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Research Article

Effects of Various Soil Amendments on the Growth of Red Clover (*Trifolium pratense* L.)

Mehdi Moameri[1*], Zahra Mohammadirad[2], Ardavan Ghorbani[1], Faezeh Beyrampoor[2], Ali Shahi Gharelar[3]

[1]*Department of Range and Watershed Management, Faculty of Natural Resources, Water Management Research Center, University of Mohaghegh Ardabili, Ardabil, Iran*
[2]*Department of Range and Watershed Management, Faculty of Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran*
[3]*Department of Plant Sciences and Medicinal Plants, Meshginshahr Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran*

***Corresponding author:** : moameri@uma.ac.ir

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Abstract

Uniform and rapid seed germination is essential for successful plant establishment and growth. Previous studies have shown that both organic and mineral amendments can improve germination rates and early plant development. This study investigated the effects of zeolite, bentonite, and potassium nanochelate on the growth characteristics of *Trifolium pratense* through a factorial pot experiment based on a Completely Randomized Design (CRD). Treatments included zeolite (2, 4, and 6 g/kg), potassium nanochelate (30, 60, and 90 mg/kg), bentonite (2, 4, and 6 g/kg), and combined applications of potassium nanochelate and zeolite. Soil samples were randomly collected from the Fandqalou rangelands of Ardabil, Iran. The prepared soil was then added to pots, where 20 seeds of T. pratense were sown per pot. After the growth period, plant height, volume, dry and fresh weight, root weight (fresh and dry), root length, root volume, root surface area, establishment rate, chlorophyll content, electrolyte leakage, leaf relative water content, and membrane stability index were measured. Results indicated that the highest fresh weight of aerial parts (124.28g/pot), dry aerial weight (34.11g/pot), dry root weight (14.30g/pot), and fresh root weight (56.84g/pot) were achieved using 30mg/kg potassium nanochelate + 2g/kg zeolite, 60mg/kg potassium nanochelate + 4g/kg zeolite, 60mg/kg potassium nanochelate, as well as with 90mg/kg potassium nanochelate, respectively. The longest root length (27.33 cm) was observed with 6 g/kg zeolite, while the greatest root volume (61.67 mm^3) and root surface area (71.06 cm^2) were recorded with 6 g/kg bentonite. The combination of 60 mg/kg potassium nanochelate + 4 g/kg zeolite yielded the highest establishment rate (84%). Findings suggest that targeted use of mineral amendments can significantly improve red clover growth and may be beneficial for rangeland restoration and pasture establishment in degraded or low-yielding rain-fed areas.

Keywords: *Trifolium pratense*, Growth enhancement, Rangeland improvement, Zeolite, Bentonite, Potassium nanochelate

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1. Introduction

Natural ecosystems are fundamental pillars of sustainable development in any country and are classified as renewable natural resources. Among them, rangelands a key role as vital components, forming dynamic ecosystems that cover approximately 47% of the Earth's land surface. These landscapes are valuable national assets, playing a crucial role in forage production, particularly for small livestock that depend heavily on them for sustenance. However, these ecosystems are increasingly threatened by the environmental pressures such as overgrazing and unsustainable exploitation. These practices have led to severe degradation, pushing many rangelands toward complete destruction. This degradation can have serious consequences, including forage shortages, devastating floods, desertification, and the spread of moving sand dunes (Mesdaghi, 2015; Moghadam, 2011; Azarnivand & Zare Chahouki, 2011). Human activities, along with social, economic, and individual factors, are among the main causes of rangeland degradation. In addition, early grazing, the conversion of rangelands into low-yield rainfed farmlands, and the subsequent abandonment of these lands are also significant contributors to the deterioration of these critical ecosystems (Gholampour et al., 2012). Therefore, it is essential to seek effective solutions for managing these lands. One viable management strategy is the restoration and rehabilitation of degraded rangelands, which can prevent further degradation, soil erosion, and nutrient leaching (Sheidai Karkaj et al., 2017).

Rangeland rehabilitation involves several key measures, including biological restoration, which facilitates the establishment of valuable and native forage species on low-productivity lands through the creation of artificial or managed rangelands. To achieve this, it is crucial to implement seed enhancement techniques and methods that support initial plant establishment prior to sowing. These practices help mitigate environmental stressors and significantly improve the speed and success of plant establishment (Mehrabi et al., 2010). Uniform, timely, and rapid germination is essential for optimal plant performance and successful establishment (Janalizadeh Ghazvini et al., 2017). This, in turn, can significantly enhance the success of rangeland improvement and revegetation projects. Achieving this requires the use of certain growth facilitators that are both accessible and economically viable. Previous studies have shown that such facilitators can have a positive and meaningful effect on the germination and early growth of rangeland plants (Eskandari & Alizadeh Amraei., 2017). Moreover, since rangelands cover a vast portion of the Earth's drylands, it is essential to use facilitators that are cost-effective (Roosta et al., 2015). One such facilitator is zeolite. Rhodes (2010) described zeolites as a unique class of solid materials with diverse solvent-phase structures, capable of functioning as effective ion exchangers in aqueous environments and retaining water in the soil, thereby supporting plant water availability. Zeolites are known for their relatively low environmental impact compared to other alternatives, and they can absorb and retain water for plant use. As a naturally occurring mineral, zeolite has gained commercial significance as a superabsorbent, valued in agriculture and for enhancing soil productivity due to its ability to retain moisture over extended periods. While zeolites share similarities with clay minerals, they differ in their crystalline structure, which allows them to retain both cations and water (Houerou, 2008). Zeolites are aluminosilicate minerals formed through the alteration of volcanic rocks. Their unique physical and chemical properties, along with their abundance in sedimentary and volcanic deposits, have facilitated their extensive use in agriculture and environmental applications. These include reducing chemical fertilizer usage and enhancing fertilizer efficiency. Incorporating zeolites into plant-growing media encourages the use of affordable, locally sourced raw materials, thereby decreasing reliance on costly imported products (Mahrokh et al., 2019).

Another widely used soil amendment is bentonite, a natural superabsorbent derived from a mixture of clay minerals. Bentonite is known for its high adhesive capacity and strong swelling behavior when water is added, it transforms into a gel-like substance. It also has a relatively high water absorption capacity. This absorbent material can retain water in the planting substrate, thereby enhancing plant growth and establishment. Moreover, the minerals that compose bentonite improve soil structure by binding soil aggregates, which increases both saturation moisture content and overall soil moisture levels (Abedi Kohpayee & Sohrab, 2004). Bentonite and Nano bentonite, due to their pronounced influence on the physical rather than chemical properties of soil, -such as enhancing water retention capacity, reducing infiltration rate, and improving soil texture- can significantly enhance the performance of sandy soils. Their application supports better plant establishment and promotes sustained vegetative growth, particularly in arid and semi-arid environments where water scarcity and poor soil structure limit productivity (Khosravian Chatroodi et al., 2025). The application of bentonite leads to improved soil structure and an increased water holding capacity (Tahir and Marschner, 2016). Its low cost, high specific surface area and environmental compatibility have made it widely used in various agricultural applications (Seyed Hosseini et al., 2023). Studies have shown that when bentonite was combined with sawdust, it significantly enhanced the growth of *Capsicum annuum* plant (Barzegar Hafshejani et al., 2015).

Moreover, potassium nano-chelate is an emerging soil amendment that has recently been recommended as an alternative to, or replacement for, conventional chemical fertilizers. It enhances the uptake of micronutrients and increases nitrate accumulation in plants. In aqueous environments, it dissociates into ions, and since potassium is an essential macronutrient for plant growth, it promotes faster plant establishment (Zahedifar & Zohrabi, 2016; Ghahremani et al., 2014). Research has shown that potassium nano-chelates improve both yield and fruit quality in date palms compared to conventional potassium sulfate fertilizers (El Salhy et al., 2021). Additionally, their application has been linked to enhanced oil quality in olive trees (Rohi Vishkayi et al., 2023). These nanofertilizers have also demonstrated potential in supporting germination, growth, and plant establishment in rangeland restoration and abandoned dryland rehabilitation projects (Abbasi Khalki et al., 2019; Abbasi Khalki et al., 2021; Moameri et al., 2018; Moameri & Dadjou, 2019). Due to their superior absorption by plants, nanofertilizers are generally more efficient than traditional chemical fertilizers (Roosta et al., 2015).

Various studies have investigated the effects of soil amendments (plant growth facilitators) on the growth characteristics of different plant species. Alijafari et al. (2020) examined the impact of different growth facilitators potassium nanosilicate at concentrations of 0, 500, and 1000 mg/L; crystal water superabsorbent polymer at 0, 10, and 30 g/kg; effective microorganisms at 0%, 1% and 2% and manure fertilizer at 0, 100, and 200 g/kg on the growth traits and performance of *Onobrychis sativa* Lam. Their results indicated that the highest values for dry plant weight, root length, plant height, root volume, aerial organ volume, establishment rate, leaf area, photosynthetic rate, and chlorophyll index were achieved with the 200 g/kg manure fertilizer treatment, although other treatments also had positive effects on plant growth and performance. Paradelo et al. (2019) investigated water retention capacity and the growth of *Lolium multiflorum* in compost-based substrates amended with polyacrylamide, guar gum, and bentonite. They reported that bentonite significantly enhanced the water moisture content in the planting media. This finding suggests that bentonite can effectively be used to improve water-holding capacity in plant growth substrates.

Yar Ahmadi & Akhzari (2021) investigated the interactive effects of zeolite and pitting on the concentration of elements, root length, and stem length in *Agropyron elongatum* L. under various drought treatments. They found that after rangeland improvement operations (pitting) and zeolite Application, the concentration of soil elements-including calcium, copper, potassium, manganese, sodium, zinc, and phosphorus, as well as stem and root biomass and the stem to root length ratio, all increased significantly. Balidakis et al. (2022) investigated the effects of sewage sludge amended with clay minerals and biochar on soil properties, the growth of *Trifolium repens* L. (white clover), and the colonization of arbuscular mycorrhizal fungi. Their findings indicated that sewage sludge treated with bentonite, vermiculite, and biochar served as effective soil amendments, significantly promoting white clover growth. Beyrampoor et al. (2022) investigated the effects of several growth facilitators on the growth characteristics of *Trifolium repens* L. and found that combinations of growth facilitators such as potassium nano-chelate and zeolite were the most effective treatments in improving growth traits and increasing the yield of *T. repens*. Agooshi et al. (2025) investigated the effect of natural zeolite and potassium-enriched zeolite on the performance, yield, and potassium concentration in soil and Brassica napus. They found that the amendment treatments had a significant impact on all the studied traits, and the interaction between treatment and soil type significantly affected seed yield.

The increasing population, improper land use, poor management, recent droughts, and the expansion of abandoned drylands have tensified the focus on restoring and rehabilitating degraded rangelands, aiming to convert them into productive pasturelands. Consequently, numerous studies in recent years have emphasized the importance of using accessible and effective soil amendments as a cost-efficient, low-risk strategy for restoring these valuable ecosystems. On the other hand, red clover (*Trifolium pratense*) is a forage legume cultivated in the temperate world, known for its high-protein feed. It is an herbaceous, perennial plant belonging to the Fabaceae family. It thrives in a wide range of soil types, thanks to its deep taproot, which enhances drought tolerance and contributes to soil structure improvement. Highly palatable, red clover is an important forage crop for cattle and sheep. It is known for its high forage yield and is widely utilized in pastures and crop rotation systems, particularly in Europe and the United States. So, this species is commonly cultivated in mixtures with plants from the Poaceae family. Crop rotation systems in pastures involve the strategic alternation of different forage crops, sometimes combined with cash crops or fallow periods, to enhance soil health, optimize grazing management, and increase livestock productivity. Its roots contain nitrogen-fixing nodules, which enhance soil fertility (Bohrani, 2013; Jouri & Mahdavi, 2011). Red clover is among the oldest cultivated forage species, with a history of widespread use across various regions,

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including cold climates where it is sown in both autumn and spring (Khodabandeh, 2010). Red clover exhibits a sporadic distribution, predominantly inhabiting cold and moist montane grasslands, open woodlands, field margins, and roadsides within the Irano-Turanian and Hyrcanian phytogeographical regions. The species thrives in cold to semi-humid climates, favoring deep to moderately deep soils that are nutrient-rich (Moghimi, 2005). In Ardabil Province, red clover is commonly found across diverse grassland habitats, including ecotonal meadows between Ardabil and Gilan provinces, foothill grasslands surrounding Mount Sabalan, the meadows of Khalkhal, and various other mountainous rangelands throughout the province (Samadi Khanghah et al., 2022). So, this research aims to assess the effects of zeolite, bentonite, and potassium nano-chelate amendments on the growth characteristics of *Trifolium pratense* L. If these amendments demonstrate a positive impact on the species' growth, they could be utilized for rangeland restoration, Pastureland establishment, and the conversion of low-yield drylands into sites for forage or medicinal plant cultivation.

2. Materials and methods

This study was conducted in the greenhouse of the University of Mohaghegh Ardabili as a factorial experiment using a completely randomized design with three replications. To obtain the soil required for red clover cultivation, samples were randomly collected from five locations within the root zone (0–30 cm depth) of red clover plants in the Fandghloo rangelands, located 35 km far from Ardabil city and 10 km south of Namin County. In this region, red clover grows as an indicator species within plant communities, thriving in the grasslands of Fandoghlu (Samadi Khangah et al., 2021). *Trifolium repens, Trifolium resupinatum, Leucanthemum vulgare, Poa pratensis, Plantago Lanceolata, Trisetum flavescense, Alchemilla caucasia, Sanguisorba minor, Dactylis glomerata* are some of the species associated with this plant.

Red clover seeds were collected from the Fandghloo grasslands during the plant's seeding stage, sourced from various plant bases. Efforts were made to collect seeds from strong and healthy plants. Robust plants produce healthy, large, and vigorous seeds, which contribute to higher rates of successful germination and improved plant establishment upon sowing. In the next step, the collected soils were homogenized, and coarse particles-such as stones, pebbles, and roots-were removed using a 4-mm sieve. Additionally, after thorough drying in the laboratory, the seeds were cleaned, and plant residues were removed from the seeds. The seeds of this species has not dormancy. The red clover seeds had a purity of 99%, a germination rate of 92%, and a thousand- grain weight of 5.5 grams (figure1).

In the next stage, the growth facilitators of zeolite and bentonite, (with an approximate diameter of 3 mm) were obtained from the Zaminkav Company in Tehran, and nano-potassium chelate (Khazra Nano Fertilizer containing 27% potassium chelate) was sourced from Golbaran Sabz Company in Tehran. Zeolite and Bentonite were ground into a powder using a mill before application. The operation of preparing the potting soil and adding the treatments to the soil was carried out as follows: Each pot contained 3 kg of soil. The treatments were as follows: zeolite and bentonite at concentrations of 0, 2, 4, and 6 g/kg of soil, which were thoroughly mixed with the soil (Ahmadiazar et al., 2015). Additionally, nano-chelated potassium was applied to the potting soil at concentrations of 0, 30, 60, and 90 mg/kg, in accordance with the experimental design (Larki et al., 2015). The zero-percentage treatment was considered as the control. The combined treatments consisted of nano-potassium chelate at 30 mg/kg with zeolite at 2 g/kg, nano-potassium chelate at 60 mg/kg with zeolite at 2 g/kg, and nano-potassium chelate at 90 mg/kg with zeolite at 6 g/kg. These components were precisely measured using a digital scale, thoroughly mixed with the soil, and subsequently added to the pots.

After preparing the treatments and adding the soil and treatments to the pots, 20 seeds of red clover were sown at the appropriate depth in each pot. The seeds were disinfected with sodium hypochlorite solution before the experiment and then washed with distilled water after disinfection (Moameri et al., 2020). At the end of the growing period (6 months), plant growth characteristics were determined. At this stage, various factors were measured, including the number of surviving plant bases (establishment rate), plant height, plant volume, dry and fresh weight of the plant, leaf relative water content (%), chlorophyll content, root length, root volume, root surface area, dry and fresh root weight, electrolyte leakage percentage, and membrane stability. To assess plant establishment rate, the number of surviving plants in each pot was recorded. The establishment rate percentage was calculated as the ratio of the number of seeds that successfully germinated and survived to the total number of seeds initially planted. The fresh and dry plant weight was determined using a digital weight scale (0.001 g). Root length, plant height, and plant volume were measured using ruler (mm). Root volume was measured by the displacement method after immersing the roots in water using a 1000 cc graduated cylinder (Moameri & Abbasi Khalaki., 2019). The establishment rate was calculated based on the number of established individuals. The chlorophyll content of leaves was measured using a

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SPAD chlorophyll meter (SPAD-502 Plus, Konica Minolta, Japan). Total chlorophyll measurements were conducted with this device at the Laboratory of Mohaghegh Ardabili University, Iran. The leaves Relative Water Content (RWC) was determined by selecting five Fresh leaves at random and Weighing them (FW). These leaves were then submerged in distilled water and stored in a dark environment at 4°C for 24 hours and being re-Weighed (TW). Subsequently, the Dry Weight (DW) of the leaves was measured after drying them in an oven at 60°C for 24 hours. Finally, the relative water content was calculated using (Equation 1) (McDonald, 2000):

$$RWC\% = \frac{FW-DW}{SW-DW} \times 100 \quad (1)$$

To measure electrolyte leakage, five leaf samples were collected from the base of each plant in every treatment. Each sample was placed separately in a 20 mL Falcon tube containing distilled water and stored in the laboratory. After 24 hours, the electrical conductivity (EC1) of each sample was measured using an EC meter. To assess the total electrolytes released due to cell death, the Falcon tubes were autoclaved at 120°C for 40 minutes. After autoclaving, the samples were returned to laboratory conditions, and their electrical conductivity was measured again (EC2). The percentage of electrolyte leakage was then calculated using (Equation 2). Then, the membrane stability index (Curtain Stability Index) was determined by evaluating the electrolyte leakage of the leaves (Equation 3) (Mohsenzadeh et al., 2006).

$$EL\% = [EC1/EC2] \times 100 \quad (2)$$

$$CSI\% = [1 - (EC1/EC2)] \times 100 \quad (3)$$

Where
EL: Percentage of electrolyte leakage.
CSI: Membrane stability index,
EC1: Initial electrolyte leakage.
EC2: Secondary electrolyte leakage.

2.1. Statistical analysis

At the end of the plant growth period, the collected data were analyzed using SPSS software (version 24) to assess the effects of facilitators on the growth characteristics of *T. pratense*. The normality of the data was examined using the Kolmogorov-Smirnov test, while Levene's test was applied to evaluate the homogeneity of variances. To determine the impact of different treatments on red clover growth, a one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test for mean comparisons.

3. Result

3.1. Morphological traits

The analysis of variance revealed significant effects of treatments on the fresh weight of aerial parts, plant height, dry weight, fresh root weight, plant volume, root length, and root volume ($p<0.5$ and $p<0.01$). However, their effects on root dry weight, root surface area, and plant establishment rate were not statistically significant (Table 1).

The mean comparison results presented in Table (2) indicate that the combination of potassium nanochelate and zeolite had the most significant positive effect on plant growth. According to the findings, the highest fresh weight (124.28 g) showed a 93.04% increase compared to the control, while the highest dry weight (34.11 g) exhibited a 50.4% increase. The highest dry root weight (14.30 g) demonstrated a 35.01% increase, and the highest fresh root weight (56.84 g) showed a 34.7% increase in the treatments with potassium nanochelate at 30 mg/kg + zeolite at 2 g/kg and potassium nanochelate at 60 mg/kg + zeolite at 4 g/kg. Additionally, the highest root volume (61.67 mm), with a 68.1% increase compared to the control, was observed in the treatment with bentonite at 6 g/kg. The combination of potassium nanochelate at 60 mg/kg + zeolite at 4 g/kg also resulted in the highest plant Establishment rate (84%). Furthermore, the largest root surface area (71.06 cm²), with a 24.3% increase compared to the control, was recorded in the treatment with bentonite at 6 g/kg

3.2. Physiological traits

The results of the analysis of variance for the treatments under study (zeolite, potassium nanochelate, bentonite, and the combination of potassium nanochelate with zeolite) revealed that there was no significant effect on the chlorophyll index, relative water content of leaves, electrolyte leakage percentage, and membrane stability index, as shown in Table (3).

The results of the comparison of the means for the effect of the studied treatments (zeolite, potassium nanochelate, bentonite, and the combination of potassium nanochelate with zeolite) on the physiological characteristics of red clover are presented in Table (4). Although no significant differences were observed between the various treatments statistically, the SPAD chlorophyll index in the bentonite treatment at 6 g/kg showed the highest value compared to the control. The leaves relative water content in the zeolite treatment at 6 g/kg had the highest value at 92.68%. The electrolyte leakage percentage was highest in the bentonite treatment at 2 g/kg, with a value of 18.89% (Table 4).

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Figure 1. A view of *Trifolium pratense* L. cultivation in the pots in greenhouse

Table 1. Analysis of variance of the effect of the studied treatments on the morphological characteristics of *T. pretense*

Variable	Sources of Variation	df	Mean Square	F value
Plant height	Treatment	12	26.63	2.33*
	Error	26	11.41	
Plant fresh weight	Treatment	12	726.55	7.72**
	Error	26	94.03	
Plant volume	Treatment	12	95574541	1.18 ns
	Error	26	80798065	
Plant dry weight	Treatment	12	28.66	3.46*
	Error	26	8.28	
Root fresh weight	Treatment	12	59.55	2.15*
	Error	26	27.64	
Root dry weight	Treatment	12	3.30	1.53ns
	Error	26	2.15	
Root height	Treatment	12	8.01	3.99*
	Error	26	2.00	
Root volume	Treatment	12	118.59	2.25*
	Error	26	52.56	
Root surface area	Treatment	12	38.20	1.69ns
	Error	26	22.54	
Establishment rate	Treatment	12	96.34	1.02ns
	Error	26	94.35	

** $p < 0.01$, * $p < 0.05$, ns is no significant

Table 2. Mean comparison effect of the treatments on the morphological characteristics of *T. pretense*

Treatment	Wet weight (gr/pot)	Dry weight (gr/pot)	Plant volume (cm³)	Plant height (cm)	Root dry weight (gr/pot)
Control	78.34 d	22.67 d	36086a	24.89b	10.85 ab
Zeolite 2 (g/kg)	94.26 b	26.72 cd	38086a	29.82ab	10.90 ab
Zeolite 4 (g/kg)	92.28 b	30.24 abc	54694a	30.21ab	13.15 a
Zeolite 6 (g/kg)	113.70 a	29.68 abc	42233a	33.62ab	12.50 ab
Bentonite 2 (g/kg)	93.32 b	30.21 abc	4140a	32.28ab	13.35 a
Bentonite 4 (g/kg)	83.27 cd	28.87 abc	48144a	34.99a	13.19 a
Bentonite 6 (g/kg)	85.11 cd	27.82 bc	43893a	34.05ab	13.97 a
Potassium nanochelate 30 (mg/kg)	93.51 b	30.04 abc	53625a	34.51ab	13.45 a
Potassium nanochelate 60 (mg/kg)	111.59 a	31.77 abc	39620a	33.76ab	14.30 a
Potassium nanochelate 90 (mg/kg)	116.03 a	32.51 ab	44000a	31.74ab	13.83 a
Potassium nanochelate 30 + Zeolite 2	124.28 a	32.93 ab	43375a	29.58ab	12.45 ab
Potassium nanochelate 60 + Zeolite 4	118.86 a	34.11 a	44103a	30.64ab	12.92 ab
Potassium nanochelate 90 + Zeolite 6	116.30 a	23.00 ab	49920a	27.48ab	12.75 ab

Different letters within columns indicate significant difference between treatments

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Table 2. (continued)

Treatments	Root fresh weight (g/pot)	Root length (cm)	Root volume (mm)	Root surface area (cm)	Establishment rate (%)
Control	42.17 ^{d}	28.83 ^{a}	36.67 ^{cd}	51.77 ^{c}	69.33 ^{bc}
Zeolite 2 (g/kg)	45.06 ^{bcd}	26.33 ^{abc}	43.33 ^{bcd}	59.78 ^{bc}	66.67 ^{bc}
Zeolite 4 (g/kg)	45.33 ^{bcd}	26.67 ^{abc}	48.33 ^{bcd}	63.33 ^{ab}	70.67 ^{bc}
Zeolite 6 (g/kg)	45.33 ^{bcd}	27.33 ^{ab}	45.00 ^{bcd}	62.13 ^{ab}	74.67 ^{ab}
Bentonite 2 (g/kg)	48.67 ^{abcd}	26.33 ^{abc}	53.33 ^{abc}	66.33 ^{ab}	65.33 ^{bc}
Bentonite 4 (g/kg)	48.67 ^{abcd}	26.17 ^{abc}	50.00 ^{abcd}	64.06 ^{ab}	69.33 ^{bc}
Bentonite 6 (g/kg)	50.84 ^{abc}	26.33 ^{abc}	61.67 ^{a}	71.06 ^{a}	77.33 ^{ab}
Potassium nanochelate 30 (mg/kg)	51.60 ^{abc}	24.33 ^{cd}	53.33 ^{abc}	63.76 ^{ab}	66.67 ^{bc}
Potassium nanochelate 60 (mg/kg)	52.78 ^{abc}	25.00 ^{bcd}	53.33 ^{abc}	64.63 ^{ab}	72.00 ^{bc}
Potassium nanochelate 90 (mg/kg)	56.84 ^{a}	26.00 ^{abc}	56.67 ^{ab}	67.99 ^{ab}	70.67 ^{bc}
Potassium nanochelate 30+Zeolite 2	54.92 ^{ab}	23.00 ^{d}	51.67 ^{abc}	61.07 ^{ab}	81.33 ^{a}
Potassium nanochelate 60+Zeolite 4	53.85 ^{ab}	23.33 ^{d}	53.33 ^{abc}	62.48 ^{ab}	84.00 ^{a}
Potassium nanochelate 90+Zeolite 6	49.05 ^{abcd}	24.33 ^{cd}	24.33 ^{d}	61.77 ^{ab}	73.33 ^{ab}

Different letters within columns indicate significant difference between treatments

Table 3. Analysis of variance of the effect of the studied treatments on the physiological characteristics of red clover

Variable	Sources of Variation	df	Mean Square	F value
Chlorophyll index	Treatment	12	4.07	1.32 ^{ns}
	Error	26	3.07	
Leaf relative water content	Treatment	12	29.00	1.09 ^{ns}
	Error	26	26.51	
Electrolyte leakage percentage	Treatment	12	25.97	1.11 ^{ns}
	Error	26	23.40	
Membrane stability index	Treatment	12	25.92	1.10 ^{ns}
	Error	26	23.38	

^{ns} is not significant

Table 4. Comparison of the average effect of the studied treatments on the physiological characteristics of the red clover species

Treatments	Chlorophyll index	Leaf relative water content (%)	Electrolyte leakage percentage (%)	Membrane stability (%)
Control	44.63 ^{ab}	87.72 ^{ab}	11.05 ^{ab}	88.98 ^{ab}
Zeolite 2 (g/kg)	44.16 ^{ab}	80.73 ^{b}	9.98 ^{ab}	90.02 ^{a}
Zeolite 4 (g/kg)	45.06 ^{ab}	87.94 ^{ab}	10.25 ^{ab}	89.75 ^{ab}
Zeolite 6 (g/kg)	45.23 ^{ab}	92.68 ^{a}	8.94 ^{ab}	91.05 ^{a}
Bentonite 2 (g/kg)	46.80 ^{a}	86.44 ^{ab}	18.89 ^{a}	81.11 ^{b}
Bentonite 4 (g/kg)	46.80 ^{a}	86.91 ^{ab}	10.35 ^{ab}	89.64 ^{ab}
Bentonite 6 (g/kg)	47.83 ^{a}	82.58 ^{b}	8.42 ^{ab}	91.75 ^{a}
Potassium nanochelate 30 (mg/kg)	45.83 ^{ab}	83.76 ^{b}	7.97 ^{ab}	92.02 ^{a}
Potassium nanochelate 60 (mg/kg)	46.43 ^{a}	83.30 ^{b}	7.84 ^{ab}	92.15 ^{a}
Potassium nanochelate 90 (mg/kg)	45.03 ^{ab}	85.98 a^{b}	8.19 ^{ab}	91.80 ^{a}
Nano-Potassium Chelate 30 + Zeolite	47.50 ^{a}	87.38 ^{ab}	7.78 ^{b}	92.20 ^{a}
Nano-Potassium Chelate 60 + Zeolite 4	45.46 ^{ab}	85.83 ^{ab}	8.16 ^{ab}	91.83 ^{a}
Nano-Potassium Chelate 90 + Zeolite 6	47.13 ^{a}	82.67 ^{b}	9.57 ^{ab}	90.42 ^{a}

Different letters within columns indicate significant difference between treatments

4. Discussion

The findings of this study indicate that increasing the concentration of zeolite enhances key growth and establishment rate in red clover, including plant height, fresh weight, dry weight of aerial parts, and establishment percentage. This effect can be attributed to the superabsorbent properties of zeolite, which enable it to retain water and gradually release it into the soil solution, thereby improving moisture availability for plant uptake and fostering growth. In this context, Beyrampoor et al. (2022) found that applying a zeolite treatment positively influenced some morphological traits in white clover (*Trifolium repens*), demonstrating that higher zeolite concentrations led to improved plant growth and development. Similarly, Dehdari et al. (2017) reported that the application of 4 g/kg of zeolite increased the performance of traits such as fresh shoot length, plant fresh and dry weight, and fresh root weight in Medicago scutellata. Akbari et al. (2010) also highlighted the role of zeolite as a superabsorbent, emphasizing its effectiveness in retaining soil moisture and facilitating water transfer to plants. Moreover, Karimzadeh Asl et al. (2018) showed that applying a zeolite treatment at a rate of 2 g/kg enhanced plant height, leaf area, dry matter accumulation, and flowering performance in *Dracocephalum moldavica*. Moreover, Tavakoli et al. (2024) demonstrated that the use of a combination of biochar and bentonite reduces soil evaporation by 7 to 14%. Additionally, soil moisture increased significantly in all treatments containing biochar and bentonite, making it beneficial for improving irrigation water use and reducing costs.

The results of this study indicate that bentonite significantly influenced several plant characteristics, including fresh and dry weight, root volume, root area, and establishment rate. Under the bentonite treatment (6 g/kg), root volume increased by 1.68%, while root area expanded by 3.24% compared to the control group. Balidakis et al. (2023) reported that white clover plants exhibited higher uptake of nitrogen, phosphorus, and potassium in sludge treated with bentonite compared to sludge treated with vermiculite and biochar. Similarly, Beyrampoor et al. (2022) found that bentonite treatment (2 g/kg) significantly influenced key growth parameters in white clover, including dry weight, fresh root weight, root volume, root area, and establishment percentage. Shirzadi et al. (2020) investigated the effects of bentonite and superabsorbent polymer on the growth of Cichorium intybus and observed that bentonite improved plant performance and enhanced resistance to water stress. Additionally, Paradelo et al. (2019) noted that hydraulic conductivity increased in bentonite-treated substrates, as bentonite reduced water movement and retained moisture within soil pores. The SPAD chlorophyll index in the bentonite treatment (6 g/kg) recorded the higher value compared to the control, while the electrolyte leakage percentage was higher in the bentonite treatment (2 g/kg), reaching 18.89%. Overall, although bentonite had no significant effect on the physiological indices of red clover, most of these indices showed an upward trend under bentonite treatment. This improvement may be attributed to bentonite's high water retention capacity, which increases soil moisture availability and creates a more favorable environment for plant growth. In this context, Paradelo et al. (2019) also reported that bentonite enhances the water retention capacity of the substrate for cultivating *Lolium multiflorum*. Additionally, Youssef (2013), in his study on the effects of bentonite and mineral zeolite on potato yield, found that bentonite (at rates of 4, 5, and 6 ton/ha) and zeolite (at rates of 1, 2, and 3 ton/ha) promoted growth traits and increased potato yield. The effect of the studied treatments on the physiological characteristics of the plant was not statistically significant. However, in general, the treatments performed better than the control and contributed to improvements in the physiological characteristics of red clover. Safikhan et al. (2019) demonstrated that zeolite had a significant effect on membrane stability index of *Silybum marianum*.

The results indicate that potassium nanochelate significantly influenced several growth and establishment parameters in red clover. The plant's fresh weight showed the greatest increase (48%), dry weight (43.40%), and root volume (54.54%) compared to the control treatment. Similarly, most of the plant's growth indices increased with the concentration of this treatment. It seems that since potassium enhances root growth, it improves the absorption of water and nutrients by the roots, which directly impacts plant performance and growth. In the potassium nanochelate treatment (90 mg/kg), the highest values for fresh root weight (7.34%) and dry root weight (1.35 %) were observed. According to Barzegari (2021), potassium nanochelate (0, 35, and 65 mg/L) mitigated the effects of drought stress on wheat's vegetative traits at the higher concentration (65 mg/L). The research by Baratzadeh et al. (2019) on the effect of potassium nanochelate, ascorbic acid, and their combination on the performance of black-eyed pea (Kamran variety) showed that increasing the concentration of potassium nanochelate and ascorbic acid (4 L/ha + 30 mL/ha) led to both quantitative and qualitative yield improvements, showing the highest harvest index at this concentration. The highest number of pods per plant was observed in the potassium nanochelate treatment at 4 L/ha. The study by Rohi Vishkayi et al. (2023) showed that potassium nanochelate and potassium nitrate fertilizers improved leaf nutrient content in olive trees compared to the control samples. Similarly, Safavi Gerdini (2016) stated that increasing the concentration of potassium nanochelate enhanced the growth characteristics of pumpkin plants under drought conditions, where a 2.5% nano potassium solution at 1,000 ml led to an increase in stem diameter (96.6 cm). Additionally, the highest number of branches and leaves was observed at the same concentration.

The results of the current study showed that the combined treatment of potassium nanochelate + zeolite had a significant effect on certain growth and establishment traits of red clover. Since both potassium nanochelate and zeolite are made available to the plant in the combined treatments, the plant benefits from the positive effects of both substances. It appears that potassium enhances the root penetration of the plant, thus increasing its ability to absorb water and nutrients. Meanwhile, zeolite, as a superabsorbent material, absorbs water and gradually releases it to the plant. These

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combined effects can play an effective role in enhancing plant growth and performance. In this regard, Beyrampoor et al. (2022) reported that the use of the combined treatment of 90 mg/kg potassium nanochelate + 6 g/kg zeolite in a pot experiment, improved the growth characteristics of white clover, including parameters such as fresh weight, dry weight, root volume, root area, and plant establishment percentage. Additionally, the highest root fresh weight was observed in the treatment of potassium nanochelate + zeolite (60 mg/kg +4 g/kg). Rohi Vishkayi et al. (2023) investigated the impact of foliar application of different fertilizer sources (nanochelates of nitrogen and potassium) and chemical fertilizers (urea and potassium nitrate) in a field spray experiment on the yield and oil quality properties of the yellow olive cultivar, showing that fruit yield was affected by higher concentrations of urea and potassium nitrate treatments, while the oil quality characteristics were influenced by lower concentrations of the nanochelate treatments.

5. Conclusion

Overall, the facilitators of zeolite, bentonite, and potassium nanochelate significantly and positively influenced most growth and establishment rate of red clover. Among these, the combined treatment of potassium nanochelate and zeolite, particularly at higher concentrations, had an even greater impact on the plant's growth characteristics. Given the beneficial effects of these treatments-especially when applied together with red clover growth, they are expected to be useful in rangeland restoration efforts, particularly in degraded areas. Additionally, these amendments could support the establishment of pastures in abandoned or low-yield, rain-fed fields, enhancing red clover growth either as a monoculture or in mixed cropping systems with other species. To validate the effectiveness of these treatments, it is strongly recommended to conduct pilot cultivation under natural conditions. The treatments can be initially applied and their effects assessed on a small scale before being tested more broadly.

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Authors Contribution
Authors have contributed equally in preparing and writing the manuscript.

Conflict of interests

Authors declared no conflict of interest.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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