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Mitigate nitrate contamination in potato tubers and increase nitrogen recovery by combining dicyandiamide, moringa oil and zeolite with nitrogen fertilizer

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ABSTRACT

Potato is considered a nitrogen (N) intensive plant with a low N use efficiency (NUE). The current study introduced an excellent approach by combining dicyandiamide (DCD), moringa seed oil (MSO), or zeolite (ZE), with N fertilizer for maximizing potato tuber yields and NUE as well as minimizing tubers nitrate (NO_3^-) accumulation. The impact of these materials on soil N availability and gaseous emissions (NH_3, and N_2O) was investigated under incubation conditions. A 2-year field experiment were carried out with seven treatments [without N (control), N fertilizer (350 kg N-urea ha^{-1} as a recommended dose; $Urea_{RD}$), 75% of N recommended dose with DCD ($Urea_{75\%RD}$+DCD), $Urea_{75\%RD}$ with 2% MSO ($Urea_{75\%RD}$+$MSO_{2\%}$), $Urea_{75\%RD}$ with 4% MSO ($Urea_{75\%RD}$+$MSO_{4\%}$), $Urea_{75\%RD}$ with 0.5 Mg ZE ha^{-1} ($Urea_{75\%RD}$+ZE_{R1}), and $Urea_{75\%RD}$ with 1.0 Mg ZE ha^{-1} ($Urea_{75\%RD}$+ZE_{R2})]. We also conducted a 40-days incubation trial with the same treatments; however, urea was added at the rate of 200 mg N kg^{-1} soil for all treatments, excluding the control. The addition of DCD, MSO, and ZE with urea under incubation conditions delayed the nitrification process, thereby causing a rise in NH_4^+-N content and a decrease in NO_3^--N content. Ammonia-oxidizing bacteria (AOB) was inhibited ($p \leq 0.01$) in treatments Urea+DCD, Urea+$MSO_{4\%}$, and Urea+ZE_{R2}. The highest NUE indexes were recorded in treatment $Urea_{75\%RD}$+DCD. The highest NO_3^- accumulation (567 mg NO_3^- kg^{-1}) in potato tubers was recorded in treatment $Urea_{RD}$. Whilest, the lowest NO_3^- content (81 mg NO_3^- kg^{-1}) was in treatment $Urea_{75\%RD}$+DCD. The lowest cumulative N_2O emissions and highest cumulative NH_3 volatilization were observed in the treatment Urea+DCD under incubation conditions. Our findings demonstrated that N fertilizer rate could be reduced by 25%, while the tuber yields increased with an acceptable limit of NO_3^- content, resulting in economical, agronomical, and environmental benefits.

1. Introduction

Potato (*Solanum tuberosum* L.) is an essential food for human consumption worldwide after rice and wheat. Potato is a nitrogen (N) intensive plant having a low N-uptake efficiency (Gao et al., 2015; Elrys et al., 2019a). Egypt's potato production increased from 0.39 Tg (10^{12} g) in 1961 to 4.9 Tg in 2018, and N fertilization was the chief management factor for increasing potato tuber yields (FAOSTAT, 2019). The N management recommendation for potato production in Egypt comprises a split application of the highest rate of 350 kg N ha^{-1} (Elrys et al., 2018a). High N concentration and favorable costs make urea the preferred N fertilizer for the traditional cultivation of crops worldwide (FAOSTAT, 2019). In Egypt, urea is the main N source used (71% of the total Egyptian N fertilizer used) (Elrys et al., 2019a). However, because

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of the fast hydrolyses of urea, that generates ammonia (NH_3) and then oxidization of NH_3 to nitrate (NO_3^-) by ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Beeckman et al., 2018), there is a risk of N loss to decrease N availability during potato N demand (Souza et al., 2020). Potato N recovery in Egypt is less than 40% (Elrys et al., 2019a). Thus, potato cultivation under Egyptian condition has a high potential for the N loss. On the other hand, the high concentration of NO_3^- in potato tubers is a significant problem for potato exporters and farmers in Egypt (Elrys et al., 2018a). For example, based on German and Polish standards, if the content of NO_3^- in tubers exceeds 200 and 180 mg NO_3^- kg^{-1} fresh weight, they are not suitable for human consumption (Gorenjak et al., 2014). The increased accumulation of NO_3 in potato tubers causes many diseases for humans and poses a threat to society's overall health (Chen et al., 2016, 2017). Consequently, finding new and different approaches to reduce the NO_3^- accumulation to the acceptable limit in tubers is an important issue.

Nitrification inhibitors (NIs) are common methods used as N stabilizers. The products are formulated to block the activity of nitrifying bacteria on the conversion of NH_4^+ to NO_3^- (Ruark et al., 2018), slowing NO_3^- release into the soil. Dicyandiamide (DCD) was reported to be active in retaining N in a less mobile NH_4^+ form in the soil, lowering soil NO_3^- leaching and enhancing N use efficiency (NUE) by plants (Ning et al., 2018). Application of DCD inhibited the AOB growth (Elrys et al., 2020). There has been a better agronomic performance when potato crop was supplied with N fertilizer blended with NIs (Souza et al., 2020). However, no study examined the indirect effect of DCD on minimizing NO_3^- accumulation in tubers. Therefore, we hypothesize that using DCD with N fertilizer will be highly useful in minimizing NO_3^- accumulation in tubers and increasing potato NUE by reducing the release of NO_3^- into the soil. Besides using chemical compounds as NIs, it is also essential to look for other natural compounds that are less expensive and environmentally friendly. For example, Elrys et al. (2019b) found that using moringa (*Moringa oleifera*) seed extract to inhibit AOB abundance in the soil is an important strategy to decrease N loss soils, and thus improve NUE and decreasing NO_3^- content in tubers. Also, Ashraf et al. (2019) reported that using moringa oil coated urea seemed highly effective in reducing N losses and sustaining better crop production. Nonetheless, to date and to the best of our knowledge, no studies have assessed the effect of moringa seed oil (MSO) on potato agronomic performance, tubers NO_3^- accumulation, AOB inhibition, and gaseous emissions. Here, we hypothesize that using MSO with N fertilizer will reduce the NO_3^- accumulation in potato tubers and increase potato NUE by inhibiting AOB and minimizing gaseous emissions.

Another approach to improve N efficiency in the urea is using of polymers, such as zeolite (ZE) mineral, with urea fertilizer to slow the release. ZE has an extensive surface area that enables it to bind NH_4^+ within its pore structure. Moreover, ZE can decrease NH_3 volatilization due to its high cation exchange capacity (CEC) and affinity for NH_4^+ (Jumadi et al., 2020). However, no one has ever studied the effect of ZE on enhancing potato NUE. Furthermore, ZE is a source of silicon (Si), which is a useful nutrients for crop growth (Ashfaque et al., 2017). Even though Ashfaque et al. (2017) stated that Si fertilizer application highly improved the nitrate reductase (NR) activity in plants, there is no study on the effect of ZE on reducing NO_3^- accumulation in potato tubers. Therefore, we assume that using ZE with N fertilizer will reduce the NO_3^- content in potato tubers and increase potato NUE.

To address our hypotheses, we conducted a two-year field trial and one incubation trial to: 1) assess the effect of DCD, MSO, and ZE on NO_3^- accumulation in tubers and NR activity, 2), determine the effect of these treatments on potato agronomic performance, potato NUE, and physiochemical properties of potato, and 3) verify the effect of DCD, MSO, and ZE with N-urea fertilizer on soil N availability, AOB abundance, and gaseous emissions (NH_3, and N_2O) under incubation conditions.

2. Materials and methods

2.1. Field experiment

2.1.1. The experimental site, study design, and cultivation practices

A two-years field trial was conducted in two seasons (October 2018 and October 2019) in Al-Husayniyah City, El-Sharkia Governorate, Egypt (31° 45′ 51.2″N, 30° 56′ 19.34″ E WGS). The study area's climate during the experimental duration was characterized by a semi-arid with average rainfalls and temperatures of 128 mm and 16.1 °C, respectively (Abdo et al., 2020). Before planting season, representative samples were collected from the 0.2 m depth of soil prior to planting to analyze their textural and chemical traits according to Piper (1951); Black (1968); and Jackson (1973). The soil was classified as a loam, with organic matter content of 8.2 ± 0.25 and 7.73 ± 0.34 g kg^{-1}, available N-NH_4^+ of 11.6 ± 0.43 and 13.4 ± 0.52 mg kg^{-1}, available N-NO_3^- of 7.53 ± 0.49 and 5.81 ± 0.74 mg kg^{-1}, available P of 15.4 ± 0.65 and 17.4 ± 0.22 mg kg^{-1}, available K of 134 ± 4.61 mg kg^{-1} and 147 ± 7.11 mg kg^{-1}, pH of 7.94 ± 0.03 and 8.01 ± 0.04 in soil-water suspension (1:2.5), EC of 1.22 ± 0.08 and 1.18 ± 0.06 dS m^{-1} in soil-water suspension (1:5), and available Fe of 4.13 ± 0.31 and 3.82 ± 0.07 mg kg^{-1} for both seasons, respectively.

The field was divided into rows (0.8-m width) after plowing utilizing a moldboard plow to a depth of 0.4 m and divided into plots (4.0-m length) and 4 rows for every plot with 2 rows among the plots as a buffer. Potato (*Solanium tuberosum* L. cv. Spunta) seed tubers were planted with a 0.3 m spacing between plants. Traditional furrow flood irrigation method was used where 51 mm was applied before potato seeding as sufficient water amount to ensure uniform soil moisture before planting. The total amount of 240 mm of irrigation water was used at 8 irrigation intervals (30 mm each interval). According to guidelines of water quality for irrigation presented by the FAO (1985), the pH, EC, Na^+, Cl^-, and NO_3^- were found to be at levels characterized as safe for irrigation purposes. The values were 7.13, EC = 0.48 dS m^{-1}, 2.72 (expressed as the SAR), 2.54 mg L^{-1}, 3.76 mg L^{-1} for pH, EC, Na^+, Cl^-, and NO_3^-, respectively. Starter fertilizer consisted of $Ca(H_2PO_4)_2$ (7% P), providing 65.5 kg P ha^{-1}. Additionally, 166 kg K-K_2SO_4 (40% K) ha^{-1} was applied for all treatments at two equal doses (after 30 and 60 days from sowing). Fertilizers chosen for the P and K fertilization (rates sufficient for potato demand) are the main sources of P and K for potato cultivation in Egypt (Elrys et al., 2018a, 2018b).

Plots were laid out in a randomized complete block design with three replicates of 7 treatments. The treatments consisted of a control (without N), N fertilizer (350 kg N-urea ha^{-1} as a recommended dose; Urea $_{RD}$), 75% of N recommended dose with DCD (Urea $_{75\%RD}$+DCD), 75% of N recommended dose with 2% MSO (Urea $_{75\%RD}$+$MSO_{2\%}$), 75% of N recommended dose with 4% MSO (Urea $_{75\%RD}$+$MSO_{4\%}$), 75% of N recommended dose with 0.5 Mg ZE ha^{-1} (Urea $_{75\%RD}$+ZE_{R1}), and 75% of N recommended dose with 1.0 Mg ZE ha^{-1} (Urea $_{75\%RD}$+ZE_{R2}). Urea fertilizer was applied on three equal doses, which were added on days 1, 30, and 60 of planting. DCD was added with a rate of 10% of urea fertilized rate with each dose of urea. Zeolite (ZE; 75.9% SiO_2, 1.32% Fe_2O_3) was added one week before potato planting. The zeolite XRD analysis indicated that it is 100% Clinoptilolite-Ca. The available Si and Fe concentrations of the ZE used was 379 and 18.6 mg kg^{-1}, respectively. Urea was developed as moringa oil coated urea. For this, each kg of granular urea was coated with 20 mL and 40 mL of moringa seed oil (MSO) extract representing 2% and 4% coating on v/w basis. Then moringa oil coated urea was allowed to dry at room temperature. Chemical components of MSO were 3.50 g kg^{-1} of myristic (C 14:0), 77 g kg^{-1} of palmitic (C 16:0), 11 g kg^{-1} of palmitoleic (C 16:1), 76.7 g kg^{-1} of stearic (C 18:0), 695 g kg^{-1} of oleic (C 18:1n-9), 22 g kg^{-1} of linoleic (C 18:2), 6.0 g kg^{-1} of linolenic (C 18:3), 6.0 g kg^{-1} of arachidic (C 20:0), 155 mg kg^{-1} α-tocopherol, 5.5 mg kg^{-1} β-tocopherol, 68.9 mg kg^{-1} γ-tocopherol, and 65.2 mg kg^{-1} δ-tocopherol.

2.1.2. Moringa seed oil (MSO) extraction, fatty acids and tocopherols analysis by gas liquid chromatography (GLC) and high-performance liquid chromatography (HPLC)

Moringa seeds were dried in a vacuum oven (50 °C), ground to a fine powder, and extracted at room temperature with *n*-hexane using a magnetic stirrer followed by filtration using Whatman No. 1 filter paper. The extraction ratio was 1 g seed to 10 mL *n*-hexane. The combined filtrate was evaporated in a rotary evaporator (BÜCHI- Rotavapor R-124 & water bath-B-480) at 40 °C. The MSO, after evaporation, was weighed to measure the yield and stored at − 20 °C until further use. According to Arens et al. (1994), fatty acids in MSO were transesterified to FAME (fatty acids methyl esters) using N-trimethylsulfonium hydroxide (Macherey-Nagel, Germany) and analyzed by Shimadzu GC-14A. MSO solution (250 mg) in 25 mL *n*-heptane was used directly to determine tocopherols using the Merck Hitachi HPLC (Ramadan, 2013).

2.1.3. Agronomic measurements

Plants were collected at tuber maturation (86 days after planting) and the final harvest sampling. At the beginning of the tuber maturation, five whole plants from each plot were collected. Plants were then divided into stems, leaves, and tubers weighed and oven-dried at 70 °C. Dry plant tissues were weighed to measure dry matter accumulation and ground to pass through 1-mm sieve. Tissue N concentrations were measured as reported by Chapman and Pratt (1982). The accumulated N quantities in each part were computed by multiplying the N concentration by the accumulated dry matter. The values for each part were summed to record the total dry matter accumulation and total N uptake. The final harvest of the tuber was carried out on the 115th day after planting. Tubers were manually harvested, counted, and weighed to measure the average tuber weight, and total fresh tuber yield. To estimate tuber dry matter content, five tubers were washed, sliced, and then weighed before and after four days drying in an oven (70 °C). The tuber dry matter yield was computed as the product of total fresh tuber yield and dry matter content.

Dry tuber samples were ground, and the N concentration was measured (Chapman and Pratt (1982). Tuber N uptake at harvest was calculated by the multiplication of N content and dry matter of the tubers. According to Baker and Smith (1969), tubers NO_3^- accumulation was determined by $Al_2(SO_4)_3$ method. The activity of the NR enzyme was determined based on the procedure of Jaworski (1971). Chlorophyll a, chlorophyll b, and carotenoids were extracted by pure acetone from potato leaves (Fadeel, 1962). The extracted chlorophyll a, chlorophyll b, and carotenoids were measured based on Wettstein (1957). Fe content was measured using atomic absorption spectrophotometer (AOAC, 1984).

2.1.4. NUE metrics at maturation

Some components of the NUE by potato crop were studied at the beginning of tuber maturation, including:

The N-uptake efficiency (NUPE), obtained as a result of total N uptake at maturation (TNUM) per unit of N application rate (NAR) (Milroy et al., 2019);

$$NUPE(\%) = \frac{\text{TNUM}}{\text{NAR}} \times 100$$

Partial nutrient balance at maturation (PNBm), calculated as the tuber N uptake at maturation (TuNUM) per unit of N application rate (NAR) (Bero et al., 2014);

$$PNBm(\%) = \frac{\text{TuNUM}}{\text{NAR}} \times 100$$

Apparent crop recovery at maturation (CRECm), that refers to the increase in total N uptake at maturation (TNUM) per unit of N application rate (NAR) (Cambouris et al., 2016);

$$CRECm(\%) = \frac{(\text{TNUM with N application–TNUM without N application})}{\text{NAR}} \times 100$$

Apparent crop removal efficiency at maturation (CREMm), representing the increase of tuber N uptake at maturation (TuNUM) per unit of N application rate (NAR) (Bero et al., 2014);

$$CREMm(\%) = \frac{(\text{TuNUM with N application–TuNUM without N application})}{\text{NAR}} \times 100$$

2.1.5. NUE metrics at harvest

Additional metrics of NUE were studied based on fresh tuber yield and tuber N uptake at final harvest, including:

N surplus was computed as the difference among mineral N input through fertilization and tuber N uptake at the final harvest (Elrys et al., 2019b). The partial factor productivity (PFP; kg tuber kg^{-1} N applied), a result of fresh tuber yield at final harvest (FTYH) per unit of N application rate (NAR) (Bero et al., 2014);

$$\text{PFP} = \frac{\text{FTYH}}{\text{NAR}} \times 100$$

Agronomic efficiency (AE; kg tuber increased kg^{-1} N applied), fresh tuber yield at final harvest (FTYH) per unit of N application rate (NAR) (Souza et al., 2020);

$$\text{AE} = \frac{(\text{FTYH with N application–FTYH without N application})}{\text{NAR}} \times 100$$

Partial nutrient balance at harvest (PNBh), obtained as the tuber N uptake at final harvest (TuNUH) per unit of N application rate (NAR) (Bero et al., 2014);

$$\text{PNBh}(\%) = \frac{\text{TuNUH}}{\text{NAR}} \times 100$$

Apparent crop removal efficiency at final harvest (CREMh), representing the increase of tuber N uptake at final harvest (TuNUH) per unit of N application rate (NAR) (Bero et al., 2014);

$$\text{CREMh}(\%) = \frac{(\text{TuNUH with N application–TuNUH without N application})}{\text{NAR}} \times 100$$

N use efficiency (NUE), indicating the increase of total N uptake at final harvest (TNUH) per unit of N application rate (NAR) (Elrys et al., 2019b);

$$\text{NUE}(\%) = \frac{(\text{TNUH with N application–TNUH without N application})}{\text{NAR}} \times 100$$

2.2. Incubation trail setup

The soil samples utilized in the current study were collected from the upper 0.2 m depth from Yangling (34°18′N, 10°85′E) that located at the Loess Plateau of Shaanxi province, China in a typical semi-humid climate. The annual air temperature, precipitation, and evaporation were 12.9 ℃, 575 mm, and 993 mm, respectively. According to the USDA system, the soil was classified as an Udic Haplustalf (Dai et al., 2016). Representative fresh soil samples were stored at a temperature below 4 °C to estimate mineral N. In contrast; some soil was dried in the air to determine various physicochemical properties. The soil in this study was classified as a loam, with organic matter content of 15.5 ± 1.32 g kg^{-1}, available N-NH_4^+ of 7.11 ± 0.24 mg kg^{-1}, available N-NO_3^- of 6.84 ± 0.36 mg kg^{-1}, available P of 9.10 ± 0.72 g kg^{-1}, available K of 143 ± 8.53 g kg^{-1}, pH of 7.72 ± 0.01 in soil-water suspension (1:2.5), EC of 191 ± 4.2 μS cm^{-1} in soil-water suspension (1:5).

To stabilize the microbial activity, the field soil was crushed and sifted (2 mm sieve), and then 2-weeks pre-incubation was conducted in the dark with 50% water-filled pore space (WFPS) at 25ºC. After a 2-weeks pre-incubation, soil WFPS percentage has been raised to 60% with the same treatments as a field experiment; however, urea fertilizer was applied at the rate of 200 mg N-urea kg^{-1} soil for all treatment, excluding the control treatment. Urea were dissolved in distilled water and mixed in soil symmetrically and adequately. All treatments were laid out in a randomized block with repeated measures design. Water was added every two days to maintain 60% soil moisture during the incubation period. Three incubation trials were conducted for 40 days with the same treatments under the same conditions, with each trail having different aims.

2.2.1. Measurements of mineral N, pH and the bacterial amoA gene abundance

Soil samples (300 g) were placed in round plastic boxes and kept at 25 °C under dark conditions in the incubation chambers. For aeration, the jars were sealed with parafilm and 8 pores on the top were created. The soil was sampled after the treatments were applied at different intervals (2, 4, 6, 8, 10, 15, 20, 25, 30, 35, and 40 days) to estimate mineral N and pH changes. Using 1.0 M KCl (soil: solution ratio of 1:10), the NH_4^+ and NO_3^- content was extracted and later measured using a continuous flow analyzer (AA3; Bran and Luebbe). The soil pH was determined at a 1:2.5 (soil: water ratio) by a pH meter.

Half gram of soil was used to extract DNA after 6, 12 and 18 days incubation period by the protocol of manufacturer (FastDNA™ Spin Kit for Soil, MP Biomedicals, USA). DNA purity and quality were verified by the spectrophotometer (NanoDrop2000, Thermo Fisher Sci., USA). The extracted DNA was kept at − 20ºC. According to Tao et al. (2017), AOB gene copy was determined using qPCR on an Applied Biosystems ABI 3730XL thermal cycler. SYBR green-based detection was utilized with amoA1F/amoA2R primer pairs for AOB (Rotthauwe et al., 1997).

2.2.2. NH_3 volatilization

Soil sample (100 g) was placed in 500-mL screw-top jar (2 cm depth and 70 mm diameter), and 20 mL of 2% boric acid (H_3BO_3) was utilized as a NH_3 absorber in a 50-mL vial and analyzed by titration with a 0.005 M H_2SO_4 (Soares et al., 2012). NH_3 volatilization rate was determined at different intervals (2, 4, 6, 8, 10, 15, 20, 25, 30, 35, and 40 days) during the experiment.

2.2.3. Measurement of N_2O emission

Erlenmeyer flasks (250-mL) containing 60 g soil each were utilized. To stop the evacuation of gases, the open end of the flask was closed with a rubber stopper. To collect gaseous samples, a hole was made in the stopper, and a tube with a 3-way valve at the exterior end was inserted (Plaza-Bonilla et al., 2014). Gas samples were collected in gas collection bags at different intervals (2, 4, 6, 8, 10, 15, 20, 25, 30, 35, and 40 days) after applying the treatments. Collected gas was analyzed using GLC technique (Plaza-Bonilla et al., 2014). The system was calibrated using standards (Carburos Metálicos, Barcelona, Spain) as reported (Holland et al., 1999), then the soil N_2O production in the flask headspace was calculated.

2.3. Statistical analysis

All field experiments results were analyzed *via* the one-way analyses of variance (ANOVA) for randomized complete block design and error variances homogeneity using COSTAT software. Whilest the incubation experiment data were statistically analyzed *via* a one-way randomized block with repeated measures ANOVA design. Combined data analysis with the least significant difference (LSD) was carried out at a probability level of 99% ($p \leq 0.01$) for each treatment by Duncan's multiple range test (Steel and Torrie, 1997).

3. Results

3.1. Incubation experiment

3.1.1. Changes in soil NH_4^+-N, and NO_3^--N

Soil NH_4^+-N content stayed low during 40-day incubation in the control treatment (Fig. 1a). Compared with control, the initial content of soil NH_4^+-N significantly ($p \leq 0.01$) increased under the treatment of urea with and without DCD, MSO, and ZE (Fig. 1a). Compared with urea treatment, the content of soil NH_4^+-N increased significantly under all N fertilizer management treatments. The content of soil NH_4^+-N decreased to the initial control concentration on day 10 under urea and Urea+$MSO_{2\%}$ treatments. However, it reduced to the initial control level on days 15, 20, 25, and 40 for the treatments of Urea+$MSO_{4\%}$, Urea+ZE_{R1}, Urea+ZE_{R2}, and Urea+DCD, respectively (Fig. 1a). Based on the mean during different timing estimates, the highest NH_4^+-N content during 40-days incubation was recorded in Urea+DCD, while the lowest content (excluding control) was observed in the urea treatment.

Based on the mean during different timing estimates, soil NO_3^--N level was 18.1 mg kg^{-1} in the control treatment, and a significant ($p \leq 0.01$) increase of soil NO_3^--N level for urea treatment with and without DCD, MSO, and ZE was recorded (Fig. 1b). A higher soil NO_3^--N level was observed in urea treatment during 40-days incubation. After deducted soil NO_3^--N in the control, NO_3^--N content was 127, 34.5, 120, 111, 106, and 87 mg kg^{-1} for urea, Urea+DCD, Urea+$MSO_{2\%}$, Urea+$MSO_{4\%}$, Urea+ZE_{R1}, and Urea+ZE_{R2} treatments, respectively (Fig. 1b).

3.1.2. Soil pH, and the bacterial amoA gene abundance

Soil pH provisionally increased during 3-days after urea addition compared with control treatment, and after that, reduced (Fig. 2a). The decline was continuous and rapid in treatments urea and Urea+$MSO_{2\%}$. At the same time, there was an abrupt decrease in soil pH values after day 6 in treatment Urea+$MSO_{4\%}$ (Fig. 2a). However, there was a progressively reduced soil pH of Urea+ZE_{R1} and Urea+ZE_{R2} treatments within 40-days incubation. There was significant difference between both treatments, where the soil pH decline in Urea+ZE_{R1} treatment was greater than Urea+ZE_{R2}. Maximum soil pH was observed in treatment Urea+DCD within 40-days incubation (Fig. 2a).

The AOB abundance was in the range of 5.11×10^4 to 2.69×10^6 copies g^{-1} soil (Fig. 2b). On days 12 and 18, the AOB abundance was ($p \leq 0.01$) greater than that on day 6. The AOB abundance has increased very significantly in urea treatment. This effect was inhibited ($p \leq 0.01$) in treatments Urea+DCD, Urea+$MSO_{4\%}$, and Urea+ZE_{R2} on days 6 and 12, and in treatments Urea+DCD, and Urea+ZE_{R2} on day 18. The AOB abundance did not differ significantly between Urea+$MSO_{2\%}$ and Urea+ZE_{R1} treatments (Fig. 2b). The DCD application was more effective in inhibiting AOB.

3.1.3. Emissions of NH_3 and N_2O

NH_3 volatilization immediately occurred after the urea fertilizer application (Fig. 3a). Massive NH_3 volatilization losses occurred during the first week and gradually decreased to the end of the experiment. In comparison with the control, urea application with and without DCD, MSO, and ZE significantly ($p \leq 0.01$) increased NH_3 volatilization, where it increased by 1.10-, 9.95-, 0.88-, 2.7-, 1.0-, and 0.9-fold for urea, Urea+DCD, Urea+$MSO_{2\%}$, Urea+$MSO_{4\%}$, Urea+ZE_{R1}, and Urea+ZE_{R2} treatments, respectively (Fig. 3a). Cumulative NH_3 volatilization increased by 422%, and 76% in treatments Urea+DCD and Urea+$MSO_{4\%}$ compared to urea treatment alone. Cumulative NH_3 volatilization did not differ significantly between urea, Urea+$MSO_{2\%}$, Urea+ZE_{R1}, and Urea+ZE_{R2} treatments (Fig. 3a).

N_2O emission was very low in the control (Fig. 3b). The urea treatment significantly ($p \leq 0.01$) increased the N_2O emissions, reaching a peak at the start of the experiment then sharply decreased to a low level (Fig. 3b). The lowest cumulative N_2O emissions were observed in

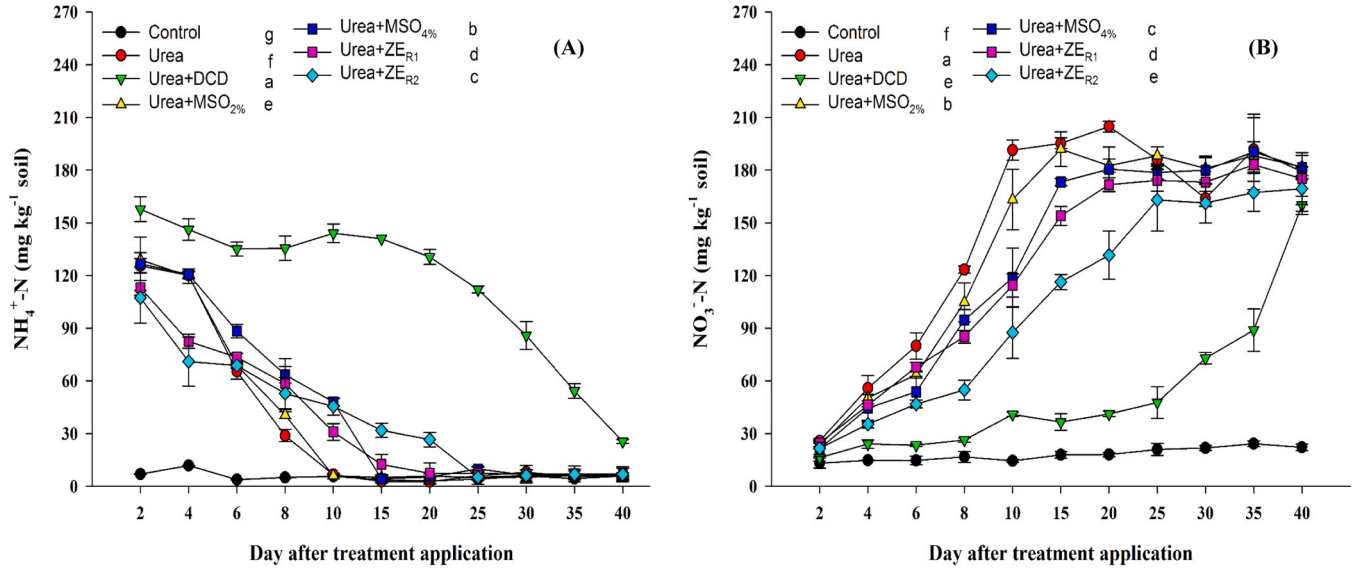

Fig. 1. N management effect on NH_4^+-N (A) and NO_3^--N (B) contents under incubation conditions at various intervals. Data in the figure represent means ± SD ($n = 3$). Different letters next to the legends indicate significant differences between the treatments at $p \leq 0.01$.

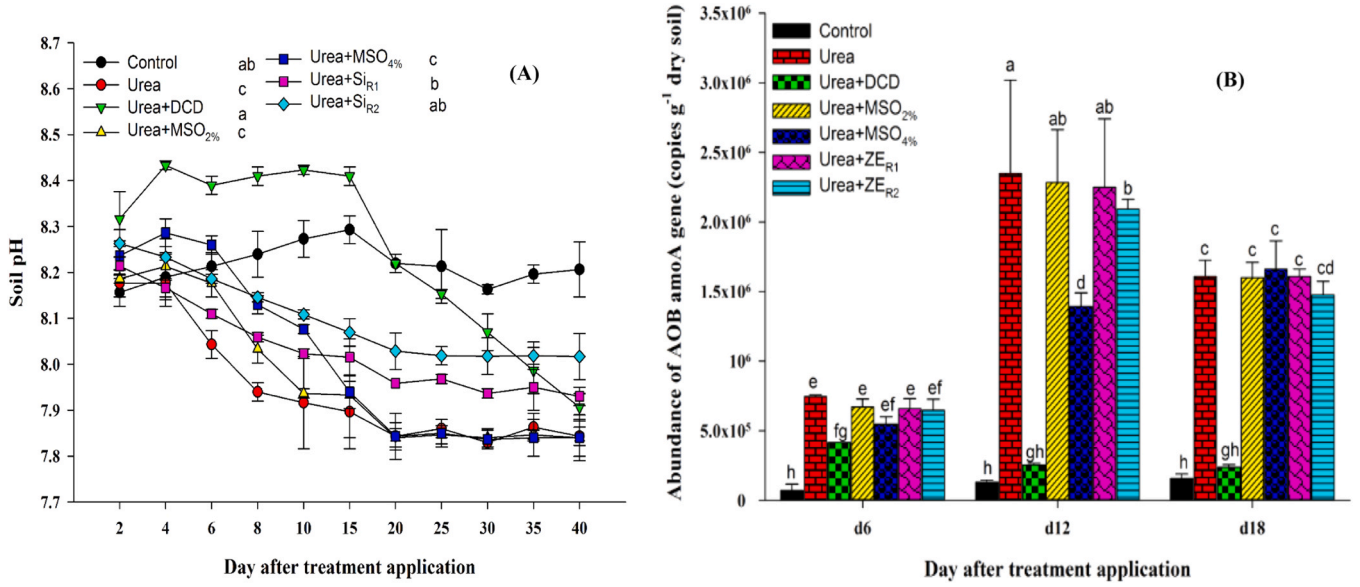

Fig. 2. N management effect on the soil pH (A) and the abundance of ammonia-oxidizing bacteria (AOB) (B) under incubation conditions at various intervals. Data in the figure represent means ± SD ($n = 3$). Different letters next to the legends (A) and above the columns (B) indicate significant differences between the treatments at $p \leq 0.01$.

Urea+DCD treatment, followed by Urea+ZE_{R2} treatment. Cumulative N_2O emissions did not differ significantly between urea and Urea+$MSO_{2\%}$ and between Urea+DCD and control treatments (Fig. 3b).

3.2. Field experiment

3.2.1. Tubers nitrate accumulation and the activity of nitrate reductase (NR)

A significant ($p \leq 0.01$) difference in tubers NO_3^- accumulation was recorded in treatment $Urea_{RD}$ with and without DCD, MSO, and ZE (Table 1). The highest NO_3^- accumulation (567 mg NO_3^- kg^{-1}) was recorded when the urea's recommendation rate (350 kg N ha^{-1}) was applied. While, NO_3^- contents were 80.6, 290, 178, 213, and 151 mg NO_3^- kg^{-1} in treatments $Urea_{75\%RD}$+DCD, $Urea_{75\%RD}$+$MSO_{2\%}$, $Urea_{75\%RD}$+$MSO_{4\%}$, $Urea_{75\%RD}$+ZE_{R1}, and $Urea_{75\%RD}$+ZE_{R2}, respectively as an average of 2 seasons (Table 1). The NO_3^- accumulation values in treatments $Urea_{75\%RD}$+DCD, $Urea_{75\%RD}$+$MSO_{4\%}$, and $Urea_{75\%RD}$+ZE_{R2} were lower than the maximum acceptable level of potato (180 and 200 mg NO_3 kg^{-1} in Poland and Germany, respectively) (Table 1). On the contrary, a significant ($p \leq 0.01$) increase in NR activity was noted in treatments $Urea_{75\%RD}$+$MSO_{4\%}$, and $Urea_{75\%RD}$+ZE_{R2} compared with the other treatments. The highest NR activity was recorded in the treatment of $Urea_{75\%RD}$+ZE_{R2} (Table 1). The NR enzyme activity did not differ significantly ($p \leq 0.01$) between $Urea_{RD}$, $Urea_{75\%RD}$+DCD, $Urea_{75\%RD}$+$MSO_{2\%}$, and $Urea_{75\%RD}$+ZE_{R1} treatments (Table 1).

3.2.2. NUE metrics

There was a significant N management effect on NUPE, CREMm, PNBm, CRECm, PFP, AE, PNBh, CREMh, NUE, and N surplus estimated at maturation and final harvest (Tables 2, and 3). Averaged across two-

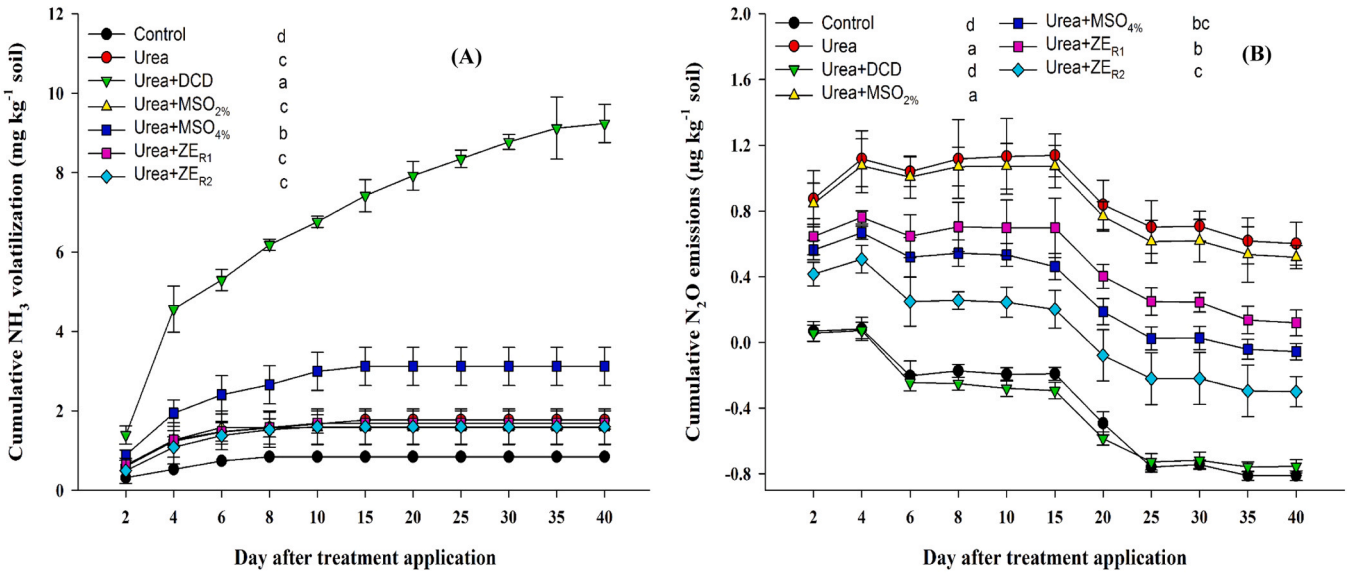

Fig. 3. N management effect on the cumulative of NH_3 (A) and N_2O (B) emissions under incubation conditions. Data in the figure represent means ± SD ($n = 3$). Different letters next to the legends indicate significant differences between the treatments at $p \leq 0.01$.

Table 1
N management effect on tuber N uptake at maturation, tuber N uptake at harvest, NO_3^--N accumulation, and nitrate reductase (NR) activity of plants during two seasons field experiment.

Treatment	Tubers N uptake at maturation (kg ha^{-1})	Tubers N uptake at harvest (kg ha^{-1})	NO_3^--N accumulation (mg kg^{-1})	NR activity (µmol NO_2 mg protein^{-1} h^{-1})	Tubers-Fe accumulation (mg kg^{-1})
1st season					
Control	12.9 ± 0.6^{f}	17.1 ± 0.5^{f}	93.5 ± 8.3^{f}	2.52 ± 0.42^{d}	2.55 ± 0.10^{f}
Urea $_{RD}$	130 ± 2.0^{c}	143 ± 6.0^{c}	561 ± 25.7^{a}	4.36 ± 0.18^{c}	5.71 ± 0.55^{c}
Urea $_{75\%RD}$+DCD	141 ± 2.0^{a}	164 ± 8.1^{a}	80.8 ± 9.5^{f}	4.54 ± 0.20^{c}	3.51 ± 0.80^{e}
Urea $_{75\%RD}$+$MSO_{2\%}$	99.5 ± 3.1^{e}	119 ± 4.2^{e}	286 ± 12.2^{b}	4.82 ± 0.29^{c}	4.22 ± 0.10^{d}
Urea $_{75\%RD}$+$MSO_{4\%}$	130 ± 2.3^{c}	145 ± 5.3^{c}	179 ± 10.0^{d}	5.35 ± 0.44^{b}	4.48 ± 0.37^{d}
Urea $_{75\%RD}$+ZE_{R1}	111 ± 1.2^{d}	127 ± 7.0^{d}	215 ± 8.0^{c}	4.96 ± 0.68^{c}	10.5 ± 0.61^{b}
Urea $_{75\%RD}$+ZE_{R2}	136 ± 2.0^{b}	153 ± 2.0^{b}	155 ± 4.0^{e}	6.41 ± 0.17^{a}	15.1 ± 0.52^{a}
2nd season					
Control	12.6 ± 0.8^{f}	17.5 ± 0.4^{f}	89.0 ± 7.1^{f}	2.63 ± 0.32^{d}	2.15 ± 0.52^{f}
Urea $_{RD}$	129 ± 2.7^{c}	145 ± 6.3^{c}	573 ± 44.5^{a}	4.57 ± 0.45^{c}	6.37 ± 0.24^{c}
Urea $_{75\%RD}$+DCD	139 ± 3.8^{a}	162 ± 8.7^{a}	80.3 ± 8.6^{f}	4.75 ± 0.18^{c}	3.60 ± 0.58^{e}
Urea $_{75\%RD}$+$MSO_{2\%}$	101 ± 2.9^{e}	118 ± 5.0^{e}	294 ± 15.4^{b}	5.03 ± 1.1^{c}	4.50 ± 0.27^{d}
Urea $_{75\%RD}$+$MSO_{4\%}$	134 ± 2.6^{c}	149 ± 5.1^{c}	176 ± 6.4^{d}	5.61 ± 0.41^{b}	4.61 ± 0.36^{d}
Urea $_{75\%RD}$+ZE_{R1}	109 ± 2.8^{d}	129 ± 7.4^{d}	211 ± 11.4^{c}	4.92 ± 0.55^{c}	10.1 ± 0.48^{b}
Urea $_{75\%RD}$+ZE_{R2}	138 ± 2.0^{b}	154 ± 2.4^{b}	146 ± 7.2^{e}	6.72 ± 0.83^{a}	14.9 ± 0.53^{a}
p value					
1st season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)
2nd season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)

Data are means ($n = 3$) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

seasons, these indexes were consistently greater in Urea$_{75\%RD}$+DCD, Urea$_{75\%RD}$+$MSO_{4\%}$, Urea$_{75\%RD}$+ZE_{R1}, and Urea$_{75\%RD}$+ZE_{R2} treatments than Urea$_{RD}$ treatment. The treatment Urea$_{75\%RD}$+DCD resulted in the greatest NUPE, PNBm, CRECm, CREMm, PFP, AE, PNBh, CREMh, and NUE compared to other treatments, followed by Urea$_{75\%RD}$+ZE_{R2} and Urea$_{75\%RD}$+$MSO_{4\%}$ treatments, respectively (Tables 2 and 3). There were no significant differences in NUPE, PNBm, CRECm, CREMm, and PFP between Urea$_{RD}$, and Urea$_{75\%RD}$+$MSO_{2\%}$ treatments. Averaged across two-seasons, the lowest N surplus (72.8 kg N ha^{-1}) was noted under the treatment of Urea$_{75\%RD}$+DCD, followed by Urea$_{75\%RD}$+ZE_{R2} (85.4 kg N ha^{-1}), and Urea$_{75\%RD}$+$MSO_{4\%}$ (94.0 kg N ha^{-1}) treatments, respectively, compared with 185, 126, and 117 kg N ha^{-1} for Urea$_{RD}$, Urea$_{75\%RD}$+$MSO_{2\%}$, and Urea$_{75\%RD}$+ZE_{R1}, respectively (Table 2).

3.2.3. Agronomic responses

Tubers fresh weight, tuber dry weight, carotenoids, chlorophyll (a

Table 2
N management effect on the N-uptake efficiency (NUPE), partial nutrient balance at maturation (PNBm), apparent crop recovery at maturation (CRECm), apparent crop removal efficiency at maturation (CREMm), and N surplus of plants during two seasons field experiment.

Treatment	NUPE (%)	PNBm (%)	CRECm (%)	CREMm (%)	N surplus (kg ha^{-1})
1st season					
Urea $_{RD}$	42.4 ± 0.72^{e}	37.2 ± 0.6^{e}	37.9 ± 0.7^{e}	33.5 ± 0.6^{e}	186 ± 9.5^{a}
Urea $_{75\%\ RD}$+DCD	60.8 ± 1.4^{a}	53.7 ± 1.0^{a}	54.7 ± 1.6^{a}	48.8 ± 1.02^{a}	73.5 ± 8.2^{f}
Urea $_{75\%\ RD}$+MSO$_{2\%}$	43.4 ± 0.92^{e}	37.9 ± 1.1^{e}	37.3 ± 0.81^{e}	33.0 ± 0.90^{e}	127 ± 6.4^{b}
Urea $_{75\%\ RD}$+MSO$_{4\%}$	56.5 ± 0.81^{c}	49.5 ± 0.94^{c}	50.4 ± 0.92^{c}	44.6 ± 1.29^{c}	94.7 ± 5.0^{d}
Urea $_{75\%\ RD}$+ZE$_{R1}$	48.0 ± 0.61^{d}	42.1 ± 0.31^{d}	41.9 ± 0.72^{d}	37.2 ± 0.50^{d}	117 ± 8.0^{c}
Urea $_{75\%\ RD}$+ZE$_{R2}$	58.7 ± 0.58^{b}	51.8 ± 0.8^{b}	52.7 ± 0.81^{b}	46.9 ± 0.92^{b}	86.0 ± 3.3^{e}
2nd season					
Urea $_{RD}$	43.1 ± 0.76^{e}	36.9 ± 0.61^{e}	38.4 ± 0.73^{e}	33.8 ± 0.64^{e}	184 ± 8.32^{a}
Urea $_{75\%\ RD}$+DCD	61.6 ± 1.4^{a}	54.1 ± 1.4^{a}	55.5 ± 1.8^{a}	49.2 ± 1.23^{a}	75.4 ± 8.0^{f}
Urea $_{75\%\ RD}$+MSO$_{2\%}$	44.2 ± 0.95^{e}	38.3 ± 1.1^{e}	38.1 ± 0.72^{e}	33.4 ± 1.13^{e}	128 ± 6.43^{b}
Urea $_{75\%\ RD}$+MSO$_{4\%}$	57.3 ± 0.87^{c}	51.2 ± 0.95^{c}	51.2 ± 0.92^{c}	45.0 ± 1.37^{c}	90.1 ± 4.62^{d}
Urea $_{75\%\ RD}$+ZE$_{R1}$	48.8 ± 0.66^{d}	41.5 ± 0.68^{d}	42.6 ± 0.61^{d}	37.6 ± 0.74^{d}	115 ± 11.2^{c}
Urea $_{75\%\ RD}$+ZE$_{R2}$	59.6 ± 0.64^{b}	52.6 ± 0.95^{b}	53.5 ± 1.13^{b}	47.4 ± 0.81^{b}	84.7 ± 4.16^{e}
p value					
1st season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)
2nd season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)

Data are means ($n = 3$) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

Table 3
N management effect on the partial factor productivity (PFP), agronomic efficiency (AE), partial nutrient balance at harvest (PNBh), apparent crop removal efficiency at final harvest (CREMh), and N use efficiency (NUE) of plants during two seasons field experiment.

Treatment	PFP (kg tuber kg^{-1} N applied)	AE (kg tuber increased kg^{-1} N applied)	PNBh (%)	CREMh (%)	NUE (%)
1st season					
Urea $_{RD}$	117 ± 5.22^{d}	67.8 ± 3.31^{c}	40.8 ± 1.70^{f}	35.9 ± 1.90^{f}	41.1 ± 2.46^{f}
Urea $_{75\%\ RD}$+DCD	162 ± 6.23^{a}	96.6 ± 5.62^{a}	62.6 ± 3.36^{a}	56.1 ± 3.41^{a}	64.2 ± 3.20^{a}
Urea $_{75\%\ RD}$+MSO$_{2\%}$	111 ± 4.36^{d}	46.0 ± 3.19^{d}	45.2 ± 1.83^{e}	38.7 ± 2.03^{e}	43.9 ± 2.05^{e}
Urea $_{75\%\ RD}$+MSO$_{4\%}$	145 ± 2.44^{b}	79.8 ± 4.99^{b}	55.2 ± 1.97^{c}	48.7 ± 1.75^{c}	56.1 ± 2.16^{c}
Urea $_{75\%\ RD}$+ZE$_{R1}$	128 ± 4.70^{c}	62.1 ± 4.39^{c}	48.2 ± 2.91^{d}	41.7 ± 3.08^{d}	47.5 ± 3.00^{d}
Urea $_{75\%\ RD}$+ZE$_{R2}$	149 ± 3.54^{b}	83.8 ± 4.42^{b}	58.3 ± 1.01^{b}	51.8 ± 1.00^{b}	59.4 ± 1.21^{b}
2nd season					
Urea $_{RD}$	118 ± 3.10^{d}	68.1 ± 2.40^{c}	41.2 ± 1.80^{f}	36.3 ± 1.76^{f}	41.5 ± 2.30^{f}
Urea $_{75\%\ RD}$+DCD	163 ± 4.21^{a}	97.1 ± 4.62^{a}	63.1 ± 1.27^{a}	56.6 ± 2.13^{a}	64.7 ± 3.06^{a}
Urea $_{75\%\ RD}$+MSO$_{2\%}$	112 ± 6.47^{d}	46.2 ± 5.24^{d}	45.6 ± 1.92^{e}	39.1 ± 1.64^{e}	44.3 ± 2.43^{e}
Urea $_{75\%\ RD}$+MSO$_{4\%}$	146 ± 5.47^{b}	80.1 ± 3.06^{b}	55.6 ± 1.94^{c}	49.1 ± 1.71^{c}	56.6 ± 2.31^{c}
Urea $_{75\%\ RD}$+ZE$_{R1}$	127 ± 4.60^{c}	61.3 ± 3.45^{c}	48.6 ± 1.68^{d}	42.1 ± 1.95^{d}	47.9 ± 2.63^{d}
Urea $_{75\%\ RD}$+ZE$_{R2}$	152 ± 4.43^{b}	85.6 ± 5.33^{b}	58.7 ± 1.74^{b}	52.1 ± 2.34^{b}	59.9 ± 1.15^{b}
p value					
1st season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)
2nd season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)

Data are means ($n = 3$) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

and b), maturation tuber N uptake, and harvest tuber N uptake of potato were significantly ($p \leq 0.01$) influenced by N management treatments (Table 1, and 4). Averaged across two-seasons, fresh and dry weight (Mg ha^{-1}) of potato tubers were significantly greater in treatments Urea$_{RD}$ (41.1 and 6.91), Urea$_{75\%RD}$+DCD (42.7 and 7.53), Urea$_{75\%RD}$+MSO$_{2\%}$ (29.4 and 5.06), Urea$_{75\%RD}$+MSO$_{4\%}$ (38.3 and 6.41), Urea$_{75\%RD}$+ZE$_{R1}$ (33.5 and 5.60), and Urea$_{75\%RD}$+ZE$_{R2}$ (39.5 and 6.68) than control (17.3 and 3.40), respectively (Table 4). Maximum contents of chlorophyll a, chlorophyll b, and carotenoids were observed in DCD treatment, followed by Urea$_{75\%RD}$+ZE$_{R2}$ and Urea$_{75\%RD}$+MSO$_{4\%}$. While, excluding control, minimum contents were noted in Urea$_{75\%RD}$+MSO$_{2\%}$ treatment, followed by Urea$_{75\%RD}$+ZE$_{R1}$ treatment (Table 4). Tuber N uptake at maturation and tuber N uptake at harvest was higher in all N-fertilized treatments compared to control, where the highest value (142, and 165 kg N ha^{-1}, respectively) was observed in treatment Urea$_{75\%\ RD}$+DCD, followed by Urea$_{75\%RD}$+ZE$_{R2}$ (136, and 153 kg N ha^{-1}, respectively) and Urea$_{75\%RD}$+MSO$_{4\%}$ (131, and 145 kg N ha^{-1}, respectively) treatments (Table 1). Tuber N uptake at maturation and tuber N uptake at harvest did not differ significantly ($p \leq 0.01$) between Urea$_{RD}$ and Urea$_{75\%RD}$+MSO$_{4\%}$ treatments (Table 1). A significant ($p \leq 0.01$) increase in tubers-Fe accumulation was observed in treatments Urea$_{75\%\ RD}$+ZE$_{R1}$, and Urea$_{75\%RD}$+ZE$_{R2}$ compared with the other treatments (Table 1). There were no significant ($p \leq 0.01$) differences in tubers-Fe accumulation between Urea$_{75\%RD}$+MSO$_{2\%}$, and Urea$_{75\%RD}$+ MSO$_{4\%}$ treatments. Excluding control, the lowest tubers-Fe accumulation was recorded in Urea$_{75\%RD}$+DCD treatment (Table 1).

4. Discussion

Soil NH_4^+-N oxidation is the main gross N transformation in soil. Most soil NH_4^+ converts into highly mobile NO_3^- by nitrification when urea is added within a few days (Wu et al., 2017). As anticipated, the main impact of DCD, MSO, and ZE was to decrease the NH_4^+ oxidation and the net NO_3^- production rate (Fig. 1). Treatment of DCD inhibited NH_4^+ oxidation *via* deactivating AMO enzyme of AOB, making it unable to catalyse the first nitrification step (Dai et al., 2013). Abundance of AOB *amoA* gene copy number strongly ($p \leq 0.01$) increased when urea was applied, although this effect was suppressed after the application of DCD (Fig. 2). The result conformed to the previous report (Ning et al., 2018). Also, the use of MSO coated urea inhibits the conversion of N to NO_3^- by AOB (Fig. 2), probably due to having phenolic functional groups that play an essential role in delaying the nitrobacteria and urease activity

Table 4
N management effect on yield and chemical constituents of plants during two seasons field experiment.

Treatment	Tubers yield at harvest (Mg ha^{-1})		Chemical constituents (mg g^{-1} fresh weight)		
	Fresh weight	Dry weight	Chlorophyll a	Chlorophyll b	Carotenoid
1st season					
Control	17.8 ± 0.58^{f}	3.33 ± 0.62^{e}	0.71 ± 0.05^{f}	0.18 ± 0.03^{f}	0.23 ± 0.02^{f}
Urea $_{RD}$	40.3 ± 0.86^{b}	6.90 ± 0.79^{b}	1.63 ± 0.08^{c}	0.45 ± 0.03^{d}	0.43 ± 0.05^{d}
Urea $_{75\%\ RD}$+DCD	42.5 ± 0.74^{a}	7.86 ± 0.64^{a}	1.93 ± 0.05^{a}	0.84 ± 0.06^{a}	0.81 ± 0.07^{a}
Urea $_{75\%\ RD}$+$MSO_{2\%}$	29.2 ± 2.14^{e}	5.17 ± 0.43^{d}	1.18 ± 0.01^{e}	0.32 ± 0.03^{e}	0.35 ± 0.03^{e}
Urea $_{75\%\ RD}$+$MSO_{4\%}$	38.1 ± 0.86^{c}	6.37 ± 0.28^{c}	1.63 ± 0.13^{c}	0.52 ± 0.08^{c}	0.49 ± 0.07^{c}
Urea $_{75\%\ RD}$+ZE_{R1}	33.5 ± 1.06^{d}	5.52 ± 0.30^{d}	1.30 ± 0.03^{d}	0.48 ± 0.03^{cd}	0.50 ± 0.03^{cd}
Urea $_{75\%\ RD}$+ZE_{R2}	39.2 ± 2.21^{b}	6.72 ± 0.31^{bc}	1.75 ± 0.03^{b}	0.76 ± 0.06^{b}	0.72 ± 0.05^{b}
2nd season					
Control	17.4 ± 0.65^{f}	3.47 ± 0.25^{f}	0.76 ± 0.05^{f}	0.20 ± 0.02^{d}	0.24 ± 0.01^{e}
Urea $_{RD}$	41.2 ± 1.39^{b}	6.92 ± 0.46^{a}	1.61 ± 0.09^{cd}	0.49 ± 0.04^{b}	0.46 ± 0.05^{c}
Urea $_{75\%\ RD}$+DCD	43.1 ± 1.28^{a}	7.20 ± 0.65^{a}	2.02 ± 0.07^{a}	0.73 ± 0.05^{a}	0.73 ± 0.05^{a}
Urea $_{75\%\ RD}$+$MSO_{2\%}$	29.5 ± 1.53^{e}	4.95 ± 0.55^{e}	1.28 ± 0.05^{e}	0.35 ± 0.03$_{c}$	0.36 ± 0.08^{d}
Urea $_{75\%\ RD}$+$MSO_{4\%}$	38.1 ± 0.91^{c}	6.45 ± 0.36^{c}	1.68 ± 0.08^{c}	0.52 ± 0.13^{b}	0.50 ± 0.04^{c}
Urea $_{75\%\ RD}$+ZE_{R1}	33.4 ± 0.86^{d}	5.67 ± 0.57^{d}	1.43 ± 0.05^{d}	0.50 ± 0.04^{b}	0.47 ± 0.03^{c}
Urea $_{75\%\ RD}$+ZE_{R2}	39.8 ± 2.78^{b}	6.63 ± 0.46^{b}	1.90 ± 0.04^{b}	0.68 ± 0.08^{a}	0.64 ± 0.06^{b}
p value					
1st season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)
2nd season	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)	0.00 (***)

Data are means ($n = 3$) ± SD. Different letters within the each column denote significant differences between the treatments according to Fisher's least-significant difference test ($p \leq 0.01$).

(Ashraf et al., 2019). Tocopherols are a member of phenolic antioxidants that can inhibit autoxidation by reacting with singlet oxygen and scavenging free radicals. Oleic and linolenic acids as a component of MSO were shown to block ammonia monooxygenase (AMO) enzymatic pathway in *Nitrosomonas* (Subbarao et al., 2009). When a fatty acid-binding protein added to the *Nitrosomonas* culture, a main portion of the inhibitory impact was removed, indicating the reversible nature of the inhibitory impact from oleic and linolenic acids (Subbarao et al., 2008). However, the inhibition of AOB by MSO did not last long, as it disappeared after day 6 in treatment Urea+$MSO_{2\%}$ and after day 12 in treatment Urea+$MSO_{4\%}$ (Fig. 2). The results that MSO was less useful for inhibiting nitrification might be because of the secondary metabolites in MSO undergoing fast degradation or being used by soil microbes for ammonification to obtain NH_4^+. Later on, it continued to transform with nitrification and formed NO_3^- (Jumadi et al., 2020). On the other side, incorporation of ZE into soil enhances N assimilation, reduces N nitrification, increases soil absorption, and reduces N leaching from soil (Ahmed et al., 2008). NH_4^+-exchanged clinoptilolite acted as a slow-release fertilizer, wherein clinoptilolite acted as a trap for NH_4^+ that formed by the decomposing urea, and thus inhibited both NH_4^+ and NO_3^- accumulation by disrupting the bacterial nitrification (Goto and Ninaki, 1980). Furthermore, in the current study, the use of ZE led to a slight decrease in the abundance of AOB in soil (Fig. 2), possibly due to an increase in Fe availability in soil (Song et al., 2017), which is one of the secondary components of ZE. Noubactep (2011) reported that increasing Fe level could result in the suppression of microbial activity. Furthermore, Fe oxide significantly reduced the net nitrification rate, properly because of the increased inorganic N immobilization (Huang et al., 2016).

Oxidation of NH_4^+ into NO_3^- through the nitrification process increases protons concentration in the soil and decreases soil pH (Fig. 2). Elrys et al. (2020) reported that the soil pH was progressively reduced when urea fertilizer was applied. However, it was observed that NH_4^+ oxidation to NO_3^- was slow when DCD, MSO, and ZE were applied with urea. Proton is therefore released into the soil solution at a lower rate (Fig. 2), suggesting that DCD, MSO, and ZE addition could mitigate soil acidification. This confirms our hypothesis that the application of DCD, MSO, and ZE delays the nitrification process. However, this decrease in the nitrification rate when DCD, MSO, and ZE were applied with urea may lead to an increase in the soil NH_3 volatilization. Nitrification inhibition by DCD leads to a high NH_4^+-N content in the soil, making conditions suitable for NH_3 volatilization (Elrys et al., 2020). The addition of DCD led to the highest increase in soil pH (Fig. 2) because of the high potential of DCD on inhibition of the nitrification process, which led to the rise of NH_3 emission (Fig. 3). However, NH_3 volatilization in MSO and ZE treatments was low compared to DCD (Fig. 3). Ahmed et al. (2008) reported that application NH_3 volatilization was low when ZE was applied with urea compared with urea alone. The formation of NH_4^+ over NH_3 was increased and kept more NH_4^+ in the soil under the application of ZE as a result of its high CEC. On the other hand, application of DCD, MSO, and ZE with N fertilizer has the potential to mitigate N_2O emissions (Fig. 3). The cumulative N_2O emission under the urea fertilizer application alone was greater than in the control treatment (Fig. 3). Treatments of DCD, MSO, and ZE do not have a direct impact on N_2O emissions. However, it has an indirect role due to its direct impact on NH_4^+ oxidation (Müller et al., 2002). Applications of DCD and MSO might reduce oxygen (O_2) consumption in soil microsites by suppressing nitrification, thus suppressing the N_2O emissions *via* denitrification (Wu et al., 2017). This result is consistent with former studies reported that DCD is highly active for decreasing N_2O emissions at different soil situations (Qiao et al., 2015). There was substantial repression of N_2O flux from corn fields in soils with a combination of N fertilizer with DCD, which reduced N_2O flux compared with urea (Jumadi et al., 2008). The addition of DCD, MSO, and ZE inhibit NO_3^- production (Fig. 2) and thereby inhibits the occurrence of the denitrification, which might lower N_2O emissions (Wu et al., 2017). The retention of NH_4^+ on the cation exchange sites of the ZE might partly explain how the urea with ZE mixture in granule form could reduce N_2O production compared with urea without ZE (Jumadi et al., 2020). Park and Komarneni (1997) stated that ZE could lower the emission of N_2O up to 50% in corn fields. Another reason for the mitigation of N_2O emission by ZE is likely linked with its increased Fe availability. Zhu et al. (2013) suggested that Fe^{3+} level is a sensitive factor in regulating N_2O emissions. Consequently, the positive and significant abating effect on N_2O emissions under the application of DCD, MSO, and ZE with N fertilizer indicates the potential use of these compounds as a N_2O abating strategy.

According to our results, the positive effects of DCD, MSO, and ZE on reducing NO_3^- losses led to increased potato N uptake and, therefore, increased potato N fertilizer recovery (Tables 1, 2, and 3). The results agree with those obtained by Yang et al. (2016). They reported in a meta-analysis that DCD had a high effect on altering soil inorganic N content, thus improving NUE and plant production. Also, Ashraf et al. (2019) reported that the use of MSO coated urea increased maize N

recovery. On the other hand, the pronounced selectivity of clinoptilolite for large cations, such as NH_4^+, has been exploited to enhance the N-retention ability of the soils by promoting a slower release of NH_4^+ for uptake by plants. In the rice fields, where NUE was less than 50%, Minato (1968) stated a 63% increase in the quantity of available N in a highly permeable paddy soil four weeks after ZE addition along with N fertilizer. Moreover, the application of Si (as a component of ZE) enhanced NUE. Because of the synergistic effect, Si can raise the N uptake, resulting from enhancing plant production (Pati et al., 2016). The increased NUE indexes, including reduction of N surplus that used as an indicator of N loss as NO_3^- or N_2O in different agroecosystems (Venterea et al., 2016), further imply that applied DCD, MSO, and ZE with 75% of the recommended dose of N fertilizer could reduce reactive N loss to the environment. Use of DCD, MSO, and ZE not only reduces the N losses in terms of nitrification, but also increased the potato tuber yields as well as chemical constituents (carotenoid, chlorophyll a, and chlorophyll b) (Table 4), which was due to sufficient availability of N and/or slow release of urea at later stages of the crop which reduce the N losses and thus provide better NUE by potato crop (Ashraf et al., 2019). This finding is consistent with previous report of Pasda et al. (2001). Furthermore, tocopherols in MSO act as growth regulators that help the plant grow well and increase production (Sadiq et al., 2019). The application of α-tocopherol was reported as a potential factor of promoting plant resistance to stressful environments (Sadiq et al., 2019). For ZE, it can release nutrients, so it is expected that the nutrients given through fertilization can be bound by ZE and not easily lost before being used by plants to increase fertilizer efficiency (Widyanto et al., 2013). Furthermore, the increase in the availability of Si could increase potato yields (Table 4) by promoting photosynthesis (Table 4), improving the plant resistance to attacks by biotic and abiotic stresses (Elrys et al., 2018b). The current findings also showed that applied DCD, MSO, and ZE with urea fertilizer could improve potato quality by reducing NO_3^- accumulation in tubers (Table 1). These findings agree with the results reported by Elrys et al. (2018a). However, a significant ($p \leq 0.01$) decrease in tubers NO_3^- accumulation was recorded when DCD, MSO, and ZE were applied with urea (Table 1), and this can be attributed to preventing the process of nitrification by DCD, MSO, and ZE (Fig. 2), where NH_4^+-N retention continued in the soil under the application of DCD, MSO, and ZE. Moreover, the NR enzyme activity was promoted under the application of ZE with urea (Table 1). These findings are consistent with those approved by Ashfaque et al. (2017), who reported that Si application as a component of ZE was significantly increased the NR activity in plants. Si indirectly inhibits enzyme degradation by protein activation because the mRNA of NR enzymes are regulated by protein (Ferrario-Mery et al., 1998). Also, NR activity was increased in treatment $Urea_{75\%RD}+MSO_{4\%}$ (Table 1). This increase may be due to the secondary ingredients in the MSO that increase the NR enzyme (Elrys et al., 2019b).

5. Conclusions

Producing high quality potato for human consumption under increasing demand and to maximize profits in the agricultural sector is of major importance. This study recommends using DCD, MSO, and ZE with N fertilizer as an excellent strategy to increase the potato N recovery and minimize NO_3^- accumulation in potato. The mechanisms resulted from decreasing the NH_4^+ oxidation rate and hence the net NO_3^- production rate through suppressing AOB growth by DCD and MSO, while through the retention of NH_4^+ on the cation exchange sites of the ZE, leading to prevent the nitrification process and therefore keeping NH_4^+-N for a long time in the soil. For the highest yield of high-quality potatoes with low potential for environmental N losses, the current study recommended reducing the recommended N fertilizer rate (350 kg N ha^{-1}) for potatoes by 25%, while at the same time application of DCD or coating urea by MSO (4% of the N fertilizer use) or addition 1.0 Mg ZE ha^{-1}. According to the obtained results, the demand for Egyptian potato crops will increase in international markets, especially Europe. The agricultural, economic, and environmental benefits of DCD, MSO, and ZE could contribute to the higher sustainability of potato cropping system.

CRediT authorship contribution statement

Ahmed S. Elrys: Conceptualization, Visualization, Investigation, Supervision, Writing - review & editing. **Mohamed F. Abo El-Maati:** Methodology, Software. **Enas Mohamed Wagdy Abdel-Hamed:** Methodology, Software. **Safaa M.A.I. Arnaout:** Methodology, Software. **Khaled A. El-Tarabily**: Conceptualization, Visualization, Investigation, Supervision, Methodology, Software, Writing - review & editing. **El-Sayed M. Desoky**: Conceptualization, Methodology, Software, Visualization, Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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