Reduction of Ammonium Toxicity by Potassium in Hydroponically Cultivated Alfalfa (*Medicago sativa*): Growth and Physiological Responses

Faten Rhimi, Ichrak Essid, Chiraz Chaffei-Haouari, and Sabeh M'rah, Laboratory of Plant Productivity and Environment Constraints, Department of Biology, Sciences Faculty of Tunis, University of Tunis El Manar, 2092 Tunis, Tunisia
https://dx.doi.org/10.4314/tjpp.v20i2.2 (***Tunisia***)

ABSTRACT

Rhimi, F., Essid, I., Chaffei-Haouari, Ch., and M'rah, S. 2025. Reduction of ammonium toxicity by potassium in hydroponically cultivated alfalfa (*Medicago sativa*): Growth and physiological responses. Tunisian Journal of Plant Protection 20 (2): 43-55.

Alfalfa (*Medicago sativa*) typically thrives on nitrate nutrition, but the environmental impact of nitrate overuse necessitates alternative nitrogen sources. While ammonium (NH_4^+) is a promising candidate, it often induces toxicity in plants. This study investigates the potential of potassium (K^+) supplementation to mitigate NH_4^+ toxicity in hydroponically grown alfalfa over a 17-day period. Plants were subjected to varying nitrogen regimes: a nitrate control (3 mM NO_3^-), and two NH_4^+ concentrations (3 and 6 mM) combined with three K^+ levels (0.2, 1.2, and 3 mM). A combination of 6 mM NH_4^+ and 3 mM K^+ proved lethal, causing complete plant mortality. In contrast, the specific regimen of 3 mM NH_4^+ with 3 mM K^+ significantly alleviated toxicity, restoring plant biomass to approximately 50% of the nitrate control. This mitigation was linked to a significant reduction in toxic NH_4^+ accumulation in shoots and a 2.3-fold increase in K^+ uptake compared to low-K^+ treatments. Physiologically, this optimal treatment maintained chlorophyll content, soluble protein, and polyphenol levels comparable to control plants, while also boosting antioxidant activity by 35%. These results demonstrate that a balanced NH_4^+/K^+ nutrition, specifically at a 1:1 ratio (3 mM each), can effectively mitigate ammonium toxicity by regulating its uptake and translocation, preserving photosynthetic function, and enhancing antioxidant capacity. This strategy offers a sustainable approach for incorporating ammonium-based fertilizers in alfalfa crop.

Keywords: Alfalfa, ammonium toxicity, hydroponics, *Medicago sativa*, nitrogen nutrition, oxidative stress, phenolic compounds, potassium interaction

Alfalfa (*Medicago sativa*) is a cornerstone species within the Fabaceae family, renowned for its exceptional agricultural value. As a high-yielding perennial legume, it serves dual roles as both a premium fodder crop (Zaidi 2021) and a natural soil enricher through nitrogen fixation via Rhizobium symbiosis (Mauriés 2003). The plant's nutritional profile, characterized by 18-22% protein content and rich carotenoid concentrations (Hadidi et al. 2023), along with its documented medicinal properties (Khairy et al. 2025), underscores its economic and ecological importance in Mediterranean agroecosystems.

Corresponding author: Ichrak Essid
Email: essidichrak3@gmail.com

Accepted for publication 22 October 2025

Although alfalfa naturally acquires nitrogen through symbiosis, mineral nitrogen fertilization is often used in hydroponic systems or soils where nodulation is ineffective. Conventional agriculture relies heavily on nitrate (NO_3^-) fertilizers, but their environmental impact is increasingly problematic. Nitrate's high solubility leads to substantial leaching losses, contributing to groundwater contamination (Smith et al. 2021). Furthermore, microbial denitrification of excess nitrate generates nitrogen oxides, potent greenhouse gases that contribute to stratospheric ozone depletion. These environmental impacts have driven the search for alternative nitrogen management strategies in forage production systems.

Ammonium (NH_4^+) presents a potentially sustainable alternative due to its positive charge, which enhances soil retention compared to nitrate. However, NH_4^+ nutrition poses significant physiological challenges for alfalfa, including rhizosphere acidification, disruption of cation balance, and accumulation of reactive nitrogen species (Liu et al. 2017). Recent studies have identified several mitigation strategies for NH_4^+ toxicity, including enhanced assimilation pathway activity (Liu and Wirén 2017) and application of organic osmolytes like γ-aminobutyric acid (Ma et al. 2016). Among these approaches, potassium (K^+) supplementation has emerged as particularly promising due to its dual role as an essential macronutrient and NH_4^+ antagonist.

The competitive interaction between K^+ and NH_4^+ at transport sites (Szczerba et al. 2008) suggests that optimized K^+ nutrition could maintain alfalfa productivity under NH_4^+-based regimes. This study hypothesizes that K^+ alleviates NH_4^+ toxicity by reducing ammonium uptake and accumulation, maintaining ionic homeostasis, and protecting physiological processes. The study specifically examines: (1) the concentration-dependent effects of K^+ on NH_4^+ toxicity alleviation, and (2) the physiological mechanisms underlying this mitigation, including ion homeostasis, photosynthetic performance, and oxidative stress responses. Through controlled hydroponic experiments employing two NH_4^+ levels (3 and 6 mM) combined with three K^+ concentrations (0.2, 1.2, and 3 mM), we aim to establish practical guidelines for implementing ammonium-potassium fertilization systems in alfalfa crop.

MATERIALS AND METHODS

Plant cultivation and experimental design.

Seed sterilization and germination. Alfalfa seeds were surface-sterilized in 20% (v/v) sodium hypochlorite solution for 10 min, followed by five rinses with distilled water. Sterilized seeds were germinated on moist filter paper in Petri dishes under ambient laboratory conditions (25 ± 2°C).

Hydroponic system establishment. After 7 days, uniformly germinated seedlings were transferred to 2 L crystallizing dishes containing a modified Hoagland and Arnon (1940) nutrient solution. Plants were maintained in a growth chamber under controlled environmental conditions: a 16/8 h light/dark photoperiod, a photosynthetic photon flux density of 150 μmol m^{-2} s^{-1}, day/night temperatures of 25/18°C, and 65% relative humidity.

Experimental treatments. At 19 days' post-germination, seedlings were divided into seven treatment groups (n = 7 per group). The NH_4^+ concentrations (3 and 6 mM) were selected to represent a moderate and a high, potentially toxic dose. The K^+ levels (0.2, 1.2, and 3 mM)

represent deficient, sufficient, and high luxury consumption ranges, respectively. The control (M1) contained 0.2 mM K^+ to provide a baseline for comparison with the ammonium treatments at the same low K^+ level.

- M1: 3 mM NO_3^- + 0.2 mM K^+ (Control)
- M2: 3 mM NH_4^+ + 0.2 mM K^+
- M3: 3 mM NH_4^+ + 1.2 mM K^+
- M4: 3 mM NH_4^+ + 3.0 mM K^+
- M5: 6 mM NH_4^+ + 0.2 mM K^+
- M6: 6 mM NH_4^+ + 1.2 mM K^+
- M7: 6 mM NH_4^+ + 3.0 mM K^+

Nutrient solutions were renewed every 3 days to maintain stable ion concentrations. Plants were harvested for physiological and biochemical analyses after 17 days of treatment exposure. The complete nutrient solution composition for all treatments is detailed in Table 1.

Table 1. Composition of experimental growth media (M1–M7)

Salts	Media						
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
KNO_3	1 mM	-	-	-	-	-	-_
KH_2PO_4	0.2 mM	0.2 mM	1.2 mM	3 mM	0.2 mM	1.2 mM	3 mM
$Ca(NO_3)_2$	1 mM	-	-	-	-	-	-_
$MgSO_4$	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM	0.4 mM
NH_4Cl	-	3 mM	3 mM	3 mM	6 mM	6 mM	6 mM
$CaCl_2$	-	1 mM	1 mM	1 mM	1 mM	1 mM	1 mM

Plant growth analysis and water content determination.

Following treatment, plants were carefully separated into aerial (shoot) and root components. Each tissue fraction was gently blotted dry using filter paper to remove surface moisture before immediate weighing to determine fresh weight (FW) using an analytical balance (precision ±0.1 mg). For dry weight (DW) measurements, samples were placed in pre-weighed aluminum foil pouches and dried to constant mass in a forced-air oven at 60°C for 72 h.

Water content was calculated using the following equation:

$$\text{Water Content (\%)} = [(FW - DW)/FW] \times 100.$$

Mineral element analysis.

Potassium ion (K^+) quantification was performed following acid digestion of plant tissues in 0.5% (v/v) nitric acid (HNO_3). K^+ concentrations were determined using flame emission spectrophotometry (Jenway PFP7 Flame Photometer) with appropriate blank corrections. A five-point standard calibration curve (0-50 ppm K^+) was generated using analytical grade KCl for quantitative analysis (Jones 2001).

Ammonium (NH_4^+) content was measured according to Weatherburn's (1967) phenol-hypochlorite method. Briefly, tissue extracts were reacted with alkaline phenol and sodium hypochlorite, forming indophenol blue whose absorbance was measured at 625 nm. NH_4^+ concentrations were calculated against an ammonium sulfate standard curve (0-10 μg mL^{-1} range).

Chlorophyll quantification.

Total chlorophyll extraction was performed using fresh leaf tissue homogenized in 80% (v/v) ice-cold acetone under dim light conditions. After

centrifugation at 3,000 × g for 10 min at 4°C, the supernatant absorbance was measured at 645 nm and 663 nm using a Beckman DU 640 UV-Vis spectrophotometer (Beckman Coulter, USA). Total chlorophyll content was calculated according to Torrecillas et al. (1984) using the following equations:

Chlorophyll a (µg/mL) = 12.7(A663) - 2.69(A645),
Chlorophyll b (µg/mL) = 22.9(A645) - 4.68(A663),
Total Chlorophyll = Chl a + Chl b.

Results were normalized to fresh weight and expressed as µg chlorophyll per mg fresh tissue (µg mg^{-1} FW).

Protein extraction and quantification.

Fresh leaf tissue samples were immediately flash-frozen in liquid nitrogen and homogenized to a fine powder using a pre-chilled mortar and pestle. The frozen powder was suspended in ice-cold extraction buffer (50 mM phosphate buffer, pH 7.2, containing 5% (v/v) glycerol, 1 mM dithiothreitol (DTT), 1 mM EDTA, and 5% (w/v) polyvinylpyrrolidone (PVP)) to prevent protein degradation and phenolic compound interference. The homogenate was centrifuged at 12,000 × g for 20 min at 4°C to pellet cellular debris.

The resulting supernatant was used for soluble protein quantification via the Bradford assay (Bradford 1976). Briefly, 100 µL of protein extract was mixed with 1 mL Bradford reagent (Coomassie Brilliant Blue G-250) and incubated for 10 min at room temperature. Absorbance was measured at 595 nm using a spectrophotometer (Beckman DU 640). Protein concentrations were determined from a standard curve generated using bovine serum albumin (BSA) standards (0-50 µg mL^{-1}). All measurements were performed in triplicate, with results expressed as µg protein per mg fresh weight (µg mg^{-1} FW).

Quantification of total polyphenolic compounds.

The total polyphenol content was determined using a modified Folin-Ciocalteu assay (Dewanto et al. 2002). Briefly, 100 µL of plant extract was mixed with 500 µL of 10% (v/v) Folin-Ciocalteu reagent and allowed to react for 5 min at room temperature. Subsequently, 400 µL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated for 60 min in the dark at 25°C. The absorbance of the resulting blue complex was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800).

A standard calibration curve was prepared using gallic acid solutions (0-500 mg L^{-1}) with the same treatment conditions. The total polyphenol content was calculated from the standard curve and expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM).

Assessment of total antioxidant capacity.

The total antioxidant activity was evaluated using the phosphomolybdenum reduction assay according to Prieto et al. (1999) with modifications. Briefly, 100 µL of plant extract was combined with 1 mL of freshly prepared reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate in distilled water. The reaction mixture was incubated at 90°C for 95 min in a temperature-controlled water bath.

After cooling to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). A standard curve was generated using gallic acid solutions (0-500 µg mL^{-1}) subjected to identical treatment conditions. The total antioxidant capacity was calculated from the standard curve and

expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM).

Statistical analysis.

All experimental data were derived from seven biological replicates per treatment (n = 7), consistent with the experimental design. The study included a total of seven distinct nutrient treatments (M1-M7, with M1 serving as the nitrate-based control). However, due to complete plant mortality in the M7 treatment, no data were available for this group. Data are presented as mean values ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) implemented in Costat software (version 6.451, Cohort Software). When ANOVA indicated significant differences ($p < 0.05$), Dunnett's post-hoc test was applied to compare all treatment means against the control group (M1). The assumption of homogeneity of variance was verified using Levene's test prior to ANOVA. All statistical tests were conducted at a 95% confidence level ($\alpha = 0.05$).

RESULTS

Impact of ammonium-potassium interactions on alfalfa growth performance.

Fig. 1 presents the biomass accumulation patterns. Dry matter production in both root and shoot tissues exhibited significant variation ($p < 0.05$) across the six viable treatments. Plants grown under nitrate nutrition (M1) demonstrated maximal biomass accumulation. Among ammonium-grown plants, those receiving 3 mM NH_4^+ combined with 3 mM K^+ (M4) showed the most favorable growth response, achieving approximately 50% of control biomass levels. This contrasted sharply with other ammonium treatments (M2, M3, M5, M6), where biomass reductions were more severe. The combination of 6 mM NH_4^+ with 3 mM K^+ (M7) proved lethal, resulting in complete plant mortality; therefore, no biomass data were available for this treatment.

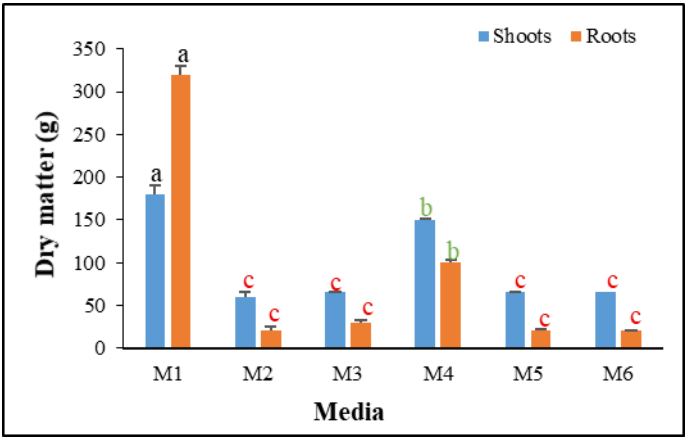

Fig. 1. Dry matter biomass partitioning in alfalfa under different nitrogen-potassium regimes after 17 days of treatment exposure. Treatments: M1 (3 mM NO_3^- + 0.2 mM K^+, control); M2 (3 mM NH_4^+ + 0.2 mM K^+); M3 (3 mM NH_4^+ + 1.2 mM K^+); M4 (3 mM NH_4^+ + 3 mM K^+); M5 (6 mM NH_4^+ + 0.2 mM K^+); M6 (6 mM NH_4^+ + 1.2 mM K^+).

Vegetative growth parameters followed similar response patterns (Fig. 2). Plants in the nitrate control (M1) developed the most extensive root systems and tallest shoots. Among the ammonium treatments, M4 plants (3 mM NH_4^+ / 3 mM K^+) showed a significant mitigation of growth inhibition, achieving intermediate values for both shoot height and root length. While all ammonium treatments significantly ($p < 0.05$) reduced these parameters relative to the M1 control, the M4 treatment consistently resulted in less severe reductions, representing a significant improvement over the other ammonium treatments (M2, M3, M5, M6).

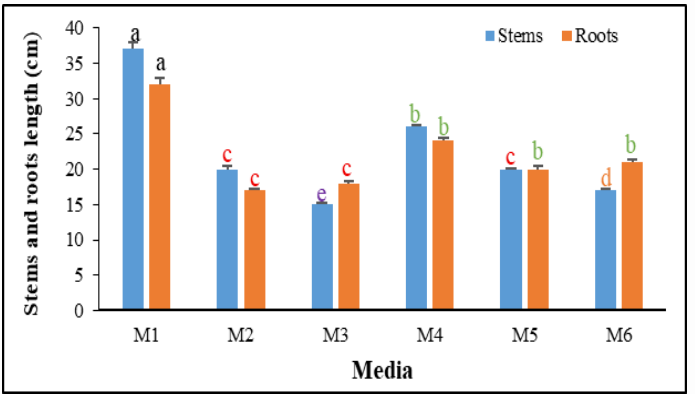

Fig. 2. Comparative stem height and primary root length measurements of alfalfa after 17-day exposure. Treatment codes: M1 (3 mM NO_3^- + 0.2 mM K^+); M2 (3 mM NH_4^+ + 0.2 mM K^+); M3 (3 mM NH_4^+ + 1.2 mM K^+); M4 (3 mM NH_4^+ + 3 mM K^+); M5 (6 mM NH_4^+ + 0.2 mM K^+); M6 (6 mM NH_4^+ + 1.2 mM K^+).

Tissue-specific mineral accumulation patterns.

The analysis of tissue mineral content revealed distinct allocation patterns for potassium and ammonium (Fig. 3). A strong positive correlation was observed between potassium concentration in the growth medium and K^+ content in both shoots and roots, with foliar tissues consistently maintaining higher concentrations than root systems (Fig. 3A). Conversely, ammonium accumulation exhibited an inverse relationship with potassium availability (Fig. 3B). Plants in low-K^+ treatments (M2, M5) showed the highest NH_4^+ accumulation, particularly in shoots. The M4 treatment (3 mM NH_4^+ / 3 mM K^+) demonstrated a significant mitigating effect, reducing shoot NH_4^+ concentration and resulting in a greater proportion of the absorbed ammonium being sequestered in the roots.

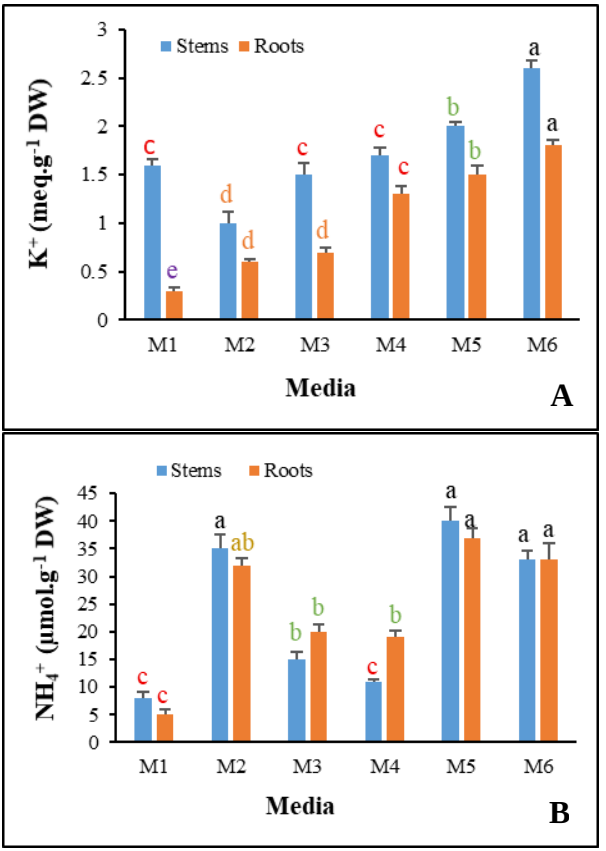

Fig. 3. K^+ (A) and $NH4^+$ (B) contents of stem and root of alfalfa after 17 days of cultivation on M1 (3mM NO^{3-} ; 0, 2 mM K^+) ; M2 (3 mM $NH4^+$; 0, 2 mM K^+) ; M3 (3 mM NH_4^+ ; 1, 2 mM K^+) ; M4 (3 mM NH4+ ; 3 mM K+) ; M5 (6 mM NH_4^+ ; 0,2 mM K^+) ; M6 (6 mM NH_4^+ ; 1,2 mM K^+).

Effects of ammonium/potassium concentration on chlorophyll and protein contents of alfalfa.

The results of the protein contents of the aerial parts and roots of alfalfa, depending on the treatments applied, are shown in Fig. 4A These concentrations reach a maximum on a strictly nitrate medium. In the presence of ammonium 3 mM and in the presence of the lowest potassium concentration, the content decreases significantly compared to the M1 medium. On the other hand, in the presence of 3 mM NH_4^+ and 3 mM K^+, the content reaches a value which is approximately equal to that obtained in the organs of plants in the M1 medium.

In the presence of 6 mM ammonium and regardless the concentration of potassium, the protein content decreases significantly. They are still highest in the aerial parts.

Fig. 4B shows the chlorophyll levels after 17 days of treatment on nitric medium or media containing different concentrations of ammonium and

potassium. Chlorophyll content was significantly altered by the growth media. Compared to the M1 control (1.00 mg g^{-1} FW), content was 25% higher in M4 but 51% lower in M6. The M2, M3, and M5 treatments showed intermediate reductions, ranging from 20% to 35% lower than the control.

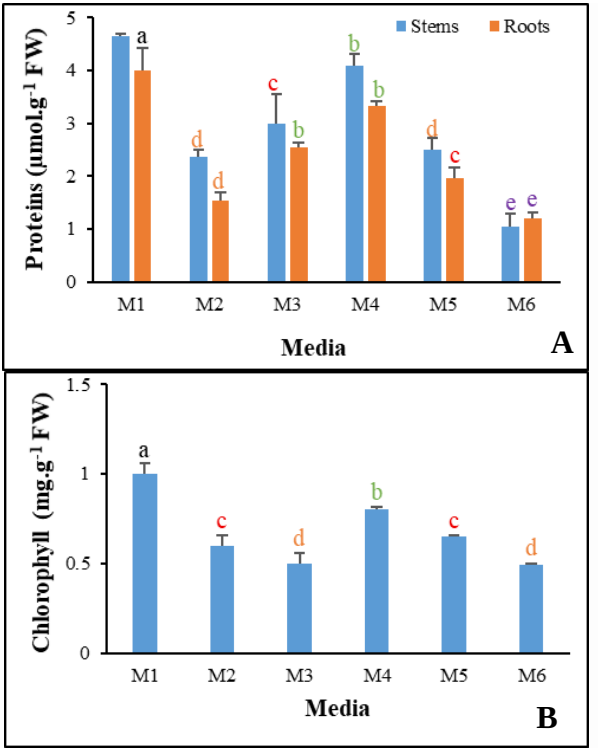

Fig. 4. Biochemical responses of alfalfa to nitrogen-potassium treatments: (A) Soluble protein content and (B) total chlorophyll concentration in aerial tissue after 17 days of cultivation on M1 (3mM NO^{3-} ; 0, 2 mM K^+) ; M2 (3 mM $NH4^+$; 0, 2 mM K^+) ; M3 (3 mM NH_4^+ ; 1, 2 mM K^+) ; M4 (3 mM NH4+ ; 3 mM K+) ; M5 (6 mM NH_4^+ ; 0,2 mM K^+) ; M6 (6 mM NH_4^+ ; 1,2 mM K^+).

Effects of ammonium/potassium concentration on antioxidant and polyphenol level.

The effects of ammonium and potassium concentrations on their accumulation were investigated alongside changes in antioxidant activity and polyphenol levels in alfalfa. As shown in Fig. 5A, total antioxidant activity reached its peak in the M4 treatment, after which it declined in M5 and M6. The lowest activity was recorded in M3 for stems and M6 for roots.

Polyphenol accumulation showed a biphasic response, with peaks at the M1 and M4 treatments (Fig. 5B). The

M4 medium yielded the highest polyphenol concentration in aerial parts, while M1 resulted in the highest concentration in roots. In contrast, plants in M2, M3, M5, and M6 exhibited substantially lower polyphenol levels, with the M6 treatment showing the least accumulation in both tissues.

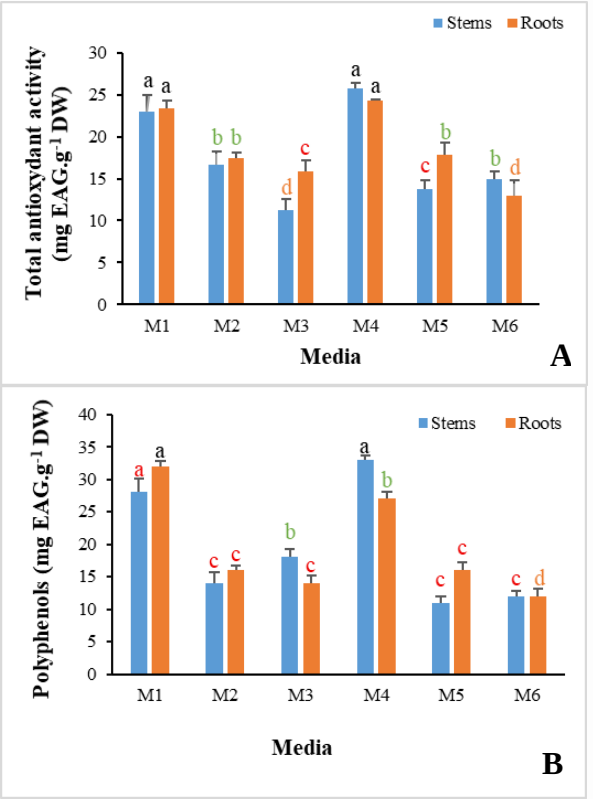

Fig. 5. Oxidative stress markers in *Medicago sativa* under varying nitrogen regimes: (A) Total antioxidant capacity (A) and (B) polyphenolic compound accumulation in shoot and root systems. of *M. sativa* after 17 days of cultivation on M1 (3mM NO^{3-} ; 0, 2 mM K^+); M2 (3 mM $NH4^+$; 0, 2 mM K^+); M3 (3 mM NH_4^+; 1, 2 mM K^+); M4 (3 mM $NH4^+$; 3 mM K+); M5 (6 mM NH_4^+; 0,2 mM K^+); M6 (6 mM NH_4^+; 1,2 mM K^+).

DISCUSSION

Nitrogen stands as an essential element for plant growth and development. While nitrate represents the preferred nitrogen source for many species, ammonium can induce toxicity symptoms when supplied as the sole nitrogen form (M'rah et al. 2010, Esteban et al. 2016).

In the present study, we specifically examined the role of potassium in alleviating ammonium toxicity in alfalfa. The results revealed complete mortality in plants grown in the

M7 medium (6 mM NH_4^+/3 mM K^+). Among the ammonium treatments, the M4 medium (3 mM NH_4^+/3 mM K^+) supported the most favorable growth outcomes. (Miller and Cramer 2004).

The beneficial effects observed in the M4 treatment can be attributed to several key mechanisms supported by potassium availability. First, these plants accumulated significantly less ammonium in their aerial tissues, aligning with findings that adequate potassium competes with NH_4^+ for uptake via transporters such as AKT1 and HAK5 (Coskun et al. 2017), thereby reducing tissue ammonium concentrations. Second, the maintained potassium levels in M4 plants support critical physiological processes including stomatal regulation and enzyme activation. Furthermore, sufficient K^+ is crucial for maintaining cytosolic pH homeostasis, which is often disrupted under NH_4^+ stress, and for facilitating the assimilation of NH_4^+ into amino acids via the GS-GOGAT cycle (Szczerba et al. 2008, Masclaux-Daubresse et al. 2020).

Notably, M4 plants preserved higher chlorophyll and protein content compared to other ammonium treatments, indicating maintained photosynthetic capacity and more efficient nitrogen assimilation. This contrasts with the photosynthetic inhibition typically associated with ammonium nutrition.

Our investigation of secondary metabolites revealed that M4 plants maintained relatively higher polyphenol and antioxidant levels. The enhanced antioxidant activity suggests that K^+ sufficiency empowers the plant's defense systems, potentially by reducing ROS generation through improved photosynthetic efficiency and by interacting with Ca^{2+} signaling pathways (Anschütz et al.,2014). This finding may have important implication for plant stress adaptation and nutritional quality.

While this study was conducted hydroponically, the implications extend to soil conditions. In the field, maintaining an adequate K^+ status is essential when using ammonium-based fertilizers to prevent toxicity, as soil cation exchange and microbial nitrification can modulate NH_4^+ availability. A balanced NH_4^+/K^+ nutrition strategy can enhance nitrogen use efficiency and sustainability in alfalfa crop (Sitienei et al. 2013).

In conclusion, our results demonstrate that a balanced 1:1 molar ratio of NH_4^+ to K^+ (3 mM each) provides optimal conditions for alfalfa to withstand ammonium toxicity, specifically under a moderate 3 mM NH_4^+ regime. Potassium exerts its protective effects through multiple interconnected mechanisms, as evidenced by our data: it competitively limits ammonium uptake and its accumulation in shoots, maintains ionic homeostasis and essential potassium-dependent physiological functions, preserves the integrity of the photosynthetic apparatus as seen in sustained chlorophyll and protein levels, and supports the production of defensive secondary metabolites, notably enhancing antioxidant capacity. These findings underscore the critical importance of maintaining adequate potassium levels in ammonium-based fertilization systems. This strategy not only improves our understanding of nutrient interactions in plant physiology but also offers a practical framework for enhancing nitrogen use efficiency and crop performance in sustainable agricultural practices that utilize ammonium fertilizers.

Acknowledgements

The work was conducted in the Laboratory of Plant Productivity and Environmental Constraints, Department of Biology, Faculty of Sciences of Tunis, University Tunis El Manar, 2092 Tunis, Tunisia (LR18ES04).

Rhimi F., Essid I., Chaffei-Haouari Ch. et M'rah S. 2025. Reduction de la toxicité de l'ammonium par le potassium chez la luzerne (*Medicago sativa*) cultivée en hydroponie: Réponses de la croissance et physiologiques. Tunisian Journal of Plant Protection 20 (2): 43-55.

La luzerne (Medicago sativa) se développe généralement grâce à la nutrition azotée, mais l'impact environnemental de la surutilisation des nitrates impose la recherche de sources d'azote alternatives. Bien que l'ammonium (NH_4^+) soit une option prometteuse, il induit souvent une toxicité chez les plantes. Cette étude examine le potentiel d'une supplémentation en potassium (K^+) pour atténuer la toxicité du NH_4^+ chez la luzerne cultivée en hydroponie sur une période de 17 jours. Les plantes ont été soumises à différents régimes azotés: un témoin nitrate (3 mM NO_3^-) et deux concentrations de NH_4^+ (3 et 6 mM) combinées à trois niveaux de K^+ (0,2, 1,2 et 3 mM). Une combinaison de 6 mM de NH_4^+ et de 3 mM de K^+ s'est avérée létale, entraînant la mortalité totale des plantes. En revanche, le traitement spécifique à 3 mM de NH_4^+ et 3 mM de K^+ a considérablement atténué la toxicité, restaurant la biomasse végétale à environ 50% du témoin (nitrate). Cette atténuation était liée à une réduction significative de l'accumulation toxique de NH_4^+ dans les parties aériennes et à une augmentation de 2,3 fois de l'absorption de K^+ par rapport aux traitements à faible concentration de K^+. Sur le plan physiologique, ce traitement optimal a maintenu les teneurs en chlorophylle, en protéines solubles et en polyphénols à des niveaux comparables à ceux des plantes témoins, tout en augmentant l'activité antioxydante de 35%. Ces résultats démontrent qu'une nutrition équilibrée NH_4^+/K^+, en particulier à un ratio de 1:1 (3 mM chacun), peut atténuer efficacement la toxicité de l'ammonium en régulant son absorption et sa translocation, en préservant la fonction photosynthétique et en renforçant la capacité antioxydante. Cette stratégie offre une approche durable pour l'incorporation d'engrais à base d'ammonium dans la culture de la luzerne.

Mots-clés: Composés phénoliques, culture hydroponique, interaction avec le potassium, luzerne, *Medicago sativa*, nutrition azotée, stress oxydatif, toxicité de l'ammonium

ملخص

رحيمي، فاتن وإشراق الصيد وشيراز شافعي-هواريو صباح مراح .2025. تخفيض سُمِّية الأمونيوم بواسطة البوتاسيوم في نبات الفصة/البرسيم (*Medicago sativa*) في الزراعة المائية: استجابات النمو والفيسيولوجيا.
Tunisian Journal of Plant Protection 20 (2): 43-55.

تزدهر الفصة/البرسيم (Medicago sativa) عادةً بالتغذية بالنترات، إلا أن الأثر البيئي بالإفراط في استخدامها يستدعي البحث عن مصادر بديلة للنيتروجين. ورغم أن الأمونيوم (NH_4^+) يُعدّ خيارًا واعدًا، إلا أنه غالبًا ما يُسبب تسممًا للنباتات. تبحث هذه الدراسة في إمكانية استخدام البوتاسيوم (K^+) كمكمّل غذائي للتخفيف من سمية الأمونيوم في الفصة المزروعة مائيًا على مدى 17 يومًا. خضعت النباتات لأنظمة نيتروجينية مختلفة: نظام شاهد بالنترات (3 ميليمول NO_3^-)، وتركيزان من الأمونيوم (3 و6 ميليمول/لتر) مع ثلاثة مستويات من البوتاسيوم (0.2، 1.2، و3 ميليمول). وقد ثبت أن مزيج 6 ميليمول من الأمونيوم و3 ميليمول من البوتاسيوم قاتل، مما أدى إلى موت النبات بالكامل. على النقيض من ذلك، أدى النظام الغذائي المحدد بتركيز 3 ميليمول من الأمونيوم مع 3 ميليمول من البوتاسيوم إلى تخفيف السمية بشكل ملحوظ، حيث استعادت الكتلة الحيوية للنباتات إلى حوالي 50% من كتلة مجموعة الشاهد المعالجة بالنترات. وارتبط هذا التخفيف بانخفاض كبير في تراكم الأمونيوم السام في الأجزاء الهوائية، وزيادة امتصاص البوتاسيوم بمقدار 2.3 ضعف مقارنةً بالمعالجات منخفضة البوتاسيوم. وعلى المستوى الفيزيولوجي، حافظت هذه المعالجة المثلى على محتوى الكلوروفيل والبروتين الذائب ومستويات البوليفينول مماثلة لنباتات الشاهد، مع تعزيز النشاط المضاد للأكسدة بنسبة 35%. تُظهر هذه النتائج أن التغذية المتوازنة بالأمونيوم والبوتاسيوم، وتحديدًا بنسبة 1:1 (3 ميليمول لكل منهما)، يمكن أن تخفف بشكل فعال من سمية الأمونيوم عن طريق تنظيم امتصاصه وانتقاله، والحفاظ على وظيفة التمثيل الضوئي، وتعزيز القدرة المضادة للأكسدة. توفر هذه الاستراتيجية نهجًا مستدامًا لدمج الأسمدة القائمة على الأمونيوم في محصول البرسيم.

LITERATURE CITED

Anschütz, U., Becker, D., and Shabala, S. 2014. Going beyond nutrition: Regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. Journal of Plant Physiology 171: 670-687. https://doi.org/10.1016/j.jplph.2014.01.009

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Coskun, D., Britto, D.T., Shi, W., and Kronzucker, H.J. 2017. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. Nature Plants 3: 1-10. https://doi.org/10.1038/nplants.2017.74

Dewanto, V., Wu, X., Adom, K.K., and Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50: 3010-3014. https://doi.org/10.1021/jf0115589

Esteban, R., Ariz, I., Cruz, C., and Moran, J.F. 2016. Mechanisms of ammonium toxicity and the quest for tolerance. Plant Science 248: 92-101. https://doi.org/10.1016/j.plantsci.2016.04.008

Hadidi, M., Orellana Palacios, J.C., McClements, D.J., Mahfouzi, M., and Moreno, A. 2023. Alfalfa as a sustainable source of plant-based food proteins. Trends in Food Science and Technology 135: 202-214.

Hoagland, D.R., and Arnon, D.I. 1940. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347: 1-39.

Jones, J.B. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton, Florida, USA, 363 pp.

Khairy, A., Hammoda, H.M., Metwally, A.M., Zaatout, H.H., and Ibrahim, R.S. 2025. Therapeutic potential of Medicago sativa (alfalfa): A comprehensive review of its bioactive compounds and pharmacological properties. Journal of Advanced Pharmaceutical Sciences 2 (2): Article 1043.

Liu, G., Du, Q., and Li, J. 2017. Interactive effects of nitrate-ammonium ratios and temperatures on growth, photosynthesis, and nitrogen metabolism of tomato seedlings. Scientia Horticulturae 214: 41-50.

Liu, Y., and von Wirén, N. 2017. Ammonium as a signal for physiological and morphological responses in plants. Journal of Experimental Botany 68: 2581-2592. https://doi.org/10.1093/jxb/erx086

M'rah Helali, S., Nebli, H., Kaddour, R., Mahmoudi, H., Lachaâl, M., and Ouerghi, Z. 2010. Influence of nitrate–ammonium ratio on growth and nutrition of Arabidopsis thaliana. Plant and Soil 336: 65-74. https://doi.org/10.1007/s11104-010-0445-8

Ma, X., Zhu, C., Yang, N., Gan, L., and Xia, K. 2016. γ-Aminobutyric acid addition alleviates ammonium toxicity by limiting ammonium accumulation in rice (Oryza sativa) seedlings. Physiologia Plantarum 158: 389-401. https://doi.org/10.1111/ppl.12473

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., and Suzuki, A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany 105: 1141-1157. https://doi.org/10.1093/aob/mcq028

Mauriès, M. 2003. Luzerne: culture, récolte, conservation, utilisation. 2e édition. Editions France Agricole, Paris, France, 310 pp.

Miller, A.J., and Cramer, M.D. 2004. Root nitrogen acquisition and assimilation. Plant and Soil 274: 1-36. https://doi.org/10.1007/s11104-004-0965-1

Prieto, P., Pineda, M., and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 269: 337-341. https://doi.org/10.1006/abio.1999.4019.

Sitienei, K., Home, P.G., Kamau, D.M., and Wanyoko, J.K. 2013. The influence of fertilizer type and application rates in tea cultivation on nitrogen and potassium efficiencies. African Journal of Agricultural Research 8: 3770-3777. https://doi.org/10.5897/AJAR2013.7337

Smith, P. 2021. How to measure, report and verify soil carbon change. Pages GC41D-01. In: AGU Fall Meeting Abstracts, Vol. 2021.

Szczerba, M.W., Britto, D.T., Ali, S.A., Balkos, K.D., and Kronzucker, H.J. 2008. NH_4^+-stimulated and -inhibited components of K^+ transport in rice (Oryza sativa L.). Journal of Experimental Botany 59: 3415-3423. https://doi.org/10.1093/jxb/ern190

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39: 971-974.

Zaidi, N., Cheima, J., Nourhene, M., Khawla, R., Bilel, K., Naceur, D., Ndiko, L., and Badri Mounawer, B. 2021. Variation of Medicago sativa varieties tolerance to Phoma medicaginis infection. Journal of Oasis Agriculture and Sustainable Development 3: 56-63. https://doi.org/10.56027/JOASD.spiss092021
