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Selenium Uptake and Maize (*Zea mays* L.) Growth as Affected by Different Rates and Forms of Nitrogen

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ABSTRACT

Background: Selenium (Se) concentration is low in animal meat and human beings due to its insufficient levels in forage and feed sources.

Aims: This study investigates the effects of nitrogen (N) rates and N forms to improve the biomass and Se concentration in maize forage.

Methods: The soil in the pots was fertilized with N at the rates of 165 and 330 mg kg⁻¹ without and with 3,4-dimethylpyrazole phosphate (DMPP). DMPP was added to soil at the rate of 0.01% of added N, whereas Se was added at the rate of 40 µg kg⁻¹. In the following experiment, the effect of N forms (NH₄⁺-N, NO₃⁻-N and NH₄⁺-N + O₃⁻-N) was evaluated on the growth and the accumulation of Se, N and phosphorus (P) in maize shoots in Se-fertilized (40 µg kg⁻¹) and control soils. The Se-fertilized and controls soils were designated as Se(+) and Se(-), respectively. The soil texture was sandy and its pH was moderately acidic (5.40).

Results: The DMPP-treated soil had 27%–42% higher NH₄⁺-N content at harvest than the non-treated soil. The DMPP treatment of soil also increased shoot Se content by 19%–23% and Se uptake by 37%–42%. A negative correlation was found between NO₃⁻-N: NH₄⁺-N ratio and shoot Se uptake, suggesting that NO₃⁻-N negatively affected Se uptake by maize. Results of second experiment depicted that applying NH₄⁺-N led to higher shoot Se content and uptake than NO₃⁻-N. In case of Se-unfertilized soil, adding NH₄⁺-N produced 14% and 56% more shoot Se content and uptake, respectively, whereas these increases were 39% and 83% on Se-fertilized soils. Similar to Se uptake, plants fed with NH₄⁺-N had higher P and N uptake by maize shoots than that fed with NO₃⁻-N. The SPAD value and shoot biomass were also higher by applying NH₄⁺-N than NO₃⁻-N.

Conclusion: These results imply that N fertilization, specifically combined application of Se and ammonium-based fertilizers could improve biomass yield and Se content of maize, which is likely to yield maize grains with improved Se contents.

1 | Introduction

Selenium (Se) is vital trace element for both humans and domesticated animals. It is a component of over 30 Se-containing

proteins (selenoproteins) that play a crucial role in the body of animals (Zarczynska et al. 2013). So far, scientists have successfully described the detailed biological functions of only 10 selenoproteins (Zarczynska et al. 2013). Selenoproteins scavenge

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free radicals, regulate thyroid hormone, maintain reproductive health, transcribe DNA, inhibit apoptosis, protect endothelial cells against oxidants, muscle differentiation, muscle development, regulate spermatogenesis, neuroprotective and muscle function (Loflin et al. 2006; Chaudière 2023; Rua et al. 2023; Zarczynska et al. 2013). Hence, appropriate quantity of Se (0.2–0.4 mg kg⁻¹ in beef, 0.3–0.5 mg kg⁻¹ in chicken, 0.2–0.4 mg kg⁻¹ in lamb, and 0.2–0.4 mg kg⁻¹ in pork) must be present in animals, whose meat is consumed by humans.

The major natural source of Se in animals is the forage and grains of crops. Among crops, maize (*Zea mays*) is one of the most extensively cultivated crops in the world, whose forage and grains are consumed by animals (Pechanova et al. 2013). The accumulation of Se in maize tissues largely depends on the Se concentration in the soil. To attain optimal Se levels in plants, a total soil Se concentration of 0.5 mg kg⁻¹ is required (Manojlović and Lončarić 2017). However, in Europe, Se-deficient soils are notably present in Germany, Denmark, Scotland, Finland and several Balkan countries. In Germany, an average Se content of 0.123 mg kg⁻¹ was found in 195 agricultural soil samples from different regions, which is regarded as deficient (Hartfiel and Bahners 1988). To correct Se deficiency, Se should be applied either directly to the soil or as a foliar spray to enhance its concentration in plants (Yan et al. 2024). Plants uptake Se as selenite (SeO₃²⁻) and selenate (SeO₄²⁻), and the latter form is more soluble in water. Although Se is not considered essential for plant growth, its low concentrations can still promote plant growth and enhance its accumulation by plant tissues.

In maize cultivation, fertilization of nitrogen (N) to soils is a very common practice worldwide to promote growth. The influence of soil-applied N on Se content of crop plants remains unclear, with contradictory reports in the literature. For instance, Teixeira et al. (2021) observed that the grain Se concentration in wheat increases was dependent on soil-applied N, whereas Grujčić et al. (2021) reported no effect of N application on Se accumulation in maize shoots. Some studies have reported a decrease in Se content in wheat and triticale plants following N application (Fan et al. 2023; Kong et al. 2023). Thus, further in-depth research is needed to understand the exact mechanism and role of soil-applied N on Se accumulation in plants. The varying concentrations of soil NO₃⁻-N and NH₄⁺-N may elicit a variable response in different crops. Previous studies have shown that various oxyanions (HPO₄²⁻, H₂PO₄⁻, and SO₄²⁻) influence Se absorbance by plants (Liu et al. 2018). Similarly, NO₃⁻ may also negatively affect Se absorption by competing with SeO₄²⁻ for plant uptake. However, the comparative effect of NH₄⁺ and NO₃⁻ forms on Se uptake by maize has not been investigated. Application of NH₄⁺-based fertilizers without and with a nitrification inhibitor can help better understand the effect of N forms on Se uptake by maize plants. Thus, we hypothesize that soil NH₄⁺-N improves Se absorption by maize more than NO₃⁻-N. Therefore, current study was conducted to determine the impact of rate and form of applied N fertilizers on soil N content, growth and selenium (Se) uptake by maize in a greenhouse pot experiment.

2 | Materials and Methods

2.1 | Experiment I: The Effect of Different Levels of N and Nitrification Inhibitor 3,4-Dimethylpyrazole Phosphate (DMPP) on Se Uptake and Growth of Maize

2.1.1 | Soil Preparation, Treatment Application and Sowing of Maize

The experiment was conducted in the greenhouse of Institute of Plant Nutrition and Soil Science at Kiel University, Germany (54.35°N, 10.11°E). The soil was air-dried, sieved and analyzed for different characteristics (Table 1). The soil was sandy in texture (90.8% sand, 3.3% clay and 5.9% silt) and moderately acidic in reaction (pH 5.4). The detailed characteristics of the soil, along with the methods used for analysis, are presented in Table S1. Approximately 96 kg air-dried soil was fertilized using a soil mixer with the following basal doses of salts (mg kg⁻¹ soil): 743 K₂SO₄, 348 KH₂PO₄, 563 MgSO₄·7H₂O, 306 CaCl₂·2H₂O, 6.5 CuSO₄·5H₂O, 19.8 Fe-EDTA, 9.5 H₃BO₃, 15 MnSO₄·H₂O, 0.67 (NH₄)₆Mo₇O₂₄·4H₂O and 0.06 Na₂SeO₄. Additionally, the soil was amended with Se at the rate of 40 µg Se kg⁻¹ soil. The aqueous nutrient solutions were added individually to the soil mixer, with a 5-min mixing time after each addition to minimize interactions between the nutrients. The nutrient-added soil was divided into four equal lots, each having 24 kg of soil, and was applied with the following four N treatments: 165 mg N kg⁻¹ soil, 330 mg N kg⁻¹ soil, 165 mg N kg⁻¹ soil + DMPP and 330 mg N kg⁻¹ soil + DMPP. Nitrogen was added as urea fertilizer. DMPP (Cayman Chemical, USA) was added to soil at the rate of 0.01% of added N (Etabo et al. 2024a, 2024b). Each treatment had four independent pot replicates.

After mixing of N and DMPP, 6 kg of soil was filled in each pot having a diameter of 20 cm. Six healthy maize seeds (cv KWS Keops, KWS SAAT SE & Co. KGaA, Einbeck, Germany) were sown per pot, and four seedlings were retained in each pot after 15 days of sowing. During the experimental period of 6 weeks, the soil was kept moist to approximately 60%–65% of water holding capacity using deionized water. In the greenhouse, photoperiod of 15 h (7 a.m. to 10 p.m.) and a day/night temperature 25/15°C were adjusted. Irradiation at the plant canopy was 250–500 µmol photons m⁻² s⁻¹ and relative humidity was maintained 50% ± 10%.

2.1.2 | Recording SPAD Value, Soil Sampling and Harvesting

SPAD values were recorded from the fully emerged fifth leaf of each plant with SPAD meter (SPAD 502, MINOLTA Co. Ltd., Japan). Overall, a total of 60 readings from 4 plants per pot, 15 readings from each plant, were taken and averaged. Afterward, three soil samples were collected with the help of a core soil sampler from each pot and uniformly mixed to prepare a composite sample. Each composite soil sample was divided into two equally weighing subsamples: One was oven-dried at 105°C for 24 h to

TABLE 1 | Effect of nitrogen levels and nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on NH_4^+ -N and NO_3^- -N concentrations in the postharvest soil.

DMPP	Nitrogen level (mg kg^{-1} soil)	NH_4^+ -N (mg kg^{-1} soil)	NO_3^- -N (mg kg^{-1} soil)	NO_3^- -N: NH_4^+ -N
DMPP(–)	165	54.9 ± 1.6 d	12.5 ± 0.68 b	0.229 ± 0.012 a
	330	119.4 ± 1.3 b	18.1 ± 0.37 a	0.15 ± 0.003 b
DMPP(+)	165	78.3 ± 2.4 c	1.9 ± 0.27 d	0.021 ± 0.003 c
	330	166.6 ± 19.3 a	2.6 ± 0.22 c	0.016 ± 0.002 c
Least significance difference		18.4	0.80	0.012
Coefficient of variance		9.09	6.10	7.59

Note: Data are shown as mean \pm standard deviation ($n = 4$). Values followed by different alphabet(s) in a column are statistically different from each other at 5% probability level. DMPP(–): no DMPP application, DMPP(+): DMPP application at the rate of 0.01% of applied nitrogen.

determine the water content, whereas the second was stored at -20°C and later used for determination of NH_4^+ -N and NO_3^- -N concentrations. The shoots were cut off at the ground level using a scalpel, chopped, placed in paper bags and dried in an oven at 65°C to a constant weight. The shoot weights of oven-dried samples were recorded. These samples were ground using a stainless-steel mill (FOSS Cyclotec 1093, Sweden) fitted with a 0.5-mm sieve. The ground shoot samples were further ground to fine powder in a ball-mill (Retsch MM400, Retsch GmbH, Haan, Germany).

2.1.3 | Soil Analysis for NH_4^+ -N and NO_3^- -N Concentration

Soil NH_4^+ -N and NO_3^- -N concentrations were determined following the method of Guo et al. (2022). For this purpose, 10 g of each soil sample was taken in 250 mL plastic bottles, mixed with 40 mL of calcium chloride solution (0.0125 M) and shaken for 1 h on an overhead shaker. The suspensions so obtained were filtered through Whatman filter paper No. 40. The NH_4^+ -N and NO_3^- -N concentrations in the extracts were determined using an autoanalyzer (SAN++ continuous flow analyzer, Skalar, the Netherlands). The autoanalyzer determines the concentrations of NH_4^+ -N and total mineral nitrogen (NH_4^+ -N and NO_3^- -N) in the soil extract separately, and NO_3^- -N is obtained by subtracting NH_4^+ -N from the total mineral N. Moreover, NO_3^- -N: NH_4^+ -N ratio was also calculated for all the samples.

2.1.4 | Shoot Analysis for Se and N Concentration and Their Uptake

To determine Se concentration in the shoot of maize, the samples were digested in HNO_3 as described by Naem et al. (2023). Approximately 200 mg of ground shoot samples was taken into the digestion tubes and 10 mL of HNO_3 (69% Suprapur) was added into it. The samples were digested in the microwave for 20 min at 190°C (MARS 6 Xpress, CEM Corporation, Matthews, NC, USA). The digests were cooled to room temperature, diluted to 100 mL with ultrapure water and filtered. The measurements of Se concentrations in aliquots were performed by an inductively coupled plasma emission spectrometer (5800 ICP-OES, Agilent

Technologies Inc., Santa Clara, CA, USA). The Se uptake by maize shoot was calculated using the following formula:

$$\text{Shoot Se uptake } (\mu\text{g kg}^{-1}) = \frac{\text{Shoot dry biomass (g)} \times \text{Shoot Se concentration } (\mu\text{g kg}^{-1})}{1000} \quad (1)$$

The total N concentration in shoot samples was determined following the method of Hussain et al. (2022). Briefly, 6–10 mg of finely ground samples was weighed into tin capsules and mixed with 10 mg wolfram-(IV)-oxide. Subsequently, N concentration was determined by a CNS elemental analyzer (Flash EA 1112 NCS, Thermo Fisher Scientific, Waltham, MA, USA). The N uptake was calculated using the following formula:

$$\text{Shoot N uptake } (\text{mg plant}^{-1}) = \frac{\text{Shoot dry biomass (g)} \times \text{shoot N concentration } (\%)}{100} \quad (2)$$

A relationship was determined between shoot Se uptake and NO_3^- -N: NH_4^+ -N ratio.

2.2 | Experiment 2: The Effect of Different Forms of N on Se Uptake and Growth of Maize

2.2.1 | Treatment Application and Sowing of Maize

Approximately 144 kg of air-dried sieved soil was mixed with the basal doses of fertilizers as described in Section 2.1.1. Simultaneous to fertilizer addition, soil was added with DMPP at the rate of 0.01% of the added N (130 mg kg^{-1}). The fertilized and DMPP-treated soil was divided into six 24-kg lots, which were fertilized with three N fertilizers (NH_4NO_3 , $\text{Ca}(\text{NO}_3)_2$, NH_4Cl), alone and in combination with $40 \mu\text{g Se kg}^{-1}$ soil. The applied fertilizers represented three different forms of N (NH_4^+ from NH_4Cl , NO_3^- from $\text{Ca}(\text{NO}_3)_2$ and NH_4^+ + NO_3^- from NH_4NO_3 salts). The N fertilizers were applied at the rate of 130 mg N kg^{-1} soil. To eliminate the effects of calcium on nitrogen uptake by maize, the

quantity of calcium added through calcium nitrate (130 mg N kg^{-1} soil) was balanced by adding CaCl_2 to other treatments. All other procedures from sowing to harvesting were the same as described in Section 2.1.1, and the experiment was harvested 6 weeks after sowing.

2.2.2 | Harvesting, Sample Collection and Recording of SPAD Value

The measurement of SPAD values, soil sampling, harvesting of plants and processing of samples were carried out as described in Section 2.1.1.

2.2.3 | Soil Analysis for NH_4^+ -N and NO_3^- -N Concentration

The collected soil samples were analyzed for NH_4^+ -N and NO_3^- -N concentrations as described in Section 2.1.3.

2.2.4 | Shoot Analysis for Se, N and P Concentration and Their Uptake

The concentrations of Se and N in shoots were determined as described in Section 2.1.4, and their uptakes were calculated using Equations (1) and (2), respectively. Phosphorus concentration was determined colorimetrically following vanadate-molybdate yellow color method (Chapmann and Pratt 1961). Equation 2 was used to calculate P uptake by maize shoots.

2.3 | Statistical Analysis

Statistix-10 program was used to perform statistical analyses. The experimental design of the both plant studies was CRD-factorial. For the first study, N and DMPP application rates were the two factors studied, whereas Se application rate and N forms were the factors in the other study. Thus, the significance of differences between treatment means was determined by two-way analysis of variance (ANOVA). Least significance difference (LSD) statistical test was employed to compare the treatment means at p value 0.05 (Steel and Torrie 1996). The descriptive statistics (means and standard deviation of means) were calculated by Microsoft Excel 2016.

3 | Results

3.1 | Growth and Leaf SPAD Value of Maize Plants

Data regarding the effect of N rates and DMPP application on leaf SPAD value and shoot biomass of maize plants are presented in Figure 1. The leaf SPAD values of plants fertilized with 330 mg N kg^{-1} soil were 10% and 17% higher than plants fertilized with 165 mg N kg^{-1} soil under DMPP(+) and DMPP(−) treatments, respectively. However, the effect of DMPP on leaf SPAD value was nonsignificant. Despite producing different leaf SPAD values, both N rates produced statistically similar shoot

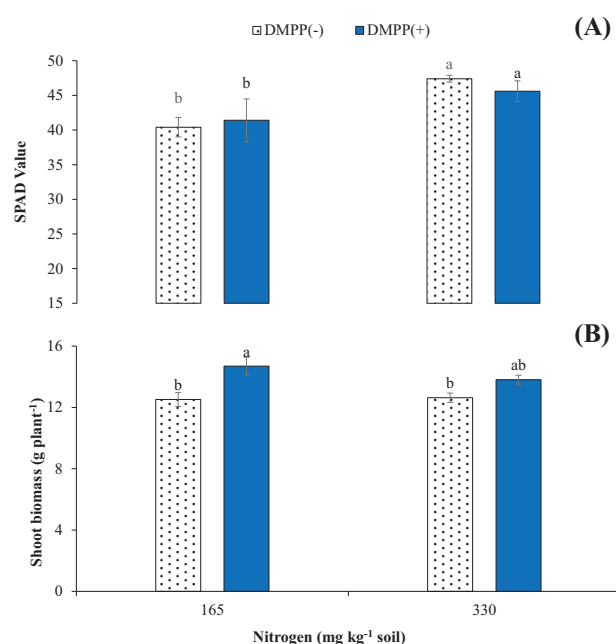


FIGURE 1 | Effect of nitrogen levels and nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on SPAD values (A) and shoot biomass (B) of maize plants. Data are shown as mean \pm standard deviation. Bar showing different alphabets are significantly different at 5% probability level. DMPP(−): no soil application of DMPP, DMPP(+): soil application of DMPP at the rate of 0.01% of applied nitrogen (LSD_A : 3.53, LSD_B : 1.37).

biomass. However, the DMPP application slightly improved the shoot biomass.

The effect of N forms on leaf SPAD values and shoot biomass in no-Se and Se-treated soil is presented in Figure 2 and Figure S1. The plants fed with NH_4^+ -N had 13% and 20% higher leaf SPAD values than those fed with NO_3^- -N in no-Se and Se-treated soil, respectively. Similarly, shoot biomass of NH_4^+ -N fed plants was 31%–34% higher than those fed with NO_3^- -N in no-Se and Se-treated soil. The fertilization of Se also slightly improved the leaf SPAD values and shoot biomass of plants fed with NH_4^+ -N.

3.2 | Soil NH_4^+ -N and NO_3^- -N Concentrations

The soil NH_4^+ -N concentration in the postharvest soil was significantly ($p < 0.05$) increased with N rate and DMPP application (Table 1). At 165 and 330 mg N kg^{-1} soil, DMPP-treated postharvest soil had 42% and 27% higher NH_4^+ -N concentration, respectively, than DMPP(−) control. Secondly, regardless of DMPP treatment, the postharvest soil added with 330 mg N kg^{-1} had 2.3-fold more NH_4^+ -N concentration than that added with 165 mg N kg^{-1} soil. In contrast to NH_4^+ -N concentration, the DMPP-treated postharvest soil had substantially lower NO_3^- -concentration than DMPP(−) control. At 165 and 330 mg N kg^{-1} soil, DMPP-treated postharvest soil had 6.6- and 6.9-fold lower NO_3^- -N concentration, respectively, than DMPP(−) control. Moreover, the highest NO_3^- -N: NH_4^+ -N ratio was recorded with the treatment DMPP(−)+ 165 mg N kg^{-1}

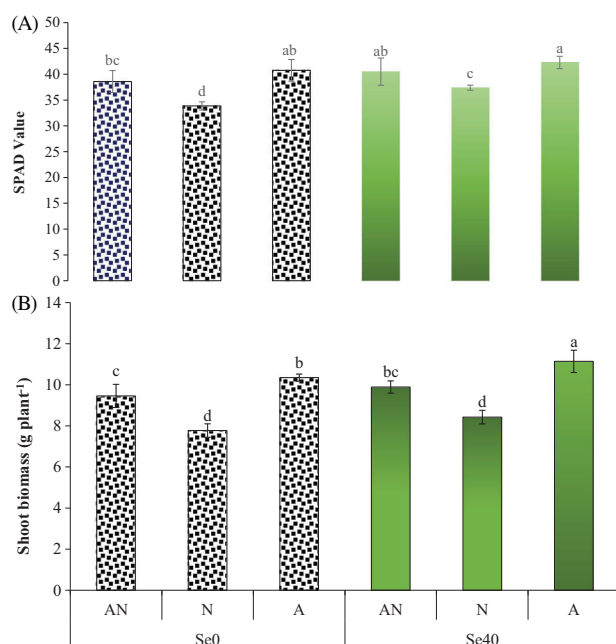


FIGURE 2 | Effect of soil-applied selenium and nitrogen forms on SPAD value (A) and shoot biomass (B) of 5-week-old maize seedlings. Data are shown as mean \pm standard deviation. Bar showing different alphabets are significantly different at 5% probability level. AN: ammonium-nitrogen + nitrate-nitrogen, N: nitrate-nitrogen, A: ammonium-nitrogen, Se0: no soil application of selenium, Se40: soil application of selenium at the rate of 40 $\mu\text{g kg}^{-1}$ (LSD_A: 3.10, LSD_B: 1.46).

soil, whereas the lowest ratio was recorded with the treatment DMPP(+)+330 mg N kg^{-1} .

3.3 | Shoot Se Concentration and Uptake

Data regarding the effect of DMPP and N rates on shoot Se concentration and uptake are presented in Figure 3. With DMPP(+) treatment, shoot Se concentration of 6 weeks old maize plants was improved by 20% and 24% at 165 and 230 mg N kg^{-1} soil, respectively, as compared to DMPP(-) control. The effect of N rates was also significant as maize shoots had 32% higher Se concentration at 330 mg N kg^{-1} soil than 165 mg N kg^{-1} soil, irrespective of DMPP treatment. Likewise, both DMPP and N rates significantly increased Se uptake by plants. Moreover, a higher soil NO_3^- -N: NH_4^+ -N ratio in DMPP-free soil was related to lower Se uptake by plants, and vice versa (Figure 4). The results of experiment with different forms of N (NH_4^+ -N, NO_3^- -N and NH_4^+ -N+ NO_3^- -N) on shoot Se concentration and uptake confirmed the negative effect of NO_3^- -N on shoot Se concentration and uptake. The application of NH_4^+ -N in the form of NH_4Cl resulted in higher shoot Se concentration and uptake, whereas NO_3^- -N in the form of $\text{Ca}(\text{NO}_3)_2$ resulted in lower Se concentration. On the Se(-) soil, the addition of NH_4^+ -N produced 14% and 56% more shoot Se concentration and shoot Se uptake, respectively, whereas these increases were 39% and 83% on Se(+) soils. Moreover, maize was found highly responsive to Se fertilization as shoot Se concentration and shoot Se uptake were increased by 4.7- and 5.0-fold, respectively, compared to no application of Se.

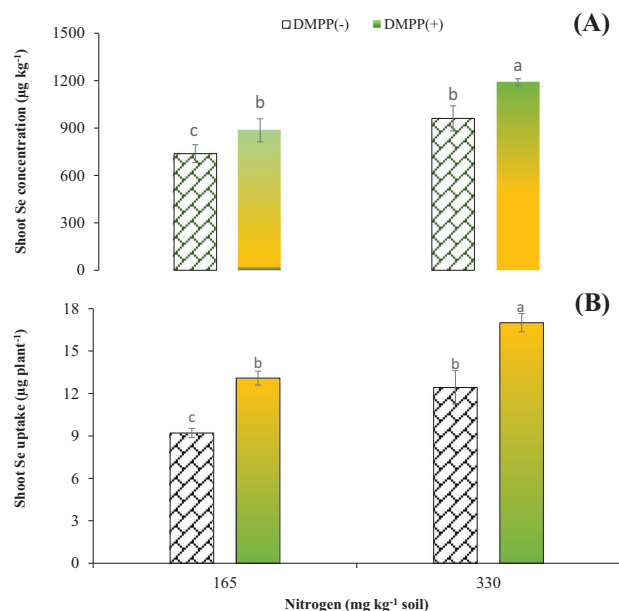


FIGURE 3 | Effect of nitrogen levels and nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on shoot Se content (A) and Se uptake (B). Data are shown as mean \pm standard deviation. Bar showing different alphabets are significantly different at 5% probability level. DMPP(-): no soil application of DMPP, DMPP(+): soil application of DMPP at the rate of 0.01% of applied nitrogen (LSD_A: 116, LSD_B: 1.39).

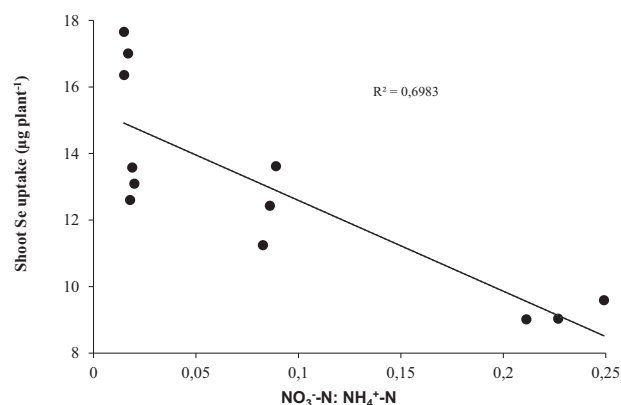


FIGURE 4 | Relationship between ammonium-nitrogen (NH_4^+ -N) and nitrate-nitrogen (NO_3^- -N) ratio and shoot selenium (Se) uptake by maize plants.

3.4 | Shoot P and N Uptake

Data regarding the effect of different forms of N (NH_4^+ -N, NO_3^- -N and NH_4^+ -N+ NO_3^- -N) on shoot N and P concentration and uptake are presented in Table 2. The application of NH_4^+ -N slightly increased the N concentration on Se(-) soils than NO_3^- -N, whereas the same N concentration was recorded on Se(+) soils with both the forms of N. The effect of N forms on P concentration and uptake in maize shoots was significant, whereas Se did not affect them. The application of NH_4^+ -N in the form of NH_4Cl resulted in a higher shoot P concentration and uptake, whereas NO_3^- -N in the form of $\text{Ca}(\text{NO}_3)_2$ produced shoots having lower P concentration and total P uptake. On Se(-) soils, the addition NH_4Cl produced 45% and 94% more shoot P concentration and

TABLE 2 | Effect of soil-applied selenium and nitrogen forms on concentration and uptake of selenium, phosphorus and nitrogen by maize shoots.

Selenium application (mg kg ⁻¹)	Nitrogen form	Shoot selenium concentration (µg kg ⁻¹)	Shoot selenium uptake (µg plant ⁻¹)	Shoot phosphorus concentration (%)	Shoot phosphorus uptake (mg plant ⁻¹)	Shoot nitrogen concentration (%)	Shoot nitrogen uptake (mg plant ⁻¹)
Control	NH ₄ ⁺ -N	151 ± 11 d	1.43 ± 0.04 e	0.136 ± 0.009 bc	12.34 ± 0.74 b	2.56 ± 0.14 a	241 ± 5 b
	N + NO ₃ ⁻ -N						
	NO ₃ ⁻ -N	170 ± 18 cd	1.32 ± 0.017 e	0.121 ± 0.006 c	9.39 ± 0.83 c	2.35 ± 0.13 b	183 ± 17 d
40	NH ₄ ⁺ -N	194 ± 10 c	2.06 ± 0.08 d	0.176 ± 0.021 a	18.24 ± 1.88 a	2.64 ± 0.11 a	273 ± 10 a
	NH ₄ ⁺ -N	868 ± 9 a	8.59 ± 0.25 b	0.147 ± 0.008 b	13.42 ± 0.06 b	2.19 ± 0.10 b	217 ± 8 c
	N + NO ₃ ⁻ -N						
	NO ₃ ⁻ -N	647 ± 28 b	5.45 ± 0.018 c	0.117 ± 0.006 c	10.11 ± 0.35 c	2.15 ± 0.03 b	182 ± 10 d
	NH ₄ ⁺ -N	899 ± 30 a	10.02 ± 0.33 a	0.187 ± 0.006 a	19.14 ± 0.50 a	2.37 ± 0.17 b	257 ± 8 ab
Least significance difference		35	0.53	0.019	1.65	0.23	18.5
Coefficient of variance		6.64	3.55	7.53	6.72	5.05	5.06

Note: Data are shown as mean ± standard deviation (*n* = 3). Values followed by different alphabet(s) in a column are statistically different from each other at 5% probability level.

shoot P uptake, respectively, compared to Ca(NO₃)₂, whereas in case of Se(+) soils, these increases were 60% and 89%, respectively. On Se(-) soils, the addition of NH₄⁺-N caused 49% more shoot P uptake than NO₃⁻-N, whereas on Se(+) soil shoot P uptake was 41% higher.

4 | Discussion

This study discusses the influence of N rates, nitrification inhibitor and N-forms on soil N content (NH₄⁺-N and NO₃⁻-N), the growth and selenium (Se) uptake by maize. Using nitrification inhibitors with N fertilizer is a highly effective method for enhancing crop growth and development (Cui et al. 2022). Applying nitrification inhibitors can slow soil nitrification, leading to increased NH₄⁺-N and reduced NO₃⁻-N content in soil. Results exhibited that the application of DMPP maintained 27%–42% higher NH₄⁺-N content in postharvest soil than DMPP-free soil. Conversely, the soil NO₃⁻-N and the ratio of NO₃⁻-N: NH₄⁺-N were drastically decreased in DMPP-treated soil (Table 2). These results align with the findings of other researchers (Cui et al. 2022; Liu et al. 2021; Zhu et al. 2019). Liu et al. (2021) who reported that the peak of NH₄⁺ for DMPP treatments was delayed by 5 days compared to the peaks for the corresponding N treatments without DMPP, and the NH₄⁺ persisted in soil for a longer duration. Moreover, we found an increase in both NH₄⁺-N and NO₃⁻-N with increase in urea levels. However, DMPP application increased NH₄⁺-N and decreased NO₃⁻-N in soils at both levels of urea. This suggests that DMPP is highly effective in slowing the soil nitrification process for maize cultivation. Thus, the objective of retaining NH₄⁺ in soil while preventing its nitrification through adding DMPP in soil was adequately met. The use of DMPP made the comparative effect of NH₄⁺ and NO₃⁻ on Se uptake more realistic, as it prevented nitrification of fertilizer NH₄⁺ that would have occurred in DMPP-free soil.

The application of N at higher rate improved the concentration and uptake of Se in maize shoots (Figure 1). This finding agrees with Reis et al. (2018), who reported an increase in Se content from 0.22 to 0.35 mg kg⁻¹ in rice grains with the increase in N application rate. Similarly, the combined application of Se and

N led to greater Se accumulation in wheat grains compared to the application of Se alone (Klikocka et al. 2017; Petković et al. 2022; Ramkissoon et al. 2019). Teixeira et al. (2021) reported that grain Se concentration of wheat was positively associated with the increased availability of N in soil. However, Grujic et al. (2021) observed that N application had no impact on Se accumulation in maize shoots. In contrast, there are a few reports of decrease in Se content in plants of wheat and triticale with the N application (Fan et al. 2023; Kong et al. 2023). This might happen due to the dilution effect of Se associated with an increase in plant biomass and grain yield. The exact mechanism explaining higher Se uptake efficiency with the N application, as observed in our study, is not known yet, but there are three possible explanations. First, consumption of H⁺ ions during urea hydrolysis increases soil pH (Kirkby and Mengel 1967). The rise in soil pH may improve Se mobility and availability for plant uptake by increasing pH-dependent negative charges on the soil colloids. Second, N application modulates the expression of NRT1.1B gene in root controlling Se uptake and transport (Teixeira et al. 2021; Zhang et al. 2019). Third, improved root growth due to N application might have increased Se absorption by maize roots. Like N application, the addition of DMPP boosted Se accumulation in maize shoots (Figure 1). This might have occurred due to lower NO₃⁻ accumulation in DMPP(+) soil (Table 1), which may compete with SeO₄²⁻ for uptake by maize roots. Accordingly, NO₃⁻-N fed plants had lower Se concentration and uptake in shoots compared to NH₄⁺-N fed plants (Table 2).

The SPAD value of leaves serves as an indicator of chlorophyll content, which is strongly related to the plant's N status. Accordingly, the leaf SPAD values of plants fertilized with 330 mg N kg⁻¹ soil were 13.6% higher than those of plants receiving 165 mg N kg⁻¹ soil (Figure 2). However, the effect of DMPP on leaf SPAD values was found nonsignificant (Figure 2). Contrary to our findings, some reports have shown a positive effect of DMPP on the SPAD value of maize leaves (Alonso-Ayuso et al. 2016; Dai et al. 2018). In our study, the most likely explanation for lack of DMPP's impact on leaf SPAD value is the similar N accumulation by maize plants (data not shown) under DMPP-treated and untreated conditions.

Along with N rates, the supply of N-forms (NH_4^+ -N and NO_3^- -N) influenced the leaf SPAD values. We found that NH_4^+ -N fed plants had higher leaf SPAD values than NO_3^- -N fed plants. Similarly, Wang et al. (2009) reported that maize seedlings treated with NH_4^+ -N [$(\text{NH}_4)_2\text{SO}_4$] had higher SPAD values compared to those treated with NO_3^- -N [$(\text{Ca}(\text{NO}_3)_2)$]. They stated that NH_4^+ is more readily absorbed by plants than NO_3^- . We also observed higher N concentration and uptake in maize shoots receiving NH_4^+ -N compared to those receiving NO_3^- -N. Likewise, shoot biomass of plants fed with NH_4^+ -N was higher than those of NO_3^- -N fed plants (Figure 3). Sabir et al. (2013) reported that among the N fertilizers, namely, urea, ammonium nitrate, ammonium sulfate, and calcium nitrate, calcium nitrate fed maize plants had the minimum shoot dry weight. The lower SPAD value and shoot dry biomass of NO_3^- -N fed plants as compared to NH_4^+ -N fed plants might be due to lower uptake of N, P and Se (Table 3). Moreover, it is likely that higher nitrogen loss occurred in soil fertilized with NO_3^- -N than in soil fertilized with NH_4^+ -N, which resulted in lower plant nitrogen uptake.

5 | Conclusions

The application of DMPP decreased NO_3^- -N content in the soil, resulting in higher selenium uptake by maize shoots as compared to DMPP-free soil, possibly due to reduced competition between nitrate and selenate. Accordingly, it was also found that soil application of NH_4^+ -N resulted in higher biomass and selenium uptake by maize than that of NO_3^- -N application. These results imply that co-application of selenium and ammonium-based fertilizers could improve biomass and selenium content of maize. Moreover, utilization of such Se-selenium enriched maize crop as forage by animals could enhance their selenium intake, thereby improving the meat quality for human consumption.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.