantly increased carbohydrate content compared with the control treatment, which recorded 8.16%, where the 3 g L^{-1} treatment achieved the highest mean value of 16.40%. Similarly, soil application of conventional potassium fertilizer showed a significant increase in carbohydrate concentration compared with the control treatment. The treatment 8 g kg^{-1} recorded the highest mean value of 12.87%. Water stress levels also significantly affected carbohydrate concentration. Carbohydrate values decreased with increasing water stress levels. The highest mean value (19.70%) was recorded at the 100% irrigation level, while the lowest mean value (9.48%) was recorded at the 50% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the nano-potassium treatment (3 g L^{-1}) at 100% irrigation level achieved the highest mean leaf carbohydrate concentration (35.64%). In contrast, the lowest mean value (3.63%) was recorded for the interaction between conventional potassium treatment (6 g kg^{-1}) and irrigation level of 25%.

Table 8. Effect of conventional and nano potassium fertilization on the mean total carbohydrate concentration (%) in alfalfa leaves under water stress conditions

Potassium treatments	100%	75%	50%	25%	Mean
Control	10.58 k	11.99 ij	4.54 no	5.51 n	8.16 f
Nano K (2 g L ⁻¹)	14.77 ef	12.39 hi	8.86 lm	5.60 n	10.41 e
Nano K (3 g L ⁻¹)	35.64 a	12.28 hi	13.25 ghi	4.43 no	16.40 a
Nano K (1 g L ⁻¹)	24.54 b	13.82 fg	10.76 jk	9.39 klm	14.63 b
K (4 g kg ⁻¹)	15.97 de	14.56 fg	9.15 lm	4.75 no	11.11 d
K (6 g kg ⁻¹)	16.29 d	13.22 ghi	10.22 kl	3.63 o	10.84 de
K (8 g kg ⁻¹)	20.12 c	13.60 fgh	9.60 kl	8.16 m	12.87 c
Mean	19.70 a	13.12 b	9.48 c	5.92 d	

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

4. Discussion

4.1 Total Chlorophyll Content in Leaves

The results of the present study revealed a significant increase in total chlorophyll content in alfalfa leaves following foliar application of nano-potassium compared with the control treatment. This increase may be attributed to potassium's essential role in chlorophyll biosynthesis and photosynthetic processes. Potassium plays a fundamental role in regulating stomatal opening, activating many enzymes associated with photosynthesis, and stabilizing chloroplast membranes, which ultimately contributes to improving photosynthetic efficiency and chlorophyll accumulation in plant tissues [41]. Under drought conditions, plants often experience a decline in chlorophyll content due to oxidative stress and damage to the photosynthetic apparatus. However, adequate potassium nutrition can mitigate these negative effects by maintaining cellular osmotic balance and improving water relations within plant tissues. Potassium also helps maintain turgor pressure and protects chloroplast structures from degradation under stress conditions [42]. The greater effectiveness of nano-potassium observed in this study compared with conventional potassium fertilizers may be explained by nanoparticles' unique physicochemical properties. Nanofertilizers possess a larger surface area and improved solubility, which enhances nutrient absorption and mobility within plant tissues. These properties increase nutrient use efficiency and improve plant metabolic activity under environmental stress conditions [43]. Furthermore, nanoparticles have been reported to enhance plant tolerance to abiotic stresses by stimulating antioxidant systems and improving physiological processes such as photosynthesis and nutrient uptake [23]. Similar effects of foliar fertilizer application on photosynthetic pigments have been documented in other crops [29]. Similar results were reported in alfalfa, where potassium fertilization significantly increased chlorophyll content, improved forage quality, and enhanced plant productivity [44].

4.2 Proline Content

Proline is considered one of the most important osmoprotectants accumulated in plants exposed to environmental stresses such as drought and salinity. In the present study, proline concentration decreased in plants treated with nano-potassium compared with the control treatment. This reduction indicates that potassium application alleviated the intensity of water stress experienced by the plants. Under stress conditions, proline accumulation helps stabilize proteins and cellular membranes, maintain osmotic balance, and protect plant cells from dehydration damage. Therefore, high proline levels are generally associated with severe stress conditions [45]. The lower proline levels observed in potassium-treated plants suggest that potassium improved plant water status and reduced the severity of stress conditions. Potassium enhances osmotic adjustment, regulates stomatal movement, and improves water-uptake efficiency, thereby contributing to maintaining plant metabolic activity under drought conditions [46]. Previous studies also indicated that potassium fertilization reduces oxidative stress by enhancing antioxidant enzyme activities and maintaining membrane stability, thereby decreasing the need for excessive accumulation of stress-related metabolites such as proline [47]. Moreover, plants with improved potassium nutrition exhibit greater tolerance

to osmotic stress and therefore accumulate lower levels of proline compared with plants exposed to nutrient deficiency [48].

4.3 Nitrogen Content

The results of the present study showed that potassium treatments did not significantly affect nitrogen concentration in alfalfa leaves, although slight increases were observed in some treatments. This result may be explained by the fact that nitrogen accumulation in alfalfa largely depends on biological nitrogen fixation through symbiotic relationships with rhizobia bacteria rather than potassium fertilization alone. Nevertheless, potassium indirectly contributes to nitrogen metabolism by activating enzymes involved in nitrogen assimilation and facilitating the transport of nitrogenous compounds throughout plant tissues. Potassium also enhances root growth and increases nutrient uptake efficiency, which may improve nitrogen accumulation under favorable conditions [49]. Water stress significantly influenced nitrogen concentration in the present study. Drought conditions reduce nutrient mobility in the soil and limit nutrient diffusion toward plant roots. Consequently, reduced soil moisture may impair nutrient uptake and decrease nitrogen accumulation in plant tissues [50]. Previous studies have demonstrated that adequate fertilization improves nutrient balance and enhances nitrogen uptake and assimilation in alfalfa, thereby improving plant growth and productivity [51]. Furthermore, environmental factors and soil fertility conditions play important roles in determining nitrogen accumulation and

4.4 Phosphorus Content

The results showed that nano-potassium application significantly increased phosphorus concentration in alfalfa leaves compared with the control treatment. This improvement may be attributed to the synergistic interaction between potassium and phosphorus in plant metabolic processes. Potassium plays an important role in energy metabolism and ATP production, which are essential for phosphorus uptake and transport within plant tissues. Adequate potassium supply, therefore, facilitates phosphorus absorption and improves its distribution within plant organs [52]. The importance of phosphorus fertilizer management in determining crop phosphorus status has also been demonstrated in other crops [53]. Additionally, nanofertilizers have been reported to enhance nutrient absorption efficiency due to their extremely small particle size and increased surface area. These characteristics enable nanoparticles to penetrate plant tissues more effectively, thereby enhancing nutrient mobility and metabolic utilization [54]. Recent studies have also highlighted the potential of nanotechnology to improve plant nutrient uptake and enhance plant tolerance to environmental stresses, including drought [55].

4.5 Potassium Content

As expected, potassium fertilization significantly increased potassium concentration in alfalfa leaves. The highest potassium content was observed in treatments receiving higher potassium fertilization rates, indicating a strong relationship between potassium supply and its accumulation in plant tissues. Potassium is involved in numerous physiological processes, including enzyme activation, regulation of osmotic potential, maintenance of cell turgor, and transport of carbohydrates within plant tissues. Therefore, increasing potassium availability directly enhances plant growth and physiological performance [56]. In calcareous and alkaline soils similar to those used in this study, fertilizer management is particularly important as soil chemistry strongly influences nutrient availability and plant uptake [14, 21]. However, water stress significantly reduced potassium concentration in plant tissues. Under drought conditions, nutrient mobility in the soil decreases, which limits potassium uptake by plant roots. In addition, reduced transpiration rates under water deficit conditions may further limit nutrient transport within the plant [17].

4.6 Total Protein Content

The results of the present study revealed a significant increase in protein content in alfalfa leaves following nano-potassium application. This increase may be related to potassium's role in nitrogen metabolism and protein synthesis. Potassium activates several enzymes involved in amino acid synthesis and facilitates nitrogen assimilation in plant tissues. As a result, improved potassium nutrition enhances protein formation and accumulation in plant tissues [57]. However, water stress may negatively affect protein

synthesis due to reduced metabolic activity and decreased availability of substrates required for protein formation. Severe drought conditions often reduce photosynthetic activity and limit the energy required for protein metabolism [58].

4.7 Total Carbohydrate Content

The results showed that nano-potassium application significantly increased total carbohydrate concentration in alfalfa leaves. This increase may be attributed to potassium's role in regulating photosynthetic processes and carbohydrate metabolism. Potassium enhances carbon assimilation by regulating stomatal conductance and facilitating the transport of photosynthates from leaves to other plant organs. Consequently, plants supplied with adequate potassium accumulate higher carbohydrate concentrations [59]. Under severe water stress conditions, carbohydrate accumulation may decline due to reduced photosynthetic efficiency and limited carbon fixation. Drought stress often leads to stomatal closure, which restricts CO₂ uptake and reduces carbohydrate production [58]. Nevertheless, nanotechnology-based fertilizers may enhance plant tolerance to drought stress by improving nutrient uptake efficiency, enhancing metabolic activity, and increasing carbohydrate accumulation in plant tissues [60].

5. Conclusions

The results of this study showed that both conventional potassium fertilization and nano-potassium application significantly affected several chemical characteristics of alfalfa (*Medicago sativa* L.) under different levels of water stress. Nano-potassium application increased chlorophyll content, protein percentage, and total carbohydrates, indicating enhanced physiological performance of plants under water-deficit conditions. In addition, potassium fertilization improved the mineral nutrient status of the plants, particularly potassium concentration in plant tissues. Water stress levels also clearly influenced most of the studied traits, and the interaction between potassium treatments and irrigation levels played an important role in regulating plant responses. Overall, potassium nutrition, especially in nanoform, can improve chemical characteristics and enhance alfalfa plants' tolerance to water stress. Specifically, nano-K at 1–2 g L⁻¹ is recommended as the optimal concentration range, and 75% field capacity is recommended as the optimal irrigation level to balance water conservation with acceptable forage quality. These results suggest that nano-K can serve as a complementary or partial replacement for conventional K fertilization under water-limited conditions. Future field-scale validation studies are recommended to extend these pot-based findings to practical agronomic applications.

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