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Effect of nitrogen forms on physiological and biochemical constituents of lettuce grown in soilless conditions during different seasons

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ABSTRACT

The effect of different nitrate to ammonium ratios (NO_3^- : NH_4^+: 100:0, 75:25, 50:50, 25:75 and 0:100 respectively) on physiological and biochemical constituents of lettuce crop raised in polyhouse and in open field conditions (control) were studied during three seasons. There were three dates of sowing *viz*; October (main season) and February and July (off-season). In each season, fresh foliage were harvested three times from each replication of respective treatments at 30 days interval starting from the date of sowing. At each harvest, fresh and dry biomass of shoot, plant height and leaf area were determined. Biochemical constituents including total chlorophyll, carotenoids, total soluble sugars, total starch, total soluble proteins, free amino acids and proline content were estimated. Activities of antioxidant enzymes *viz*; superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were also determined. In each season, plant growth, yield, biochemical constituents and antioxidants activity peaked when supplied with nutrient solution having NO_3^- and NH_4^+ in the ratio of 75:25. Even in the main season, polyhouse raised crop supplied with this solution gave better results than the crop raised in the field as per recommended practices.

Keywords: *Lectuca sativa*, ascorbic acid, biochemical parameters, growth, yield.

INTRODUCTION

Lettuce is in demand throughout the year as it is a popular vegetable salad being rich in antioxidants, calcium, iron and vitamin A and C. Cultivation of lettuce under polyhouse in soilless culture is an effective way of ensuring year round availability of this crop. For soilless cultivation, an appropriate concentration of the nutrient solution is an important aspect for successful vegetable production. Mineral nutrient supply directly affects the crop yields and quality during critical growth stages. Plants use N mostly in two forms, nitrate-N and ammonium-N, but nitrate is the preferred source for lettuce growth and development. Using ammonia as the sole source of N can be toxic to some plants including lettuce. However, N supplied either as ammonium or as a mix of both forms is known to maximize growth and yield in many other crops because of a higher rate of CO_2 assimilation per unit leaf area and also because of the savings in photo energy when N is supplied as ammonium. Assimilation of ammonium into plant metabolites needs less energy than that required for nitrate assimilation because ammonium does not have to be reduced. In ryegrass plants which were supplied with 75/25 and 50/50 (NO_3^-/NH_4^+) ratios in the nutrient solutions had the maximum shoot dry matter and also had the maximum leaf area (Cao *et al.,* 5). In previous studies on leafy vegetables, it has been shown that an appropriate ratio of NO_3^- and NH_4^+ in the nutrient solution resulted in enhanced yield and nutritional quality as compared to crop where nitrogen was supplied solely as either NO_3^- or NH_4^+ e.g., in spinach (Wang *et al*., 18), and Chinese cabbage (Hu *et al*., 9). Many researchers have indicated that N form and their varying ratios can ultimately increase the chlorophyll and carotenoid contents in leafy vegetables (Barickman and Kopsell, 3). Recently it has been reported in kale that 75/25 and 50/50 (NO_3^-/NH_4^+) ratios had resulted in achieving maximum plant growth including fresh and dry biomass, leaf area, chlorophyll content and yield (Assimakopoulou *et al.,* 2) and in pepper (Zhang *et al*., 19). To the best of our knowledge, there is very little information on effect of NO_3^- to NH_4^+ ratios on lettuce raised in different seasons in the soilless medium in north Indian conditions. So, in the present study we examined the effect of forms of N (NO_3^- or NH_4^+) on the growth, yield and biochemical constituents of soilless grown lettuce in relation to the cultivation season and sowing conditions. We hypothesize that the supply of nitrogen having appropriate concentration of nitrate and ammonium will increase the growth, yield and biochemical constituents in lettuce. Keeping all these things in mind the present study was conducted with the following objectives: 1. To standardize a suitable NO_3^- :NH_4^+ ratio in nutrient formulation for optimum growth and yield of lettuce under soilless conditions with intermittent water and nutrient supply. 2. To

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compare the growth, physiological and biochemical attributes, yield and quality traits of lettuce under different seasons and growing conditions.

MATERIALS AND METHODS

The study was conducted during the years 2016-17 and 2017-18 in a polyhouse at the College of Agricultural Engineering and Technology, Vegetable research farm at the Department of Vegetable Science and in the laboratories of the Department of Botany, Punjab Agricultural University (PAU), Ludhiana (North latitude 30°-56', east longitude 75°-52'). The seeds of lettuce (cultivar Iceberg) were obtained from the Department of Vegetable Science, PAU, Ludhiana. Three crops were raised per year which were sown in the months of October (main season), February (off- season) and July (off-season), respectively. At each date of sowing, two crops were raised simultaneously, one in open field conditions (control) and another under polyhouse. For all the three seasons, nursery was raised at a field area of the Department of Vegetable Science as per the schedule given in Table 1 and then after 45 to 55 days after sowing (DAS). Lettuce plantlets were transplanted to plastic pots [30 cm (diameter) × 30 cm (height)] on a coco peat: perlite: vermiculite (3:1:1) medium and placed in a polyhouse Under the open field conditions, the crop was raised in $2m^2$ plot as per the recommended package of practices (Anonymous, 1). Fresh foliage was harvested by cutting it close to the base with a pair of scissors. Harvesting was done 3 times in each season at 30 days interval starting from date of transplantation. The data represented for various parameters is a mean value of 2 years (6 harvestings).Inside the polyhouse heaters and coolers were used for the maintenance of optimum temperature in cool and hot months, respectively. Green net sheets were also used to shade the plants during very hot summer months. The temperatures recorded during main season (October- January) was in the range of 11.4°C to 25.2°C and in the off seasons the range was between 16.7°C to 28.1°C (February-May) and 16.9°C to 28.3°C (July-October).The basal nutrient solution was a modified Hoagland's nutrient solution (Cao *et al.,* 5) that contained all the macronutrients (Table 2). For micro-nutrients, a commercially available formulation was used. There were five treatments with varying ratios of NO_3^- and NH_4^+ while keeping the total N content constant (Table 2). The NO_3^- was supplied as calcium nitrate [Ca $(NO_3)_2$] and NH_4^+ as ammonium nitrate [$(NH_4)_2SO_4$] respectively. The pH was adjusted at 6.0 ± 0.2 for all the treatments with dilute sodium hydroxide (NaOH) or hydrochloric acid (HCl). Nutrient solution was made fresh every 10 days. Approximately, 2 and 3 L nutrient solution were added manually to each pot during the main cropping season and off season, respectively.

Leaf area from each replication was measured with a leaf area meter (LI-COR; LI-3100). Leaf discs were cut with a cork borer and leaf water potential was measured with a water potential

Table 1. Sowing and harvesting schedule of polyhouse and open field raised crop.

	Season	Off season	
	October	February	July
Raising of nursery	2nd week of September	1st week of January	2nd week of June
Transplantation of plantlets	4th week of October	2nd week of February	4th week of July
Month of harvesting	3rd week of January (2017 and 2018)	1st week of May (2017 and 2018)	1st week of October (2017 and 2018)

Table 2. Compositions (mmol L−1) of nutrient solutions with different NO_3^-/NH_4^+ ratios.

Treatment	NO_3^-/NH_4^+ ratios	Source of nutrients ($mmolL^{-1}$)						
		KNO_3	$Ca(NO_3)_2.4H_2O$	$CaCl_2.2H_2O$	$(NH_4)_2SO_4$	KH_2PO_4	$MgSO_4.7H_2O$	KCl
T1	100:0	4.8	1.6	3.4	0.0	1.0	2.0	1.2
T2	75:25	2.8	1.6	3.4	1.0	1.0	2.0	3.2
T3	50:50	0.8	1.6	3.4	2.0	1.0	2.0	5.2
T4	25:75	2.0	0.0	5.0	3.0	1.0	2.0	4.0
T5	0:100	0.0	0.0	5.0	4.0	1.0	2.0	6.0

*The N concentration was kept constant at 8 mmol L^{-1}.
*KNO_3: Potassium nitrate, $Ca(NO_3)_2.4H_2O$: Calcium nitrate tetrahydrate, $CaCl_2.2H_2O$: Calcium chloride dehydrate, $(NH_4)_2SO_4$: Ammonium sulphate, KH_2PO_4: Potassium dihydrogen phosphate, $MgSO_4.7H_2O$: Magnesium sulfateheptahydrate, KCl: Potassium chloride.

meter (PSYPRO, ELI Tech Group WESCOR ®). Plant height was measured from the base till the tip of the topmost leaf with a centimetre scale. Fresh weight (g) of head was recorded immediately upon harvest and same leaf samples were dried in an oven at 60°C for constant as well as dried weight to be recorded later. From the freshly harvested leaves, total chlorophyll, carotenoids, proline (Malik and Singh, 11), ascorbic acid (Ranganna, 14) and α- tocopherol (Jayaraman, 10) were estimated. Dried leaves were analyzed for the estimation of total soluble sugars, total starch, total soluble proteins (TSP) and total free amino acids (FAA) (Malik and Singh, 11). Activities of SOD, POD and CAT were determined from the fresh leaves (Malik and Singh, 11). Data were analyzed using SPSS (Version16.0 SPSS) statistical software.

RESULTS AND DISCUSSION

Plant growth was determined in the form of fresh and dry biomass of head (Fig.1a; 1b), plant height (Fig.1c) and leaf area (Fig.1d). Plant growth was significantly affected by the nitrate and ammonium ratios in the nutrient solutions. In each season, leaf area, plant height, fresh and dry weight of shoot was more when proportion of ammonium in the supplied nutrient solution was 25% i.e. plants supplied with nutrient solution T2. Maximum leaf area, plant height, fresh and dry weight of shoot in the plants which were supplied with T2 nutrient solution in the main season (final harvesting in January) was 396.59 cm2, 22.31cm, 253.00g and 12.70g, respectively than Feb (i.e. 199.54 cm^2, 18.23 cm, 201.00g and 9.34g) and July (i.e. 117.95 cm^2, 11.98 cm, 35.00g and 5.00g) sown crop. Lesser fresh weight, when the proportion of NH4+ was 50% or above, may be due to greater supply of carbohydrates for assimilation and detoxification of ammonium. A similar decrease in plant growth at higher concentration of NH4+ in nutrient solution was attributed to salinity of nutrient solution that caused restrictions in plant water flux. Better growth in plants supplied with nutrient solution T2 is corroborated by findings of Assimakopoulou *et al.* (2) in Kale wherein higher proportion of NH_4+ (50% to 100%) wrt NO_3- in the nutrient solution decreased plant growth parameters.

During all the three growing seasons, less leaf water potential i.e. 2.89 (more negative values) were observed in the plants which were supplied with nutrient solutions T2 and less negative leaf water potential values i.e. 1.65 (higher leaf water potential) were recorded in the plants which were supplied with higher proportion of NH_4^+ in the nutrient solutions i.e. T4 and T5 (Fig. 1e). Proline content (Fig.1f) in lettuce leaves was highest in plants supplied with nutrient solution T2 i.e. 975.09μg/g FW (Oct), 962.91 μg/g FW (Feb) and 941.89 μg/g FW (July) than soil grown crop i.e. 339.26, 788.13 and 427.30 μg/g FW in Oct, Feb and July respectively. Higher accumulation of proline in the plants provided with nutrient solution T2 can be explained by lower values (more negative) of water potential in plants supplied with this nutrient solution. Proline is an osmolyte helps in reducing the cellular water potential and gets accumulated in wide range of stresses including low water deficit, salinity, low and high temperature etc. (Hayat *et al.,* 7). In October (main season), sown crop, the proline content of the plants grown in soil-less conditions under polyhouse was more than that of soil grown plants but some deviation in trend of proline accumulation was observed in February and July sown crop (Fig. 1f) Plants supplied with nutrient solution T2 in the nutrient solution accumulated 187.42%, 22.18% and 120.43% more proline respectively than soil grown lettuce in October, February and July seasons, respectively.

In each season, plants supplied with nutrient solution T2 and T1 (having 25% and 0% NH_4^+ respectively in nutrient solution) had maximum total chlorophyll and carotenoid content as compared to other treatments however, the solution with 100% NH_4^+ had the least total chlorophyll and carotenoids content (Fig. 2a; 2b). Previous studies have also reported that high proportions of NH_4^+ in the nutrient solution suppressed the plant growth and decreased the chlorophyll content, whereas low proportions of NH_4^+ increased chlorophyll content. e.g. an increase in chlorophyll and carotenoids content in crops supplied with NO_3^-/NH_4^+ in the ratio of 75:25 have been reported in kale (Assimakopoulou *et al.*, 2) and swiss chard (Barickman and Kopsell, 3). Therefore the main season (October) crop which was supplied with nutrient solutions T1 and T2 had more total chlorophyll content than the off season (February and July) sown crop supplied with same nutrient solutions.

The T2 nutrient solution promoted the accumulation of total soluble sugars [202.81 (Oct), 210.71 (Feb) and 181.82 mg/g DW (July)] and starch content [99.23 (Oct), 106.33 (Feb) and 100.45 mg/gDW (July)] followed by total soluble sugar and starch content of treatment T1 i.e. 176.79 and 98.63 (Oct), 184.26 and 101.37 (Feb) and 175.31 and 94.72 mg/g DW respectively. A similar increase in total soluble sugars and starch content has been reported by Sun *et al.,* (16) in Chinese chive seedlings. In contrast, the plants supplied with nutrient solution T5 had the lowest soluble sugar and starch content i.e. 57.84 and 64.88 (Oct), 62.86 and 67.98 (Feb) and 65.12 and 61.47 mg/g DW respectively (Fig. 2c; 2d). The total soluble sugar content in plants supplied with nutrient solution T2 in polyhouse grown plants was 80.70%, 84.28% and 86.13% which was more than the open field sown crop in October, February and July respectively. In a previous study, it has been reported that when the ionic strength of the nutrient solution was increased, respiration rate also increased; hence reduction in sugar content in

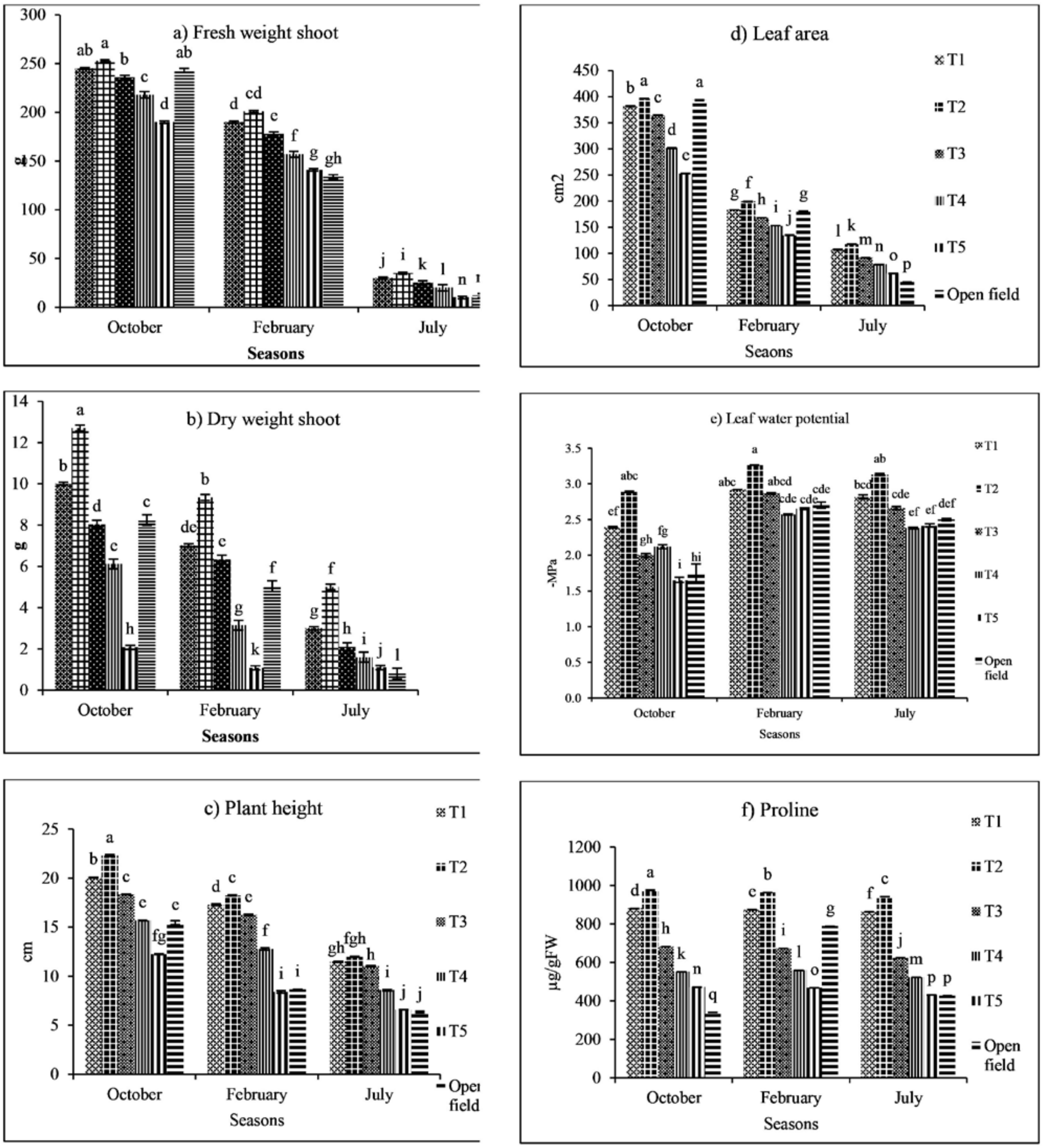

Fig. 1. Effects of different NO_3^-:NH_4^+ ratios on (a) Fresh weight shoot (b) Dry weight shoot (c) Plant height (d) Leaf area (e) Leaf water potential (f) Proline content.

T1 (NO_3^-:NH_4^+ : 100:0), T2 (NO_3^-:NH_4^+ : 75:25), T3 (NO_3^-:NH_4^+ : 50:50, T4 (NO_3^-:NH_4^+ : 25:75) T5 (NO_3^-:NH_4^+:0:100) leafy lettuce grown with high nutrient concentration could be related to an increase in tissue vegetable respiration (Fallavo *et al*., 6).

The contents of both total soluble proteins (TSP) and free amino acids (FAA) varied with the form of nitrogen used in nutrient medium (Fig. 2e; 2f). Both TSP and FAA contents generally increased with the lower proportion of NH_4^+ and decreased when NH_4^+ was above 25% in the nutrient solution. Plants supplied with nutrient solution T2 and T5

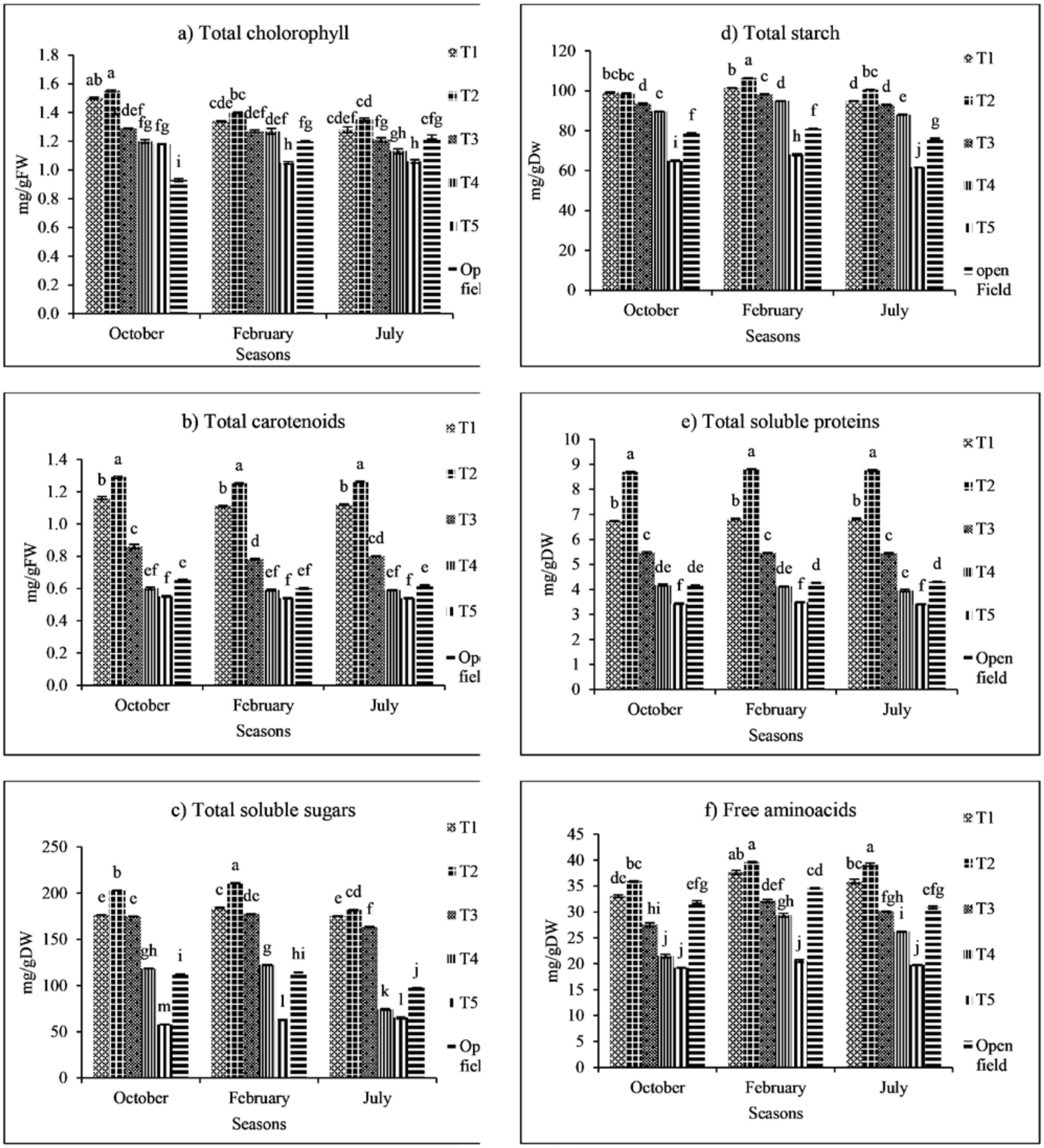

Fig. 2. Effects of different NO_3^-:NH_4^+ ratios on (a) Total chlorophyll content (b) Total Carotenoids content (c) Total soluble sugars (d) Total starch (e) Total soluble proteins (f) Free amino acids content.

T1 (NO_3^-:NH_4^+ : 100:0), T2 (NO_3^-:NH_4^+ : 75:25), T3 (NO_3^-:NH_4^+ : 50:50, T4 (NO_3^-:NH_4^+ : 25:75) T5 (NO_3^-:NH_4^+ : 0:100).

had maximum and minimum mean TSP and FAA, respectively. TSP and FAA content were more in off season crop than the main season crop and also the polyhouse crop had more FAA content than the open field grown crop. Similarly, increased TSP and FAA have been observed in Chinese kale (Zhu *et al.,* 20) supplied with nutrient solution having 20% NH_4^+ and in chives (*Sun et al.,* 16) supplied with 25% NH_4^+ in nutrient solution. Off season crop had more accumulation of both TSP and FAA and that

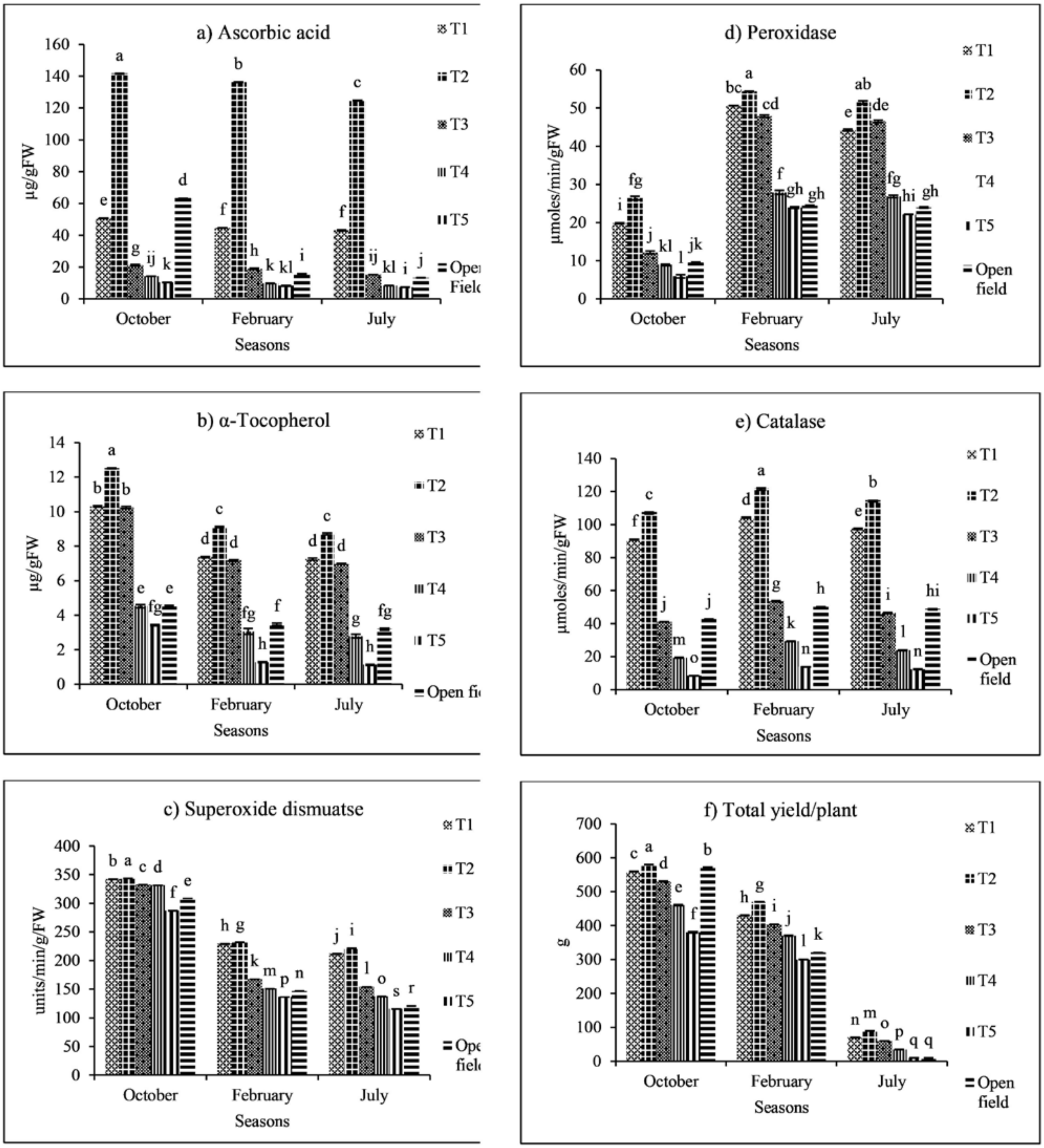

Fig. 3. Effects of different NO_3^-:NH_4^+ ratios on (a) Ascorbic acid content (b) Alpha-tocopherol content (c) SOD activity (d) POD activity (e) CAT activity (f) Total yield/plant.

T1 (NO_3^-:NH_4^+ : 100:0), T2 (NO_3^-:NH_4^+ : 75:25), T3 (NO_3^-:NH_4^+ : 50:50, T4 (NO_3^-:NH_4^+ : 25:75) T5 (NO_3^-:NH_4^+ : 0:100).

may be due to high temperature (as a stress signal) prevailing during the off season sown crop than the main season crop.

In each season, among the treatments, content of ascorbic acid and α- tocopherol were significantly higher in the plants supplied with treatment T2 in the nutrient solution than the other four treatments (Fig.3a; 3b). Previous study has reported higher amounts of both alpha and gamma-tocopherols in leaves of rapeseeds oil (Hussain *et al*., 8), when proportion of supplied NH_4^+ in the nutrient solution was lower as compared to the nutrient solution where NO_3^- was the sole source of nitrogen. This suggests that an increase in tocopherol is dependent on the nitrogen source and application rate. In all the seasons, polyhouse grown crop had higher ascorbic acid and α-tocopherol content than the soil grown crops. Results are in line with a previous study where hydroponically grown lettuce recorded more ascorbic acid and α- tocopherol content than the soil grown lettuce (Buchnann and Omaye*,* 4). Ascorbic acid and α- tocopherol content in the leaves were higher in main season crop raised under both polyhouse and open field conditions. Phillips *et al.,* (13) have also reported significantly more ascorbic acid content in winter (main season) as compared to summer and spring cultivated spinach, potatoes and oranges. Differences in vitamin C contents were observed between the seasons, which suggests that the growing conditions may also have an effect on vitamin C content and consequently on the quality and visual appearance of leafy vegetables, such as lettuce and rocket leaves (Petropoulos *et al.,* 12).High temperatures and low amounts of light resulted in low vitamin C content since vitamin C content in many vegetables is significantly affected by temperature and total available heat as well as the amount and intensity of light.

The main season sown crop showed more SOD activity than the off season sown crop (Fig.3c). Irrespective of the season, SOD activity of plants supplied with nutrient solution T2 was significantly higher than the other treatments. Similarly POD activity was maximum in the plants which were supplied with treatment T2 in the nutrient solution (Fig.3d). But unlike the activity of SOD, the activity of POD in off season sown crop was more than that of main season sown crop. CAT activity (Fig.3e) in lettuce leaves followed the same trend as POD activity. Minimum SOD, POD and CAT activity among all the treatments was recorded in the plants supplied with nutrient solution T5. Antioxidants activity in polyhouse grown crop was higher than the soil grown crop. Sofo *et al*., (15) has suggested that antioxidant properties of lettuce may be affected by the fertilizers applied and growing systems.

Among the seasons, the main season (October) sown crop had higher yield than the off season (February and July) sown crop. Irrespective of the season, yield of soil-less grown plants underpolyhouse supplied with nutrient solution T2 was more than soil grown crop (Fig. 3f). The yield enhancement in polyhouse grown crop supplied with treatment T2over open field crop was 1.75%, 46.88% and 800.00% respectively in October, February and July sown crops. Similar biomass enhancement in crop supplied with nutrient solution in the ratio of 75:25 (NO_3^-: NH_4^+) have been reported in lettuce (Wang and Shen, 17). The reason, plants did not tolerate higher proportion of NH_4^+ might be due to the unavailability of NO_3^- as a N source and the higher demand for carbohydrates channelled for NH_4^+assimilation and detoxification (Assimakopoulou *et al.,* 2).

In conclusion, in main season as well as in off season sown lettuce crop, the yield was significantly more in soil-less grown crop than open field sown crop. Further, the crop supplied with nutrient solutions having higher proportion of NO_3^- had higher values of biochemical constituents and more antioxidant activity than the open field crop. Hence providing nutrient solutions with appropriate ratios of NO_3^-:NH_4^+ improves the yield and biochemical traits in soil-less grown lettuce. It is therefore suggested that NO_3^-/NH_4^+ ratios of 75:25 could promote growth, increase yield and improve the biochemical traits in lettuce.

DECLARATION

The authors declare no conflict of interest.

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