Vegetable Crops Prefer Different Ratios of Ammonium-N and Nitrate-N in the Growth Media

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ABSTRACT

Understanding the preference of a crop for forms of nitrogen (N) is important to ensure efficient use of N-sources in agriculture. A study was conducted to assess nutrient uptake and dry matter yield of six vegetable crops under different ratios of ammonium-N (NH₄⁺-N) and nitrate-N (NO₃⁻-N) in growth medium. Seedlings of cabbage, carrot, capsicum, knolkhol, lettuce, and tomato, were supplied with five N-treatments (NH₄⁺-N: NO₃⁻-N in the ratios of 0:100, 25:75, 50:50, 75:25 and 100:0) under controlled environment in sand culture. Six weeks after initiating treatments plants were harvested and dry matter recorded. Plants were analysed for total N, P and K contents. Dry matter yield and N and K concentrations in shoot tissues in response to N-treatments were significantly different (p<0.05) among crops. Tomato and knolkhol showed high dry matter yield and N content when supplied with NO₃⁻-N than NH₄⁺-N. The highest dry matter yield for cabbage was obtained with equal supply of NO₃⁻-N and NH₄⁺-N than supplying either form alone. Dry matter yield and N concentration in shoots of lettuce, capsicum and carrot were not significantly affected (p>0.05) by N-treatment. Supplying NH₄⁺-N alone significantly reduced K concentration in shoot tissues in knolkhol, capsicum and tomato. This study revealed that performance of vegetable crops is affected by the NH₄⁺-N: NO₃⁻-N in the growth medium. This knowledge can be utilized to optimize fertilizer-N usage in vegetable cultivation.

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INTRODUCTION

Ammonium (NH₄⁺) and nitrate (NO₃⁻) are the plant available N forms in soil. Under aerobic soil condition (unsaturated soil) NH₄⁺ is readily converted to NO₃⁻ via a microbiologically mediated nitrification process. Nitrate is the predominant plant available N form under unsaturated soil conditions, which account for more than 80% of available N pool (Sylvia et al., 2005). Nitrate is an anion present mostly in soil solution and has a high potential to leach out from soil or to be lost through denitrification becoming unavailable for plant uptake (Sylvia et al., 2005). Application of N-fertilizers along with nitrification inhibitors, split application of N fertilizers and the use of slow-release N fertilizers have been practiced to retard NO₃⁻ releasing rate to soil solution and these practices have improved N use efficiency and crop productivity under field conditions (Slagen and Kerkhoff, 1984; Rodgers, 1986; Upadhyya et al., 2011; Kemal and Workie, 2015; Herrera et al., 2016). However, the nitrogen use efficiency, quality of harvest and crop productivity have been found to depend on NH₄⁺:NO₃⁻ ratio (Serna et al., 1992; Palaniswamy et al., 2002; Heebe, 2005; Omari and Nihri, 2015) and the optimum ratio appears to be crop specific (Kafkafi, 2009).

A study conducted with different ratios of NH₄⁺:N:NO₃⁻ on canola under salt stress condition revealed that supplying N with 50:50 ratio of NH₄⁺:N:NO₃⁻ protected plants from salinity stress (Bybordy, 2012). The NH₄⁺:N:NO₃⁻ ratio in the growth medium is known to affect qualitative aspects of the crop. Supplying most of the N in the form of NH₄⁺ improved sweetness, taste and flavours of tomato (Heeb, 2005), reduced oxalic acid content in purslane leaves (Palaniswamy et al., 2002), and improved fatty acid content in canola (Bybordy 2012). Serna et al. (1992) showed that when NH₄⁺ proportion in the N supply was increased, phosphorus, magnesium, ferrous and copper contents of leaves increased while calcium, potassium, manganese and zinc contents decreased. Sensitivity of crop to the NH₄⁺:N:NO₃⁻ ratio is an important factor to consider when deciding the degree of nitrification inhibition required and the type of N fertilizer to be applied for optimizing N fertilizer use efficiency. By knowing the plant preferences for NH₄⁺:N and NO₃⁻, it would be possible to develop better nutrient management practices to improve nutrient use efficiency of crops. Therefore, this study was conducted with the objective of assessing the effect of the form of N on dry matter yield and nutrient uptake of six popular vegetable crops in Sri Lanka. This was achieved by using cabbage, carrot, capsicum, knolkhol, lettuce and tomato as target crops grown at different NH₄⁺:N: NO₃⁻ ratios in a controlled environment.

METHODOLOGY

When selecting vegetable crops for the study, information was gathered for popular vegetable crops in relation to demand for N (Lathif and Yapa, 2010), N removal with harvest (unpublished data), the land extent under cultivation (Statistics of Ministry of Agriculture, 2016) and suitability of the crop to grow at Gannoruwa, Sri Lanka (WM2b agro-ecological zone). Accordingly, cabbage, carrot, capsicum, knolkhol, lettuce and tomato were selected and the varieties used for the study were F1 Hybrid AS39, Kuroda, CAB, Early White Vianna, Grand Rapid and T 246, respectively. Daily mean temperature during experimentation period was 29 ± 2 °C.

Preparing planting materials and establishing pot experiment

Acid washed and sterilized sand was used for establishing nurseries and as the anchoring medium for the pot experiment in greenhouse. Nurseries were established for cabbage, capsicum, knolkhol, lettuce and tomato in sand beds prepared in trays. In preparing planting materials, seedlings were grown supplying nutrients using quarter strength Albert’s solution®, a commercial liquid fertilizer mixture (Unipower (PVT.) Ltd. USA) which included ammonium-N (0.2 %) and nitrate-N (10.3%) as the N sources. At the end of the nursery period uniformly grown and vigorous seedlings were uprooted, the roots were immersed in Captan® (commercially available fungicide) and transplanted in the pots. The double pot method was used for the pot experiment. A 500 ml pot, which was perforated on sides and the bottom served as the planting compartment and placed in a pot with a larger diameter, which served as the nutrient reservoir. The planting compartment was filled with 500 g of 2 mm sieved, acid washed and sterilized sand. The liquid fertilizer mixture was applied to the nutrient reservoir and the perforated walls facilitated free nutrient movement into planting compartment. Two selected seedlings from nurseries were transplanted per pot in sand filled planting compartment. For each crop 25 pots were prepared. For all crops, except carrot, 500 ml of half strength Albert’s solution® was applied per pot for one-week period until plants got stabilized in the pots. Then plants were thinned out leaving one plant per pot and N treatments were imposed. Unlike other crops, carrot was not transplanted. Hence, carrot was direct seeded in the sand filled planting compartment of each pot at the rate of 10
seeds /pot at the time of establishing nurseries for other crops. Then pots containing carrot seeds were initially supplied with quarter strength Albert’s solution® for two weeks followed by half strength Albert’s solution® for 4 weeks. At this stage, carrot seedlings were thinned out leaving one healthy, uniform seedling per pot and the N treatment was commenced in parallel to other crops.

**N treatment**

Hoagland fertilizer solution (Hoagland and Arnon, 1950) was used as the basis for preparing liquid fertilizer medium. The composition of the Hoagland solution was 200 ppm of N, 235 ppm of K, 200 ppm of Ca, 31 ppm of P, 64 ppm of S, 48 ppm of Mg, 0.5 ppm of B, 1.0 ppm of Fe, 0.5 ppm of Mn, 0.005 ppm of Zn, 0.02 ppm of Cu, and 0.01 ppm of Mo. Five N treatments were developed to supply 200 ppm of N either using NH₄NO₃ or NO₃-N as the sole form, or as mixtures of both NH₄+ and NO₃- in different proportions, while keeping all other nutrients at constant levels. The five N treatments corresponding to the percentage of each form to supply 200 ppm of N when presented as a ratio of NH₄+NO₃ were N₀:100, N₂5:75, N₅₀:50, N₇5:25 and N₁₀₀:0. Potassium nitrate, ammonium hydroxide and ammonium nitrate of analytical reagent (AR) grade were used to prepare the N-treatments. All five fertilizer mixtures were supplemented with dicyandiamide (DCD), a synthetic nitrification inhibitor, at a rate of 10 mg/kg of sand culture to ensure that the plants experienced the intended NH₄+:NO₃ ratio in each treatment. The pH of the liquid fertilizer medium was adjusted between 6.5 to 7.0 using diluted sodium hydroxide and hydrochloric acid. Nutrient solution compartment of each pot was filled with 500 ml of Hoagland solution with respective N treatment and this was renewed twice a week to supply the plants with intended NH₄+:NO₃ levels for a period of 6 weeks for every crop. The solution level was topped-up regularly with distilled water to make it 500 ml until the next turn of re-fill. The N-treatment was applied in five replicates per crop and pots were arranged in a complete randomized design (CRD) in the greenhouse. Pots were randomly re-arranged once a week to avoid environmental variables in the greenhouse.

**Crop harvesting, data collection and plant sample analysis**

Crops were harvested after the vegetative phase. Shoots and roots were carefully harvested and gently washed with tap water to remove soil and dust particles. Then they were dried in distilled water, drained and extra moisture was removed using paper towels. Shoots and roots were separated from all crops except for lettuce, in which whole plant was used for the analysis. The samples were oven dried at 60 °C until a constant weight and brittle texture was gained. Finally, dry weights of all plant samples were measured. Dried samples were ground to a fine powder using a mortar and a pestle, labelled and stored for further analysis in sealed polythene bags. Before nutrient analysis, all plant samples of cabbage were destroyed due to a malfunctioning of the thermal control of the oven. Hence, only the shoot dry weight of cabbage crop is reported herein.

Shoot and root samples were analysed separately for total N for knolkhil, carrot, capsicum, and tomato and as the whole plant for lettuce after wet acid digestion following Kjeldahl method (Jackson, 1958). Remaining plant samples were digested with nitric acid (Jackson, 1958; Huang, 2004) and the extract was used to determine P colourimetrically (Olsen and Sommers, 1982) at 470 nm absorption using a spectrophotometer (Jenway 6305 UV/VIS Spectrometer), and K following the method described by Baruah and Barthakur (1997) using flame photometer (BWB Technologies-Flame photometer). Parameters related to nutrient concentrations, uptake and recovery for N, P and K were calculated using following equations (equations 1 to 5).

\[
\text{Nutrient concentrations (X)} = \text{mass of nutrient per unit dry matter (mg/kg)}
\]

**equation 1**

\[
\text{Nutrient content in shoots (Y)} = X \times \text{shoot dry matter of a plant (mg/plant)}
\]

**equation 2**

\[
\text{Nutrient content in roots (Z)} = X \times \text{root dry matter of a plant}
\]

**equation 3**

\[
\text{Total nutrient uptake (mg/plant)} = Y + Z
\]

**equation 4**

\[
\text{Nutrient recovery} = \frac{(\text{Total nutrient uptake} - \text{Total nutrient added to plant}) \times 100}{\text{Total nutrient added to plant}}
\]

**equation 5**

**Statistical analysis**

A two factor factorial design with generalized linear model (GLM) procedure was used in conducting analysis of variance (ANOVA) to assess the significance of the effect of N form/s in the growth medium (N treatment) on dry matter yield and nutrient uptake of crops. Crop and N treatment were used as the two groups in factorial analysis. When the main effect or the interaction effect was
significant the mean comparison was performed with Tukey mean separation technique. The correlations between different parameters were assessed using Pearson’s correlation test at $p<0.05$. Statistical analyses were performed with Minitab 17 Statistical Software (2010) [State College, PA: Minitab, Inc. (www.minitab.com)].

RESULTS AND DISCUSSION

Plants assimilate nitrogen in the forms of NH$_4^+$ and/or NO$_3^-$. The dry matter yield of roots and shoots, nutrient accumulation and tissue nutrient concentration in studied crops were significantly affected by the NH$_4^+$:NO$_3^-$ ratio in the growth medium (Table 1). Changes in dry matter yield and uptake of N and K with the ratio of NH$_4^+$:NO$_3^-$ in the growth medium has been reported previously for vegetable crops (Ikeda and Osawa, 1981; Marti and Mills, 1991; Song et al., 2000; Tian et al., 2003; Zhang et al., 2010; Nasraoui et al., 2011). According to previous studies, preference of a crop for NH$_4^+$ and NO$_3^-$ could change with crop growth stage (Ganmore-Neumann and Kafkari, 1985; Marti and Mills, 1991; Cui et al., 2017). It should be noted that in this study all crops reached the end of vegetative period. In lettuce, the crop is considered matured and harvested at the end of vegetative phase. Performance of a crop during vegetative stage is important for its performance at reproductive stage.

Dry matter yield

The effect of N treatment on dry matter yield was not consistent among the studied crops at the end of the six-week period as indicated by the significant interaction effect between N-treatment and crop type (Table 1 and Figure 1). Total dry matter yield of lettuce and carrot during vegetative stage were not significantly affected by NH$_4^+$:NO$_3^-$ in the growth medium. Scaife et al. (1986) showed that lettuce can be grown satisfactorily at very low NO$_3^-$ content while supplying ammonium sulphate and inhibiting nitrification. In the same study, they stated that tip burn was less serious with plants grown with NH$_4^+$:N nutrition than those with NO$_3^-$. However, Tian et al. (2003) reported that NO$_3^-$ is the most suitable form of N for lettuce. Differences in growth conditions, especially the temperature and growth duration and varietal differences may have contributed to contrasting observations related to lettuce (Ganmore-Neumann and Kafkafi, 1985; Song et al., 2011; Cui et al., 2017). Ganmore-Neumann and Kafkafi (1985) observed that preferential uptake of NH$_4^+$:N and NO$_3^-$ change with root temperature in addition to the crop growth stage. In the present study, dry matter yield of knolkhol and tomato were high under high NO$_3^-$ (NH$_4^+$:N:NO$_3^-$:N, 0:100) in the growth medium, whereas cabbage had the highest dry matter yield at 50:50, NH$_4^+$:N to NO$_3^-$N ratio. Nasraoui et al. (2011) observed that tomato plants preferred NO$_3^-$ while NH$_4^+$ inhibited its growth. Song et al. (2001) reported that supplying N with 25% NH$_4^+$ in a nutrient solution increased plant height, stem diameter and dry matter of Chinese cabbage (Brassica pekinensis) (Brassica oleracea) and therefore, this suggests that there can be differences among crop species on how they respond to NH$_4^+$:N:NO$_3^-$N in the growth medium. In the present study, although the treatment effect was not significant ($p>0.05$) the dry matter yield of capicum grown under 50% and 75% NH$_4^+$:N in the growth medium resulted in numerically higher dry matter yield than other treatments (Figure 1).

Nitrogen nutrition of plant

Nitrogen nutrition of different crops varied with NH$_4^+$:N:NO$_3^-$ ratio in the growth medium. Tissue N concentration was significantly affected by crop type, N-treatment and their interaction while N content in shoot and total N uptake were significantly affected only by N-treatment and interaction effect (crop type and N-treatment) (Table 1). Nutrient accumulation is governed by two main factors that are growth status or dry matter yield and nutrient availability (Devine-Barret et al., 2000). In the present study, the treatment effects on nutrient uptake by crops were further analysed by considering the nutrient accumulation in shoot. Nitrogen content in shoots of carrot, lettuce and knolkhol were not significantly affected by NH$_4^+$:N:NO$_3^-$ ratio (Table 2). Tomato and capsicum showed variation in shoot N content according to NH$_4^+$:N:NO$_3^-$ ratio in the growth medium (Table 2). The concentration of N in shoot was affected only in tomato in which the highest concentration was achieved with 50:50 of NH$_4^+$:N:NO$_3^-$ ratio (Figure 2 a.).

However, shoot N content in tomato was highest under 0:100 of NH$_4^+$:N:NO$_3^-$ ratio and it was lowest under 100:0 of NH$_4^+$:N:NO$_3^-$ ratio when compared to other treatments (Table 2). The highest N content in capicum shoots was observed in 100:0, NH$_4^+$:N:NO$_3^-$ ratio (Table 2). Ikeda and Osawa (1981) showed that the preference between NH$_4^+$:N and NO$_3^-$N in N absorption was different among vegetables in a study conducted with 20 different vegetables. In their study, lettuce assimilated more N in the form of NH$_4^+$ while cabbage and tomato assimilated NO$_3^-$ more dominantly.
Table 1: Analysis of variance of the effects of crop type, nitrogen form and their interaction on dry matter yield, nitrogen, phosphorus and potassium nutrition of the six crops considered.

| Dry matter yield |             |             |             |
|------------------|-------------|-------------|-------------|
| Effect           | Shoot dry weight | Root dry weight | Total dry weight |
| Crop             | ***         | ***         | ***         |
| N-treatment      | ***         | ***         | ***         |
| Crop×N-treatment | ***         | ***         | ***         |
| CV%              | 97.2        | 93.9        | 75.4        |
| R² (%)           | 85.2        | 68.2        | 83.4        |

| N nutrition      | Tissue N Concentration | N content in shoot | Total N Uptake |
|------------------|------------------------|--------------------|---------------|
| Effect           |                        |                    |               |
| Crop             | ***                    | ***                | ***           |
| N-treatment      | ***                    | ***                | ***           |
| Crop×N-treatment | ***                    | ***                | ***           |
| CV%              | 23.4                   | 97.8               | 73.1          |
| R² (%)           | 80.9                   | 88.4               | 80.9          |

| P nutrition      | Tissue P Concentration | P content in shoot | Total P Uptake |
|------------------|------------------------|--------------------|---------------|
| Effect           |                        |                    |               |
| Crop             | ***                    | ***                | **            |
| N-treatment      | ***                    | Ns                 | Ns            |
| Crop×N-treatment | ***                    | *                  | Ns            |
| CV%              | 79.9                   | 100.8              | 178.0         |
| R² (%)           | 73.6                   | 53.3               | 38.1          |

| K nutrition      | Tissue K Concentration | K content in shoot | Total K Uptake |
|------------------|------------------------|--------------------|---------------|
| Effect           |                        |                    |               |
| Crop             | ***                    | Ns                 | *             |
| N-treatment      | ***                    | Ns                 | Ns            |
| Crop×N-treatment | ***                    | Ns                 | Ns            |
| CV%              | 27.4                   | 70.4               | 65.3          |
| R² (%)           | 91.5                   | 63.55              | 46.4          |

The effects followed by *, **, and *** are significant at p value <0.05, <0.01 and <0.001, respectively. Ns=Not significant (p>0.05). R² indicates goodness of fit of model.

A study by Heeb (2005) suggested that NH₄⁺-N as an equivalent N source for tomato compared to NO₃⁻-N and NH₄⁺-N improved sweetness, acidity and flavo of tomato. A study conducted by Scaife et al. (1986) indicated that lettuce absorb more N in the presence of high concentration of NH₄⁺-N. Similar observations have been made with rice varieties and strawberry indicating presence of NH₄⁺-N in the growth medium promoting NO₃⁻-N uptake by plant (Ganmore-Neumann and Kafkafi, 1985; Kronzucker et al., 1999).
Phosphorus and potassium contents in tissue

N treatment, crop type and their interactions differently affected contents and concentrations of P and K in shoots (Table 1). Significant effect of N treatments on shoot P content was observed for tomato and carrot crops only (Table 2 and Figure 2b). Total K uptake was significantly affected by crop type only (Table 1). Tissue K concentration and K content in shoot tend to be higher when plants were supplied with NO$_3$- in the growth medium for knolkhol and tomato (Figure 2c and Table 2). Antagonistic interaction between K$^+$ and NH$_4$-N during crop nutrient uptake is well established (Marti and Mills, 1991; Zhang et al., 2010). However, K accumulation in shoots of capsicum (Table 2) and tissue K concentration of carrot (Figure 2c) indicated that there may be exceptions for the interaction between K$^+$ and NH$_4$-N in nutrient uptake of crops during vegetative growth. Ammonium-N inhibit the activity of high affinity K$^+$ transport system but not the low affinity K$^+$ transport system in plant roots during K$^+$ uptake. Looking into partitioning of N, P and K into shoots and roots with different N-treatments should be done to arrive at valid conclusions. More K$^+$ translocation to leaves under NH$_4$-N nutrition compared to that under NO$_3$-N nutrition has been observed previously (Zhang et al., 2010). Due to limitation of sample weights K and P analyses could not be performed for root samples in the present study.

In general, the nutrient recovery in this study remained less than 28 % with average recovery of N, P and K amounting to 5 %, 8 % and 5 % respectively. There was no significant effect of crop type, N-treatment or their interaction on nutrient recovery (p >0.05). The nutrient recovery values suggest that nutrient supply was not limited for crop throughout the duration of the pot experiment. Therefore, the observed effects could be attributed to the effect of crop type, N-treatment (form/s of N supplied) and their interaction effect.
Table 2: Nitrogen, phosphorus and potassium content in shoot with different nitrogen treatments

| NH₄⁺:NO₃⁻ | Lettuce* | Tomato | Carrot | Capsicum | Knolhhol |
|-----------|---------|--------|--------|----------|----------|
| 0:100     | 29.9 a  | 167.6 a | 36.1 a | 26.8 ab  | 124.9 a  |
| 25:75     | 53.1 a  | 80.0 b  | 42.7 a | 38.4 ab  | 150.2 a  |
| 50:50     | 48.7 a  | 101.6 b | 30.4 a | 13.3 b   | 44.8 a   |
| 75:25     | 49.8 a  | 73.1 b  | 32.7 a | 15.9 b   | 45.1 a   |
| 100:0     | 42.1 a  | 36.5 b  | 49.1 a | 49.4 a   | 18.4 a   |

CV%        | 15.1    | 78.3    | 13.09  | 38.12    | 48.2     |

Coefficient of variation (CV%) and goodness of fit of model (R²) are also provided. *Lettuce analysed as whole plant
Figure 2: Concentration of (a) N, (b) P and (c) K in shoot tissues of four vegetable crops and whole plant tissues of lettuce at the end of vegetative stage as affected by proportions of ammonium-N and nitrate-N in the growth medium.
CONCLUSIONS

Six crops used in this study showed different responses towards N, P and K nutrition of plants upon subjecting to different ratios of NH₄NO₃-N in the growth medium. The performance of carrot, capsicum and lettuce did not change with the form of N in the growth medium during vegetative growth of the crop. However, tomato, knolkhol and cabbage were sensitive to varying proportions of NH₄NO₃-N levels in the growth medium. Tomato and knolkhol performed better under higher NO₃-N levels. Cabbage preferred equal supply of NH₄-N:NO₃-N for dry matter yield.

It can be concluded that different vegetable crops respond differently towards NH₄-N:NO₃-N in the growth medium during vegetative phase. It is important to further investigate how this response during vegetative phase could impact crop yields and the nutritional quality of the yields and the nitrogen use efficiency of the crop.

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