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Research article

Nitrogen use efficiency and critical leaf N concentration of *Aloe vera* in urea and diammonium phosphate amended soil

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ABSTRACT

Aloe vera L. is widely cultivated in many countries due to its importance as an all-purpose herbal or medicinal plant. The growth and yield of this plant can be enhanced by application of fertilizer. It is expected that a higher and balanced nutrient supply will result in higher crop production maintaining soil health, which is possible when the applied fertilizers are done in way that is efficient. So, there is a need to understand the amount of applied and type of fertilizer that will give the best output for farmers and to formulate economical market products. This study was conducted to investigate the effect of N fertilizer on leaf yield, its uptake and requirement, critical concentration, use efficiency and economics of *Aloe vera* L. Plants were grown at six levels of N: 0, 40, 80, 100, 150 and 200 kg ha^{-1} from urea and diammonium phosphate (DAP) following completely randomized design with three replicates under field condition. The highest values of yield and yield attributes and profit based on benefit cost ratio (3.81 for urea and 2.91 for DAP) were obtained with 150 kg N ha^{-1} (urea) and 100 kg N ha^{-1} (DAP). Leaf biomass yield increased by 18–128 % in urea-N and 30–139 % in DAP-N fertilized plant over control while DAP > urea by 7.59 %. Sucker production (mean number) was urea-N (4.95 $Plant^{-1}$) > DAP-N (2.28 $Plant^{-1}$). Both gel and leaf N concentration and uptake was highest at 200 kg ha^{-1} for both sources. For 80 % leaf biomass yield, minimum requirement of N was *ca* 74.90 (urea) and 89.60 kg ha^{-1} (DAP). Growth and yield parameters to N application exhibited significant and positive correlations. Critical leaf N concentration was *ca* 0.88% (DAP) and 0.90% (urea) while mean and maximum NUE was 34% and 64 % (urea) and 43% and 69% (DAP), respectively. Farmers can be advised to apply N at the rate of 150 kg ha^{-1} from urea for producing economically higher yield and better-quality *A. vera* leaves.

1. Introduction

Aloe vera L. is an important member of Liliaceae family and genus *Aloe* (Nie et al., 2018; Cock, 2015; Hasanuzzaman et al., 2008; Reynolds, 2004) widely cultivated in many countries due to its importance as an all-purpose herbal or medicinal plant. Due to this, the plant is sometimes referred as a “miraculous plant”, ‘the wonder plant’, ‘plant of immortality’ and ‘nature powder’ (Lanka, 2018; Akev et al., 2015). It leaves of this plant contain fat compounds, carbohydrates, proteins, lipids, and 18 essential amino acids, vitamins (e.g., A, C, E, vitamin B12, folic acid), minerals, glycoprotein, C-glucosylchromone, anthraquinones, emodin, salicylic acid and various kinds of enzymes (Lanka, 2018; Hamman, 2008; Surjushe et al., 2008). Also contained in the plant are secondary metabolites including alkaloids, aloins, lectins, lignin, saponins, tannins, phenolic and glukomannan are also present in the plant (Boudreau and Beland, 2006; Darini et al., 2013). *A. vera* is familiar for using as functional food supplement and preservative of foods for the presence of antioxidant molecules, high amount of carbohydrates, and vitamins as its constituents (Gupta et al., 2020a). The plant has wide use in cosmetology and medicine (Lanka, 2018; Cock, 2015; Akev et al., 2015; Eshun and He,

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2004; Hamman, 2008). *A. vera* extract can also be used for the perturbation of enzymatic and nonenzymatic antioxidative indices levels in rats (Gupta et al. 2019, 2020b).

As the nutrient uptake and crop yields are the principal factors that determine optimal fertilization practices (Ju and Christie, 2011), it is expected that a higher and balanced nutrient supply will result in higher crop production maintaining soil health. This is possible when the applied fertilizers are done in way that is efficient way. Thereby minimizing loss of nutrient and improving its efficiency (Li et al., 2009). Nitrogen (N) is a key nutrient for plant growth and playing vital role in plant biochemical processes associated with amino acid, protein, enzymes, and chlorophyll molecule (Abbas and Fares, 2009). In soil, nitrogen enhancing plant growth will result in increase in leaf area and number of leaves and having direct impact on vegetative and reproductive phases of plants (Zhang et al., 2014; Ibrahim et al., 2011; Acquaah, 2005; Fageria and Baligar, 2005). In the atmosphere, by composition N is the largest and yet remains the most limiting in most of the plants. The mechanism by which N is imputed in soil from the atmosphere includes non-symbiotic and symbiotic fixation, and rainfall in addition (Sullivan et al., 2014). The natural soil N input mechanism does not significantly support food production globally as there is a continuous population growth (Crews and Peoples, 2004), therefore, the use of N from synthetic fertilizer sources is highly necessary. Nitrogen generally determines crop yield and when applied in excess, will lead to low N use efficiency (NUE). Researchers such as Fageria and Baligar (2005), Hirel et al. (2007) and Garnett et al. (2009) have developed methods in improving the low NUE beyond the environmental, agronomic, and breeding perspectives. These methods involved genetic variability and quantitative genetics as well as using specific root phenotypes such as root morphology; root to shoot ratios; root vigour, root length density; and root N transport and metabolism. Two widely used source of N include Urea and DAP. They are preferable by farmers for their crop cultivation. These fertilizers when applied in soil are hydrolyzed to produce NH_4^+. Though DAP is known to give poor yields in calcareous soils where a greater portion of insoluble reaction products may be prevalent (Tisdale and Nelson, 1970), soil released substantial amounts of NH^+-N for relatively longer period from DAP than urea amended soil (Mohiuddin et al., 2006). The ammonium ion present in DAP enhances phosphorus (P) uptake by altering soil pH and P solubility near the plant root (Soon and Miller, 1971). Albeit the huge potential of commercial cultivation of *A. vera* in Bangladesh for both domestic and international market, efforts is still very limited, due to information gap regarding fertilizer requirement, nutritional values, and marketing. Critical leaf N concentration, NUE and N requirement for the cultivation of *A. vera* is yet to be reported in Bangladesh. The comparative performance of urea and DAP as a source of N for *A. vera* cultivation with respect to leaf biomass yield, its content and use efficiency have also not been reported. The objectives of the study were to determine N requirement, NUE and critical leaf N concentration and economics for the growth and yield of *A. vera* in urea and DAP amended soil.

2. Materials and methods

2.1. Experimental site

The pot experiment was carried out with *A. vera* in the farmer's nursery, *Kashiganj*, Tarakanda, Mymensingh, Bangladesh during September 2018 to May 2019. Geographically the experimental site was located at 24°75′26.5″ N latitude and 90°50′12.2″ E longitude at an elevation of 18 m above the sea level. The site belonged to the Non-calcareous Dark Grey Floodplain soil under the Agro-Ecological Zone of Old Brahmaputra Floodplain and classified as Cambisols according to World Reference Base (AEZ-9) (IUSS WG WRB, 2015). The climate of the experimental area is under the sub-tropical climatic zone, which is characterized by moderate to high temperature, heavy rainfall, high humidity and relatively long day during *kharif* (April to September) and scanty rainfall, low humidity, low temperature and short-day period during *rabi* season (October to March).

2.2. Experimental set up

Non-calcareous soil was collected from 0-15 cm depth of the selected area, made free from plant residues and other extraneous materials, air dried, grinded, and sieved through a 2 mm sieve, 500 g was separated and preserved. Soil pH was measured using a glass-electrode pH meter, the soil water ratio being 1: 2.5 as described by Saha et al. (2018). Organic C was determined following wet digestion method (Nelson and Sommers, 1982). The amount of organic matter was calculated by multiplying the per cent organic carbon with the van Bemmelen factor 1.73 (Piper, 1950). Micro-Kjeldahl method (Bremner and Mulvany, 1982) was used to measure soil total N. Soil available P was extracted by $NaHCO_3$ (pH 8.51) solution and determined colorimetrically using molybdate blue ascorbic acid method (Olsen and Sommers, 1982). Exchangeable K, Ca and Mg was extracted by ammonium acetate extraction method (Coleman et al., 1959) and determined by flame photometer as outlined by Knudsen et al. (1982). Calcium and Mg concentration was determined by complexometric method of titration using Na_2EDTA as a complexing agent (Page et al., 1982). Available S was extracted by $CaCl_2$ solution and determined turbidimetrically using $BaCl_2$ crystals (Fox et al., 1964). Available B was extracted following a hot water extraction method (Page et al., 1982) and determined by spectrophotometer using azomethine-H (Keren, 1996). Available Cu, Fe, Mn and Zn was extracted following DTPA extraction method and measured by atomic absorption spectrophotometer (Model UNICAM 969, England) (Lindsay and Norvell, 1978). The soil was silty loam in texture, bulk density, particle density and field capacity were 1.46, 2.59 g cm^{-3} and 27.24%, respectively. Organic matter, pH, total N, exchangeable K, Ca and Mg, were 1.05%, 5.90, 0.06%, 0.13, 3.32 0.78, meq $100g^{-1}$, respectively. Available P, S, Zn and B were 3.00, 4.00, 1.81, 0.06 μg g^{-1} soil, respectively. Ten kg processed soil was taken in each plastic pot of 30 cm in height with 24.50 cm diameter at the top and 20 cm diameter at the bottom. The pot was filled by soil leaving 2 cm from the top and labeled with proper tagging. Six level of N was applied at the rate of 0 (N_0), 40 (N_{40}), 80 (N_{80}), 100 (N_{100}), 150 (N_{150}) and 200 (N_{200}) kg ha^{-1} from urea and DAP. Urea was applied in 3 installments (one-third during land preparation and rest at 60 days after transplanting (DAT) and 120 DAT and DAP in 2 installments (half during soil and pot preparation and half at 60 DAT). Other nutrients P, K, S, Zn and B were applied as basal dose at the rate of 80, 120, 40, 3 and 1 kg ha^{-1} from TSP, MoP, gypsum, zinc sulphate and boric acid, respectively as prescribed by Biswas (2010). For DAP fertilizer, rest amount of P was adjusted from TSP. Experiment was set up following completely randomized design (CRD) with three replications. The test crop used in the experiment was *Aloe vera* L. belonging to the family Liliaceae and sub-family Asphodeloideae. Eighteen-month-old *A. vera* seedlings were collected from *Oshudhi* village, Natore Sadar, Natore and one seedling per pot was transplanted. Intercultural operations were done as and when necessary.

2.3. Harvesting and cleaning

A. vera leaf was harvested at 14 days interval up to 178 DAT. Leaves were collected carefully and cleaned with tap water followed by distilled water to remove soil and other foreign materials. Paper towel was used to remove adhering water.

2.4. Growth and yield parameters

To understand the growth and development of *A. vera* plant, specific growth and yield parameters were studied. These have been briefly described under the following heads.

2.5. Plant height

Height of the individual leaf of each plant was measured in centimeter (cm) from ground to the apex of leaf. The average plant height was recorded at 14, 28, 58, 88, 118, 148 and 178 DAT.

2.6. Leaves plant^{-1}

Total number of leaves was counted and recorded at 14, 28, 58, 88, 118, 148 and 178 DAT.

2.7. Leaf area plant^{-1}

After harvest, 3 leaves were randomly selected from each pot during harvest and leaf area was obtained by multiplying leaf length and breadth (expressed in cm^2).

2.8. Leaf biomass yield and dry leaf weight

Fresh leaf biomass yield and weight of dried leaf after air drying, sun drying and oven drying at 60 °C for 48 h were recorded and expressed in g plant^{-1}.

2.9. Fresh and dry gel weight

Fresh and dry gel weight after air drying, sun drying and oven drying at 60 °C for 48 h were recorded and expressed in g plant^{-1}.

Preparation of *A. vera* leaf for chemical analyses: For preparing the extraction, the fresh leaf was chopped, washed, and cut from the middle. The gel was separated by scraping it with a spoon. Then the gel and chopped leaves were sun dried for 2 days. Sun dried leaf and gel was oven dried at 70 °C for 48 h and ground, preserved in polythene bag and kept in desiccators.

N determination: Total N was determined by Kjeldahl method (Page et al., 1982). Powdered leaf and gel samples were digested with conc. H_2SO_4 in presence of K_2SO_4 catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: Se = 10:1:0.1). Nitrogen in the digest was collected by distillation with NaOH followed by titration of the distillate trapped in H_3BO_3 indicator solution with standard H_2SO_4.

N uptake: Uptake was calculated from N concentration using the following formula (1) (Maniruzzaman et al., 2017)

$$\text{N Uptake (mg pot}^{-1}) = \frac{\text{N concentration (\%)}}{100} \times \text{ Dry weight (mg pot}^{-1}) \quad (1)$$

N use efficiency (NUE): NUE was calculated using the following formula (2) (Daradjat et al., 1991)

$$\text{NUE (\%)} = \frac{N\ uptake\ in\ leaf\ (gpot^{-1})}{N\ applied\ (gpot^{-1})} \times 100 \quad (2)$$

Relative yield: Relative yield was calculated using the following formula (3) (Fageria et al., 2010)

$$\text{Relative yield (\%)} = \frac{\text{Yield of control or treated pot}}{\text{Maximum yield of treated pot}} \times 100 \quad (3)$$

Critical N concentration and N requirement: The critical N concentration in *A. vera* leaf was estimated from the relative amount of leaf biomass to achieve 80% of the maximum production of leaf biomass yield (Kouno and Ogata, 1988). The relative leaf biomass yield was plotted on the ordinate (Y axis) against the respective N concentration of leaf on the abscissa (*X axis*). For N requirement estimation, the applied N was plotted on the *X axis* against the relative leaf biomass yield on the Y axis.

Economic analysis: The cost of production was analyzed per hectare basis to find out the most economic dose of N fertilizer. All input cost included the cost for lease of land and interests at the rate of 8% for one year on running capital. The market price of *A. vera* leaf and sucker were considered for estimating the cost and return. The benefit cost ratio (BCR) was calculated as follows in Eq. (4) (Tarafder et al., 2020)

$$\text{BCR} = \text{Gross return per ha}^{-1}\ \text{(Tk.)/Total cost of production ha}^{-1}\ \text{(Tk.)} \quad (4)$$

Statistical analyses: Data were tabulated and analyzed using statistical software Minitab 2017 Version 17.0 (Minitab Inc, USA). The means for all the treatments were calculated and analysis of variance (ANOVA) for all the characters under consideration was performed by Tukey's range test to determine the significant difference between groups. Overall statistical analysis of the present study was done following Gomez and Gomez (1984).

3. Results

3.1. Plant height

Different N rates had significant effect on plant height of *A. vera* (Figure 1). An increase in plant height was observed from planting stage to harvesting irrespective of treatments. Nitrogen application at all levels increased plant height by 4.91–11.13 cm and 2.56–9.91 cm in case of urea-N and DAP-N, respectively. Tallest plant was obtained when N was applied at the rate of 150 kg ha^{-1} from urea and 100 kg ha^{-1} from DAP and the shortest plant was from control. The mean plant height at harvest

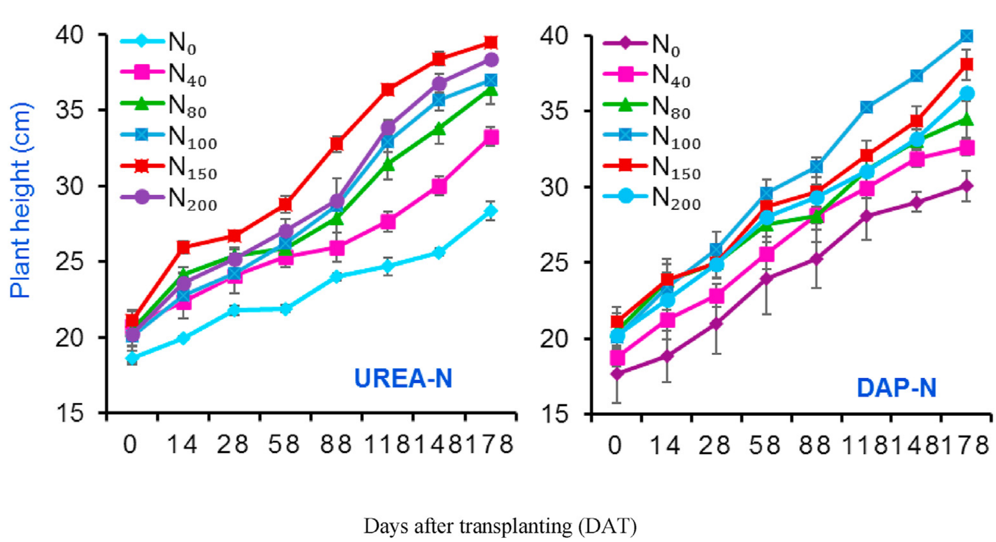

Figure 1. Effects of different levels of N from urea and DAP on the plant height of *A. vera*. Bars indicate standard error (±SE) at 0.05.

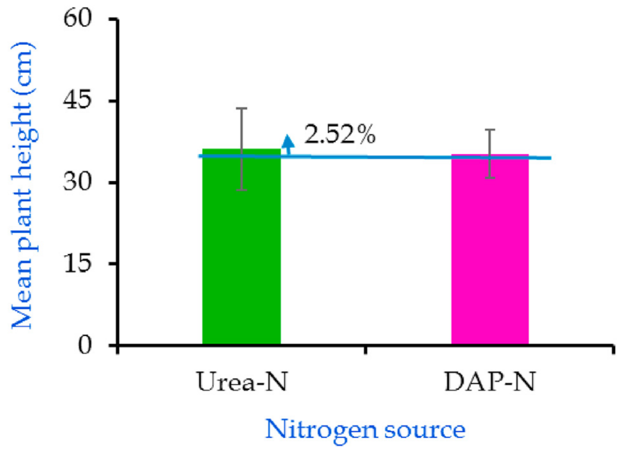

Figure 2. Comparative performance of urea-N and DAP-N on the plant height of *A. vera.* Bars indicate standard error (±SE) at 0.05.

was 36 cm in case of urea-N and 35 cm in case of DAP-N (Figure 2). However, there were no significant differences ($p > 0.05$) between them.

3.2. Number of leaves plant^{-1}

Leaf number responded significantly due to the application of different levels of N (Figure 3). The result revealed that number of leaves plant^{-1} progressively increased with the increasing levels of N application up to 150 kg ha^{-1} in case of urea-N and 100 kg ha^{-1} in case of DAP and then declined. The application of N influenced the number of leaves plant^{-1} variably from 15 to 178 DAT irrespective of fertilizers and treatments. Rapid increase in leaf number was observed between 28 and 178 DAT in both fertilizers. The highest number of leaves plant^{-1} (16.67 in urea and 18.00 in DAP) at 178 DAT was counted from the plant receiving 150 kg N ha^{-1} from urea and 100 kg N ha^{-1} from DAP. On the other hand, lowest plant height was obtained from the control treatment which might be due to no N application. Average 14.83 leaves were obtained from urea-N treated plant and 15.91 leaves were obtained from DAP-N treated plant (Figure 4).

3.3. Leaf biomass yield

The leaf biomass yield of *A. vera* varied significantly due to the application of different levels of N fertilizer (Figure 5). The highest leaf biomass yield pot^{-1} was measured from the plant receiving 150 kg N ha^{-1} from urea (1952 g) and 100 kg N ha^{-1} from DAP (2015 g) that were significantly higher than other levels of N. The lowest values were obtained from the control. Nitrogen application at all levels increased leaf biomass yield at harvest by 18–128% in urea-N and 30–139% in DAP-N, respectively over control. All doses showed significant differences ($p < 0.05$) for both urea-N and DAP-N and from the controls.

Comparatively 7.59% higher mean leaf biomass yield was obtained by the application of DAP-N than urea-N (Figure 6). Nitrogen application at all levels increased leaf biomass yield on average 92% in urea-N and 89% in DAP-N, respectively over control.

3.4. Total leaf area and number of suckers

The data pertaining to the total leaf area and number of suckers plant^{-1} at harvest as influenced by different levels of N have been presented in Table 1. Leaf area plant^{-1} responded significantly ($p < 0.05$) due to the application of different levels of N. Results revealed that the highest total leaf area plant^{-1} at harvest was measured from the plant receiving 150 kg ha^{-1} from urea-N (3714 cm^2) and 100 kg ha^{-1} from DAP-N (3994 cm^2) which was significantly ($p < 0.05$) higher than other levels of N. The lowest leaf area was found from the control treatment. The number of suckers also significantly and progressively increased with the increased levels of N application up to 200 kg ha^{-1} in urea (9.67) and 150 kg ha^{-1} in DAP (4.00) and then declined with further addition. The lowest number of suckers was found from the control. Mean number of sucker production was more than double in urea-N fertilized soil (4.95 Plant^{-1}) than DAP-N (2.28 Plant^{-1}). Comparatively 117% higher number of suckers was obtained by the application of urea-N than DAP-N (Figure 7).

3.5. Fresh and dry gel weight

The fresh gel weight of *A. vera* plant^{-1} at harvest varied significantly due to the application of different levels of N fertilizer (Table 1). The highest fresh gel weight plant^{-1} (1252 g in urea-N and 1335 g in DAP-N) at harvest was found from the plant receiving 150 kg N ha^{-1} from urea-N and 100 kg N ha^{-1} from DAP-N which was significantly higher than other levels of N. The lowest fresh gel weight was found from the control treatment. The fresh gel weight was higher in case DAP-N applied pot than urea-N. Dry gel weight of *A. vera* plant^{-1} at harvest varied significantly (Table 1). The highest dry weight plant^{-1} (19.85 g in urea-N and 21.98 g in DAP-N) at harvest was measured from the plant receiving 150 kg N ha^{-1} from urea-N and 100 kg N ha^{-1} from DAP-N and the lowest

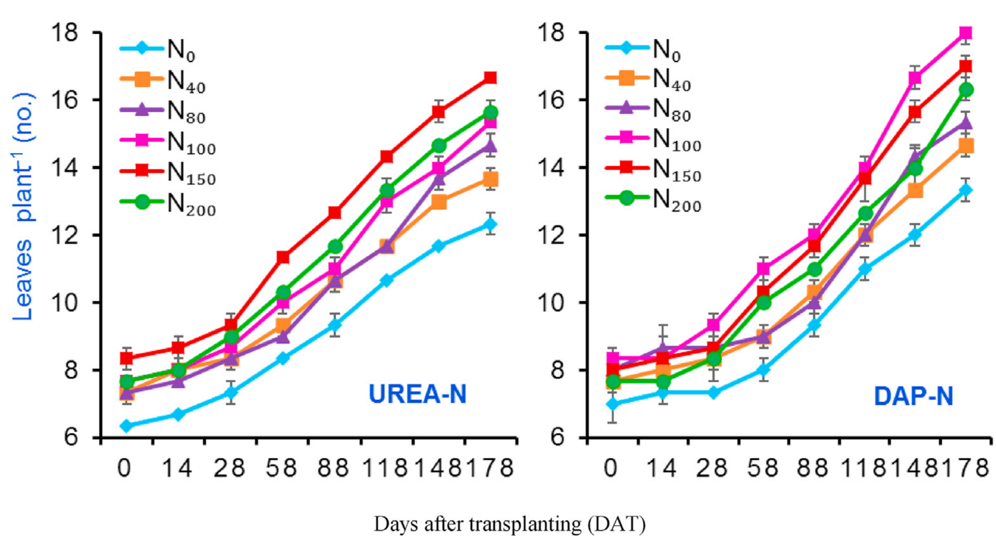

Figure 3. Effects of different levels of N from urea and DAP on the number of leaves of *A. vera.* Bars indicate standard error (±SE) at 0.05.

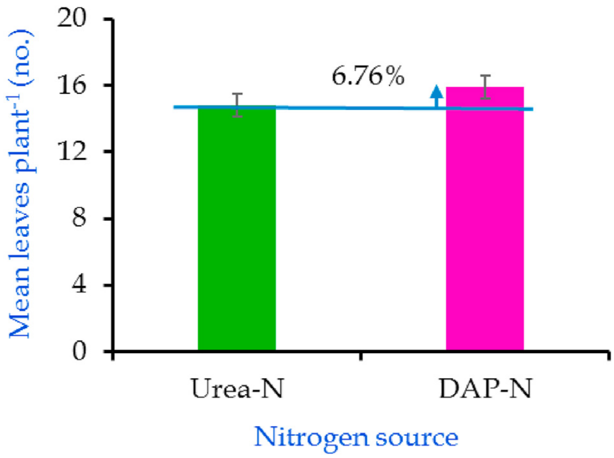

Figure 4. Comparative performance of urea-N and DAP-N on the number of leaves of *A. vera*. Bars indicate standard error (±SE) at 0.05.

values were obtained from the control treatment. The dry gel weight was higher in case DAP-N applied pot than urea-N.

3.6. Gel N concentration

There was a significant effect ($p < 0.05$) of different levels of N on its concentration in *A. vera* gel (Table 2). Nitrogen concentration of the gel was increased with the increased levels of N irrespective of fertilizers used. The highest N concentration (0.62% in urea-N and 0.68% in DAP-N) was obtained when N was applied at the rate of 200 kg ha^{-1} which was significantly higher than other levels of N. The lowest N concentration was obtained from the plants receiving no N in case of both fertilizers. The gel N concentration of the plants grown in the pot fertilized with urea was lower the N concentration of the plants grown in the pot fertilized with DAP.

3.7. Leaf N concentration and uptake

Different levels of N exerted significant influence on its concentration and uptake by *A. vera* leaf (Table 2). Nitrogen concentration of the leaf was increased with the increased levels of N irrespective of fertilizers used. The highest N concentration (1.17% in urea-N and 1.44% in DAP-N) was obtained when N was applied at the rate of 200 kg ha^{-1} which was significantly higher than other levels of N. The lowest N concentration was obtained from the plants receiving no N in case of both

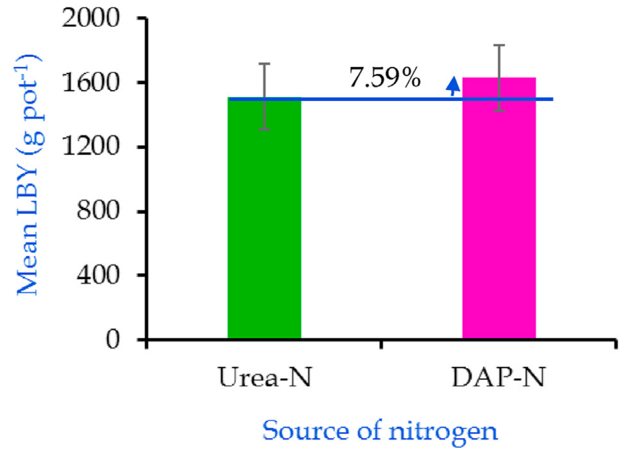

Figure 6. Comparative performance of urea and DAP on the leaf biomass yield of *A. vera*. Bars indicate standard error (±SE) at 0.05.

fertilizers. The leaf N concentration of the plants grown in the pot fertilized with urea was lower than N concentration of the plants grown in the pot fertilized with DAP. The effects of different levels of N on its uptake were significant (Table 2). The trend was similar like N concentration of *A. vera* leaf. Mean leaf N concentration was more than double (0.93%) compared to gel-N concentration (0.44%) irrespective of the fertilizer sources (Figure 8). Comparatively 110% higher N concentration was obtained in the leaf compared to gel of *A. vera*.

The N uptake was maximum (893.64 mg pot^{-1} in urea-N_{200} and 828.90 mg pot^{-1} in DAP-N_{200}) which showed no significant difference ($p > 0.05$). Similarly, when N was applied at the rate of 150 kg ha^{-1} from both urea and DAP, there were also no significant difference ($p > 0.05$). Nitrogen uptake as expected was increased with N levels up to 200 kg ha^{-1}. In contrast, the lowest content and uptake of N was obtained from control treatments.

3.8. Correlation studies among yield and yield attributes of Aloe vera L

Statistical relationships between leaf biomass yield (LBY) with plant height and leaf area, LBY and fresh gel weight of A. vera were studied (Figure 9). The results revealed that plant height and total leaf area were significantly and positively correlated with leaf biomass yield having the correlation coefficients (r) of 0.91** and 0.90**, respectively. Fresh gel weight was also significantly and positively correlated ($r = 0.98$) with LBY.

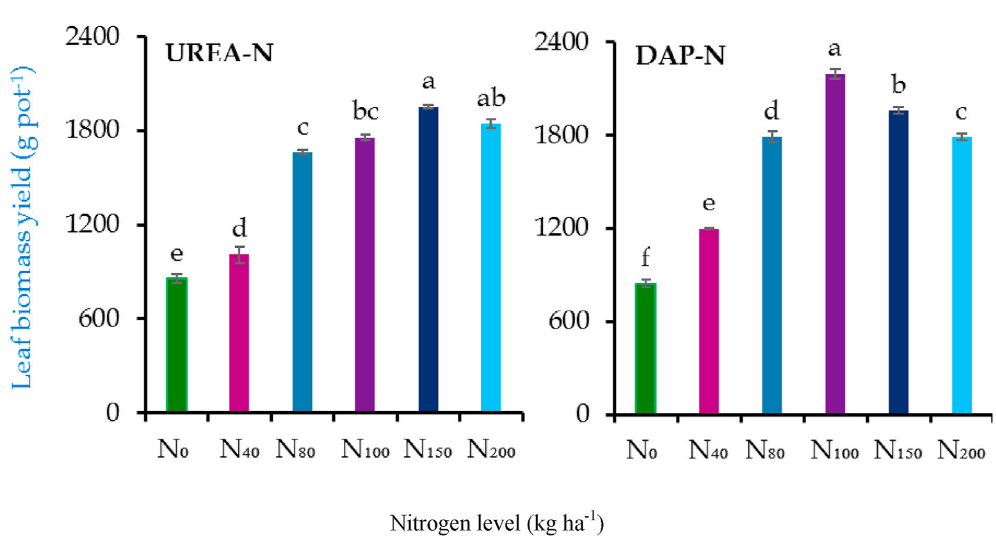

Figure 5. Effects of different levels of N from urea and DAP on the leaf biomass yield of *A. vera*. Bars indicate standard error (±SE) at 0.05.

Table 1. Effects of different levels of N from urea and DAP on total leaf area, number of suckers, fresh gel weight, dry gel weight and leaf biomass yield increase over control of *Aloe vera* L.

N level (kg ha^{-1})	Total leaf area plant^{-1} (cm^2)		Suckers pot^{-1} (No)		Fresh gel weight (g pot^{-1})		Dry gel weight (g pot^{-1})		*LBY increase over control (%)	
	Urea	DAP	Urea	DAP	Urea	DAP	Urea	DAP	Urea	DAP
N_0	1844e	1664e	1.33d	0.33c	484e	534e	7.44c	8.68f	_	_
N_{40}	2348d	2252d	2.67cd	1.00bc	671d	695d	9.14c	10.17e	18	30
N_{80}	2659cd	2614c	3.67cd	1.67b	973c	941c	14.80b	15.05d	94	63
N_{100}	3084b	3995a	6.33ab	3.67a	1088b	1335a	16.81b	21.98a	105	139
N_{150}	3714a	3182b	6.00c	4.00a	1252a	1256a	19.85a	19.73b	128	121
N_{200}	2784bc	2858c	9.67a	3.00a	1161b	1062b	17.41ab	17.41c	116	92
SE	83.89	63.93	0.70	0.17	13.45	17.87	0.45	0.24	-	-
CV (%)	5.42	4.18	33.09	37.27	3.53	4.87	7.40	3.22	-	-

* LBY = Leaf biomass yield; CV = Coefficient of variance, SE± = Standard error of means. Values with the same alphabet in column are not significantly different at 5% level of probability.

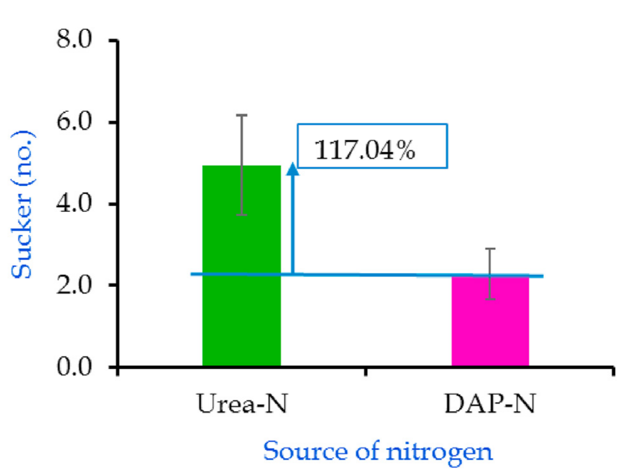

Figure 7. Comparative performance of urea and DAP on the mean sucker number of *A. vera*. Bars indicate standard error (±SE) at 0.05.

3.9. Critical leaf N concentration of A. vera

From Figure 10, the N concentration corresponding to the arbitrary point at 80% to achieve the maximum leaf biomass production was estimated by the fitted curve to be *ca* 0.90 and 0.88% in *A. vera* leaf grown in urea-N and DAP-N treated pot, respectively.

3.10. Nitrogen requirement for Aloe vera L

From the fitted curve used in determining the N requirement, it was revealed that the corresponding estimated minimum amount of N for 80% leaf biomass production in the plant grown in urea-N and DAP-N treated pot was estimated to be *ca* 74.90 and 89.60 kg ha^{-1}, respectively (Figure 11).

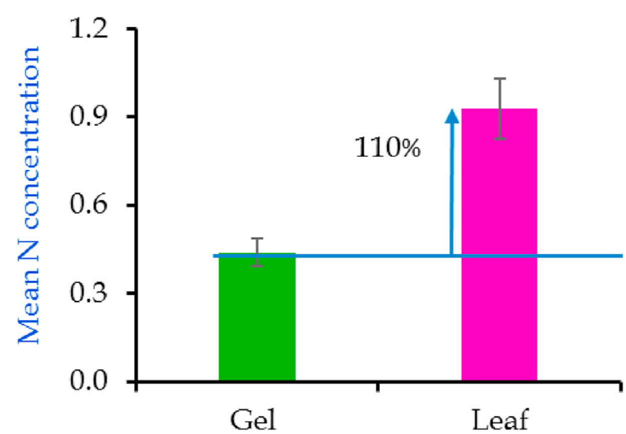

Figure 8. Mean N concentration of *A. vera* gel and leaf as influenced by urea and DAP. Bars indicate standard error (±SE) at 0.05.

3.11. Nitrogen use efficiency

The nitrogen use efficiency of *A. vera* as influenced by different levels of N is shown in Figure 12. The result revealed that, in case of DAP-N, the highest value of NUE (69%) was recorded in N_{100} and the lowest in N_{80}. But in case of urea-N, the highest NUE (55%) was obtained from 80 kg N ha^{-1} and lowest at the rate of 200 kg N ha^{-1}. Mean NUE was higher in DAP (42.47%) fertilized soil than urea (33.88%) and that was almost 25.35% higher than urea (Figure 13).

Table 2. Effects of different levels of N from urea and DAP on the gel and leaf N concentration and its uptake by *Aloe vera* leaf.

N level (kg ha^{-1})	Gel N conc. (%)		Leaf N conc. (%)		Leaf N uptake (mg pot^{-1})	
	Urea	DAP	Urea	DAP	Urea	DAP
N_0	0.17c	0.21e	0.35e	0.46d	159.41d	142.63b
N_{40}	0.38b	0.27de	0.73d	0.58cd	273.78cd	237.61b
N_{80}	0.46ab	0.36cd	0.88cd	0.74c	469.02bc	321.05b
N_{100}	0.52ab	0.49bc	1.09bc	1.11b	683.37ab	792.92a
N_{150}	0.55ab	0.57ab	1.15ab	1.20a	739.69a	739.49a
N_{200}	0.62a	0.68a	1.37a	1.44ab	893.64a	828.90a
SE	0.04	0.03	0.04	0.05	43.48	46.70
CV (%)	15.61	14.59	8.19	10.19	15.11	15.18

CV = Coefficient of variance, SE± = Standard error of means. Values with the same alphabet in column are not significantly different at 5% level of probability.

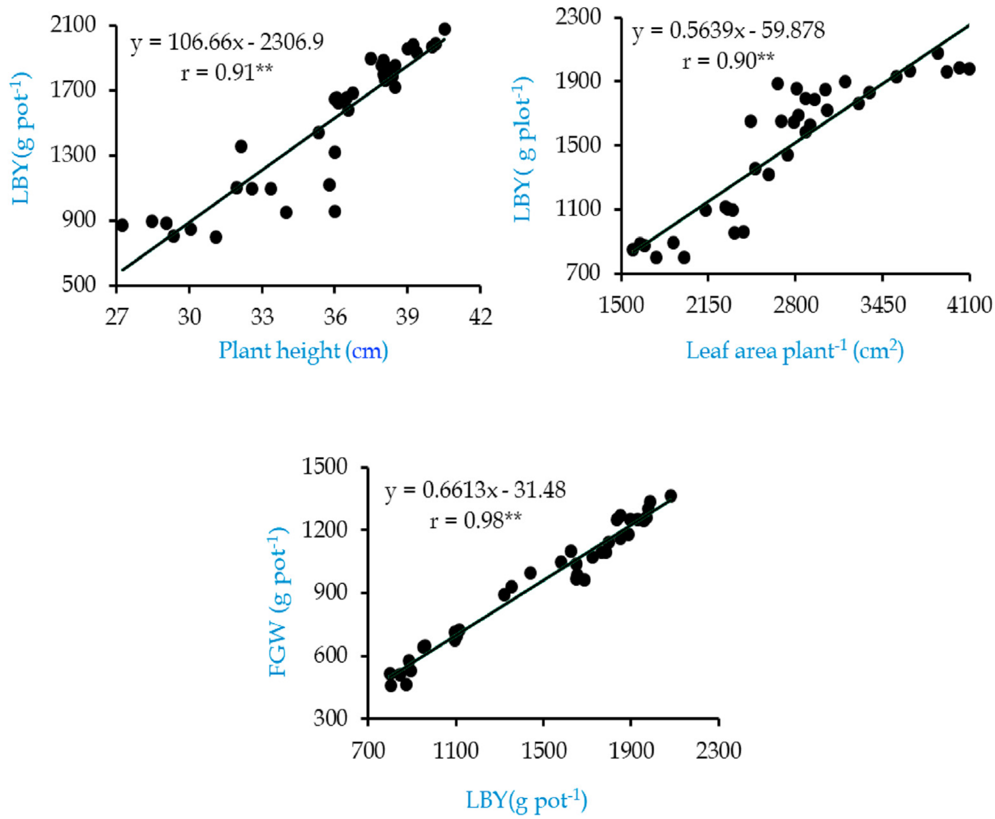

Figure 9. Correlations and regression equations between leaf biomass yield (LBY) with plant height and leaf number, fresh gel weight (FGW) with LBY as influenced by different levels of N from urea and DAP.

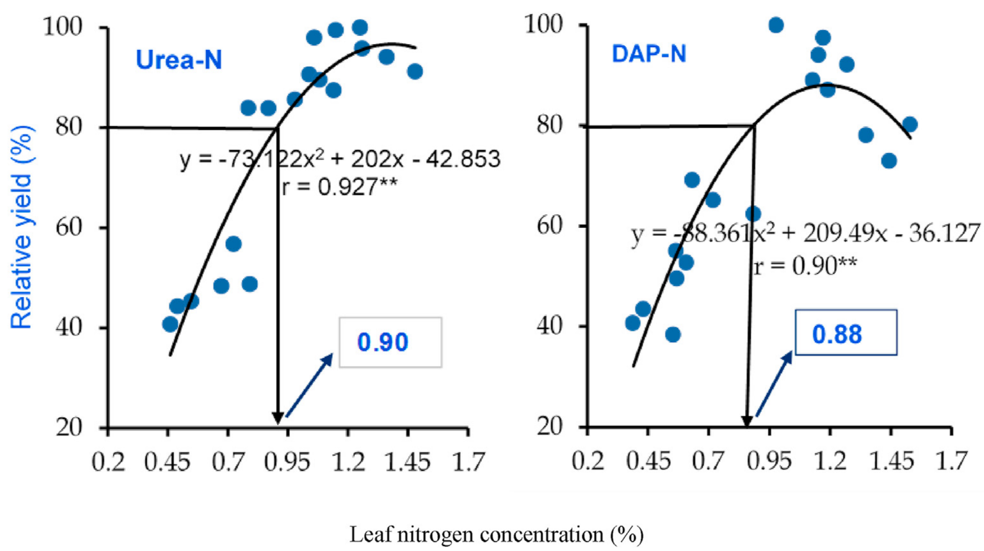

Figure 10. Correlation between leaf N concentration and relative leaf biomass yield of *A. vera*.

3.12. Economic analysis

Net return of different treatments of N from urea and DAP (Table 3) showed that net return varied significantly among N levels and source. The highest net return (Tk. 14,48,060 or USD 17,076 in Urea-N and Tk. 9,91,866 or USD 11697 in DAP-N) was found from the plant receiving 150 kg N ha^{-1} from urea and 100 kg N ha^{-1} from DAP which was significantly higher than other levels of N. The net return showed significant differences ($P < 0.05$) for different urea-N treatment except for N_0 to N_{40} while DAP showed only significant differences ($p < 0.05$) for N_0, N_{100}, N_{150} and N_{200}. In contrast, the lowest net return was obtained from the control. Mean net income of (Tk. 8,58,650) was generated from urea-N fertilized plant compared to (Tk 6,89,638) from DAP-N fertilization from one hectare of land. Comparatively 24.51% higher net return was obtained by the application of urea than DAP (Figure 14).

Data in Table 3 revealed that benefit cost ratio of *A. vera* significantly influenced by the different levels of N. In case of urea treatment, N_{150}indicated the highest benefit cost ratio (3.81). But in case of DAP treatment, N_{100} showed highest benefit cost ratio (2.91).

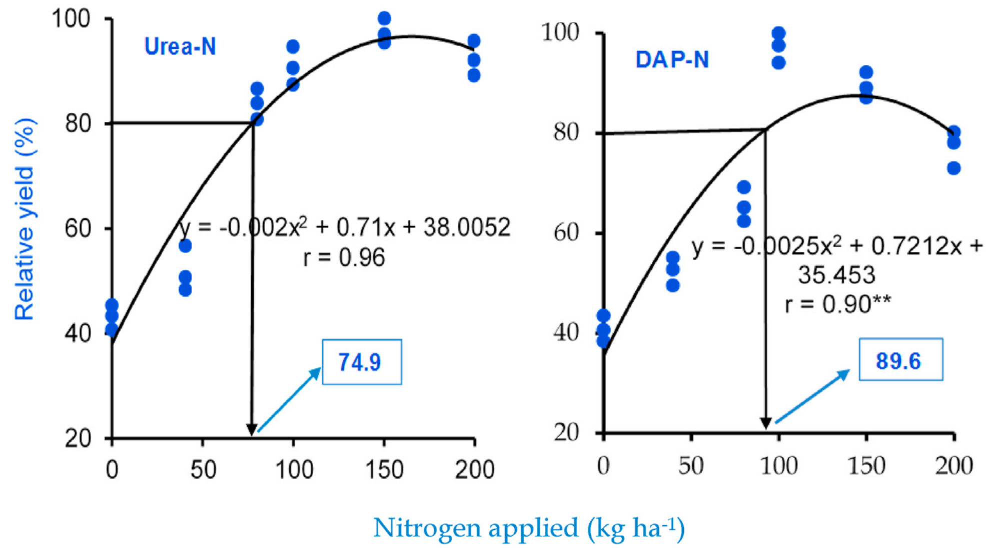

Figure 11. Correlation between applied N and relative leaf biomass yield of *A. vera* L.

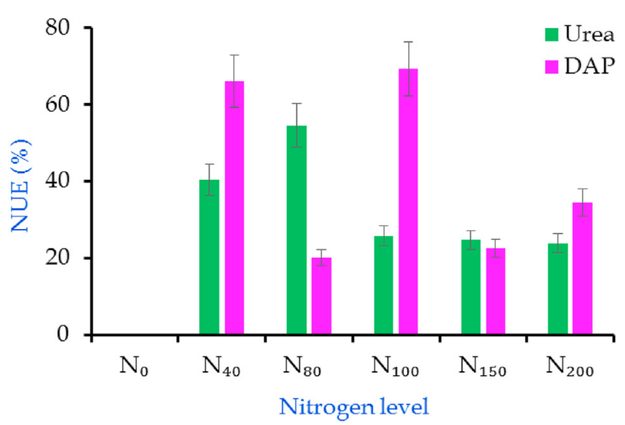

Figure 12. Effect of different levels of N from urea and DAP on NUE of*A. vera*. Bars indicate standard error (±SE) at 0.05.

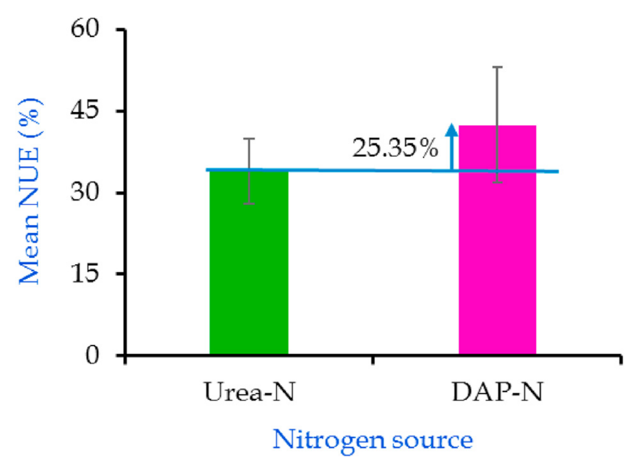

Figure 13. Comparative performance of urea and DAP on mean NUE of *A. vera*. Bars indicate standard error (±SE) at 0.05.

4. Discussion

Plants that are efficient in absorption and utilization of the absorbed nutrients greatly enhance the efficiency of applied fertilizers. It is expected that a higher and balanced nutrient supply will result in higher yield of *A. vera*. So, there is a need to understand the amount of applied and type of fertilizer that will give the best output for farmers and to formulate economical market products.

The plant height was generally higher than control (Figure 1), while urea-N > DAP-N (Figure 2). The reasons of obtaining higher plant height from urea-N than DAP-N might be due to maximum uptake of N from urea for its quick availability in these levels. This result was supported by previous studies by Jasso-Chaverria et al. (2005), Waseem et al. (2008) and (Barandozi et al., 2011), they found that the improvement of vegetative growth with increased N fertilizer rate attributed to increased uptake of N and its associated role in photosynthesis and carbon dioxide assimilation. Further, Maniruzzaman et al. (2016) reported the influence of the highest N level (N_{300}) to produce the tallest stevia plant (88 cm) in acid soil whereas N_{250} (94 cm) in non-calcareous soil. Hejazi et al. (2013) reported that 250 kg ha^{-1} N had the highest values of leaf length of Artichoke (*Cynarascolymus* L.). Egbuchua and Enujeke (2015) showed that at 50 and 75 kg N ha^{-1} application rates, leaf length of *A. vera* increased from 17.21 cm to 18.82 cm as compared to the control. However, contrasting results was obtained by Dastagir and Hussain (2015), when they found better plant height with the application of DAP than that of urea.

The treatments showed increasing number of leaves plant^{-1} due to the applied fertilizers, which could possibly be ascribed to the fact that N often increases plant growth and subsequently more production of leaves. In a related study, Egbuchua and Enujeke (2015) showed a significant influence of N application for the number of leaves of *A. vera* and the highest number of leaves (10.30) was found with highest application rate (75 kg N ha^{-1}). Almost similar result was reported by Allahdadi and Farzane (2018) who found the maximum number of leaf plant^{-1} of Artichoke plant (*Cynarascolymus* L.) was recorded by 200 kg N ha^{-1}. Maniruzzaman et al. (2016) also recorded maximum number of leaves of stevia plant^{-1} with N_{250} which was significantly higher than all other N levels. This could be due to the slow nutrient releasing capacity of DAP than urea. Succinctly, the splitting of N applications can improve N use efficiency because the greatest need is in the phase of fastest mass growth, i.e. typically well into the vegetative phase. Ideally the timing and proportion of the N split(s) matches the demand. This also limits the risk of denitrification and leaching. The plant height and leaf number were better in the application of 150 kg N ha^{-1} compared to other N application rate. This might be due to better synchronization of N supply according to plant demand which is crucial for better growth and development of plants. Moreover, the optimum supply of N from 150 kg

Table 3. Comparative economic per hectare profitability of *Aloe vera* L. as influenced by different levels of N from urea and DAP.

N level (kg ha^{-1})	Urea		DAP		BCR	
	Net return (Tk.)	Net return (USD)	Net return (Tk.)	Net return (USD)	Urea	DAP
N0	351247d	4142d	361747d	4266d	1.69d	1.71d
N40	601730d	7096d	504594c	5950c	2.18d	1.98c
N80	663214cd	7821cd	605442c	7140c	2.29cd	2.17c
N100	1050955b	12393b	991866a	11697a	3.05b	2.91a
N150	1448060a	17076a	892175ab	10521ab	3.81a	2.70ab
N200	1036664bc	12225bc	781985b	9222b	3.00bc	2.47b
SE	71642		24508		0.14	0.05
CV (%)	16.95		7.39		9.25	3.79

CV = Coefficient of variance, SE± = Standard error of means. Values with the same superscript are not significantly different at 5% level of probability.
The Tk was converted to USD according to the Bangladesh bank exchange rate accessed on 29th November 2020 (https://www.bb.org.bd/econdata/exchangerate.php).

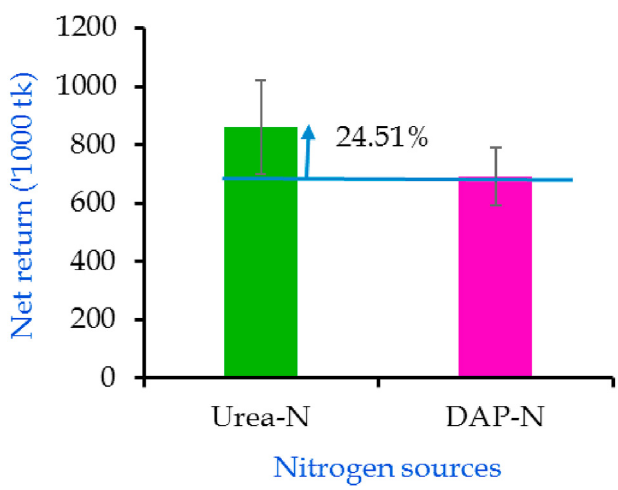

Figure 14. Comparative performance of urea-N and DAP-N on mean net return of *A. vera*. Bars indicate standard error (±SE) at 0.05.

N ha^{-1} might also play a synergistic role with other nutrients and ensure balanced nutrients supply whereas this might not be the case for lower and excess application of N.

The higher leaf number and the leaf area could be the reason for obtaining higher biomass yield compared to the control (Figure 5) while DAP had higher yield than urea. Previously, similar result was found by Dastagir*et al.* (2015) who observed that the average leaf fresh biomass yield of *A. vera* were significantly increased with the increased doses of N from urea and DAP and better result was obtained from different doses of DAP than the same doses of urea. This result is in accordance with the findings of Khan et al. (2012), Egbuchua and Enujeke (2015) and Goussous and Mohammad (2009). Khan et al. (2012) reported that availability of nutrients increased the biomass of plants. The study is also in concomitant with the findings of Egbuchua and Enujeke (2015) who reported highest fresh biomass weight of *A. vera* using highest N application rate. Goussous and Mohammad (2009) reported an increase of leaves fresh weight of *Allium cepa* due to N and P fertilizers.

The significant response of leaf area to comparatively higher rates of N-levels for both fertilizer might be considered as an indication that N was taken up by the plant and subsequently utilized in cell multiplication, amino acid synthesis and energy formation that acts as structural compound of the chloroplast which carries out photosynthesis (Ng'etich et al., 2013). Dastagir*et al.* (2015) also observed similar result that the average leaf area of *A. vera* were significantly increased with the increased doses of N from urea and DAP and they found better result from the higher dose of DAP than urea. Maniruzzaman et al. (2016) found significantly highest total leaf area plant^{-1} in acid soil and in non-calcareous soil at 60 DAT from the plant receiving 250 kg N ha^{-1}. Ng'etich et al. (2013) also found a general trend with increase in leaf area as the N-fertilizer was increased with that of 160 kg N ha^{-1} yielded the highest leaf area of 209 % at 59 DAS when evaluated against the control. Enhanced leaf parameters with increased levels of fertilizers were also reported previously (Khanom et al., 2008). The significant increase in sucker number was highest at 200 kg ha^{-1} in urea (9.67) and 150 kg ha^{-1} in DAP (4.00) and then declined with further addition. We further observed that the mean number of sucker production was more than double in urea-N fertilized soil (4.95 Plant^{-1}) than DAP-N (2.28 Plant^{-1}) (Figure 8). Higher number of sucker production in urea-N fertilized soil could be related to fastest release of N from urea compared to DAP during the early growth period of the mother plant. Interestingly leaf area and number of suckers inversely responded to N application from urea and DAP irrespective of N levels.

The fresh and dry gel weight of *A. vera* plant^{-1} at harvest varied significantly with DAP-N treatment having higher weight than urea-N (Table 1). Dastagir et al. (2015) also observed similar result for dry weight of *A. vera* which was significantly increased with the increased doses of N from urea and DAP. Chowdhury et al. (2020) found the highest gel weight plant^{-1} (2956 g) of *A. vera* at harvest measured from the plant receiving 25% inorganic fertilizer and 75% poultry manure. Allahdadi and Farzane (2018) found the highest fresh weight of Artichoke (*Cynarascolymus* L.) plant by application of 200 kg N ha^{-1}. The experiments conducted by Saha et al. (2005) and Nematian et al. (2010) confirmed that the nutrient minerals, such as N and K, increase leaf growth and lead to a substantial amount of gel in *A. vera*. Previously, many reporters found increased leaf fresh weight from the increased N application (Sheikh and Ishak, 2016; Khaghani et al., 2012; Alizadeh et al., 2010). Hossain et al. (2007) observed that N fertilizers significantly increased the dry leaf weight of *A. indica*. Allahdadi and Farzane (2018) also reported that the consumption of 200 kg N ha^{-1} had the highest dry leaf weight of Artichoke (*Cynarascolymus* L.) plant.

There was a significant and increasing trend on different levels of soil applied N on its concentration in *A. vera* gel and leaf (Table 2). Control treatments generally showed lowest content and uptake of nutrients. Nitrogen concentration of the gel and leaf increased with the increased levels of N irrespective of fertilizers used; although leaf N uptake of the plants grown in the pot fertilized with urea was lower than the N uptake of the plants grown in the pot fertilized with DAP. Higher nutrient concentration might be due to the higher rate of N application and higher nutrient uptake may be related to higher biomass yield obtained from those rates. Plants generally have the ability to uptake organic chemicals such as N from soil (Isiuku and Enyoh, 2019). Mean leaf N concentration was more than double (0.93%) compared to gel-N concentration (0.44%) irrespective of the fertilizer sources (Figure 9). It could be due to the presence of peel in leaf which is likely to contain more N than gel. Between the gel and leaf, higher concentration was observed in the later. This could be due to the highest dry leaf yield harvested from that treatment and N concentration because nutrient uptake was calculated from their concentrations and corresponding dry leaf yield. These results are in conformity with those of Angkapradipta et al. (1986) who reported that N concentration in stevia plant increased due to its increased doses. Saha *al.* (2019) concluded that increased N uptake by silver beet resulted from increased application of N fertilizer.

Correlation analysis revealed that plant height and total leaf area were significantly and positively correlated. Similarly, observation was shown by fresh gel weight and LBY (Figure 9). These results suggest that the applied fertilizers (urea and DAP) in the soils conveyed similar effects on the plants vegetative growth and biomass yield. Similar results were reported by Chowdhury et al. (2020) who found significant and positive correlation of plant height, number of leaves and fresh leaf weight with fresh gel weight of *A. vera*.

The critical nutrition concentration which estimates the critical N concentration is the minimum N concentration necessary to achieve maximum aboveground biomass at any time during the growing season. The obtained minimum concentration of N corresponding to the arbitrary point at 80% to achieve the maximum leaf biomass production, which was estimated to be *ca* 0.90 and 0.88 % in *A. vera* leaf grown in urea-N and DAP-N treated pot, respectively (Figure 10). Maniruzzaman et al. (2016) reported the critical N concentration of 1.43 and 1.50 % in the leaves of stevia plants grown in acid and non-calcareous soils, respectively under different levels of N.

Specific nutrient requirement of a crop is called “the minimum content of that nutrient associated with the maximum yield” or “the minimum rate of intake of the nutrient associated with the maximum growth rate” (Loneragan, 1968). Nitrogen requirement are crop specific. From the fitted curve, it was revealed that the corresponding estimated minimum amount of N for 80% leaf biomass production in the plant grown in urea-N and DAP-N treated pot was estimated to be *ca* 74.90 and 89.60 kg ha^{-1}, respectively (Figure 11). Higher values were obtained Maniruzzaman*et al.* (2016) estimated for stevia grown in acid and non-calcareous soils to be 273 and 257 kg ha^{-1}, respectively.

The nitrogen use efficiency (NUE) of *A. vera* informs on the ability of the plant to utilize the applied N in soil effectively. The efficiency can be considered moderate with maximum value of 69 % for DAP-N and 55 % for urea-N (Figure 13). This suggests that *A. vera* utilizes DAP-N in soil than urea-N for better growth. This could be due to the slow release of N from DAP than urea and urea showed a faster and higher rate of mineralization while DAP had a steady state and relatively slower rate of mineralization (Mohiuddin et al., 2006). On the other hand, more urea-N may not be used by the plants due to loss by leaching and volatilization than DAP-N. This result is supported by the previous reporters (Cao et al., 2018) who suggested considering the N sources to improve NUE in crop production system and reducing N loss from urea application as a first and important step. Because N response is independent of yield level and N availability is strongly dependent on the environment, optimum fertilizer N rates are often unpredictable (Dhital and Raun, 2016). However, improved N economy from reduced N fertilizer inputs must operate within acceptable crop yield levels (Hirel et al., 2007). This result is in line with a review study which indicated that NUE of cereal in 2015 was 35, 41, 30, and 21% for the world, the United States, China, and India, respectively (Omara et al., 2019).

The economic analysis for the application of DAP and urea was studied. The results presented in Table 3, showed that net return varied significantly among N levels and source but higher than control. Higher mean net income of BDT (Bangladesh Taka) was obtained from urea-N compared to DAP-N, which indicate that the better profit (24.51%) will be obtained by applying urea-N in the planting of *A. vera* (Figure 14). This could be to the high sucker production (Figure 7) in the urea-N treated plant than DAP-N which ultimately added more profit.

The results for the benefit cost ratio of *A. vera* significantly influenced by the different levels of N (Table 3), which was obtained for N_{150} and N_{100} applied urea and DAP treatment, respectively. Chowdhury et al. (2020) found the highest BCR (1.72) of *A. vera* applying 25% inorganic fertilizer along with 75% poultry manure and the lowest (1.11) from control. According to Kelly and Murekezi (2000), treatments with BCR values lower than 2 are not worthy in farmers' perspectives. The farmer cannot shift from one crop cultivation to another unless benefits are sure. According to CIMMYT (1988), marginal benefits need to be 1.18 times the marginal costs to be attractive to farmers. All fertilizer treatments did not meet this requirement. This is consistent with the result reported by Celestin (2009) in Rwanda that application of FYM and ½ DAP +½ FYM were more profitable in maize but not in common bean and soybean. A previous report (Rajkhowa et al., 2003) at Jorhat observed that application of 100% fertilizer significantly increased economics of mungbean over control. Tarafder et al. (2020) found that the application of 3 t ha^{-1} poultry manures along with 70% inorganic fertilizers showed economically better results indicating the highest BCR (BARI Mung-6: 2.47, BINA Mung-8: 2.13).

5. Conclusions

Application of different levels of N exerted significant influence on the growth, leaf yield and nutrient uptake by *A. vera*. The highest values of plant height, leaf number, leaf biomass yield and profit based on benefit cost ratio, leaf area, number of suckers, fresh and dry gel weight of *A. vera* were obtained from the plant fertilized with N at the rate of 150 kg ha^{-1} from urea and at the rate of 100 kg ha^{-1} from diammonium phosphate. Comparatively 24.50% higher net return was obtained by the application of urea than diammonium phosphate. Nitrogen application at all levels increased leaf biomass yield at harvest over control. About 7.59% higher leaf biomass yield was obtained from fertilization with diammonium phosphate than urea. Mean number of sucker production was more than double in urea fertilized pot than diammonium phosphate. Nitrogen concentration of *A. vera* gel and leaf and its uptake by leaf were also significantly influenced by their additions. Highest values were obtained from the plant receiving the highest doses of N. The applied N was more concentrated in the leaf than gel. Significant and positive correlations were found among the growth and yield parameters of *Aloe vera* L. due to N application. The minimum requirements of N to produce 80% leaf biomass yield were 74.90 and 89.60 kg ha^{-1} from urea and diammonium phosphate, respectively. Critical leaf N concentration of *A. vera* was estimated to be *ca* 0.90 and 0.88 % for urea and diammonium phosphate. The highest NUE was estimated to be *ca* 64 and 69 % for urea and diammonium phosphate, respectively. The results suggest that farmers can be advised to apply N at the rate of 150 kg ha^{-1} from urea for producing higher yield and better-quality *A. vera*. leaf. Of course, more research work is required to validate this results in various soil and climatic conditions for better production of this important industrial crops.

Declarations

Author contribution statement

Md. Akhter Hossain Chowdhury: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Taslima Sultana, Md. Arifur Rahman, Biplob Kumar Saha: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tanzin Chowdhury: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Christian Ebere Enyoh: Analyzed and interpreted the data; Wrote the paper.

Wang Qingyue: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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