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Effect of Proportion of NO_3^- and NH_4^+ in Nutrient Solution and Growing Seasons on Yield and Quality in Coriander

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ABSTRACT

The effect of nutrient solutions with varying nitrogen forms was evaluated on yield and quality of coriander (cv. Punjab Sugandh) grown in soilless medium under polyhouse conditions. Crop was sown on three dates viz., October (main season), February, and July (off-season), respectively. In polyhouse, N (Nitrogen) was supplied in variable ratios of NO_3^- to NH_4^+ viz; 100:0 (T1), 75:25 (T2), 50:50 (T3), 25:75 (T4), 0:100 (T5), respectively, keeping total N content constant. Fresh foliage was harvested 3 times in each season at 30 days interval starting from date of sowing. At each harvest, plant growth quality parameters including activity of antioxidants enzymes were determined. Irrespective of the seasons, the yield was significantly higher in polyhouse than soil-grown crop, and among the nutrient solutions, the solution of T2 recorded significantly higher yield as well as growth and quality parameters during all the season. It could be concluded that NO_3^- to NH_4^+ ratio of 75:25 in the nutrient solution improved both yield and quality and is thus most suitable for coriander crop.

Keywords: α-tocopherol, Ascorbic acid, Polyhouse conditions, Proline, Soilless medium.

INTRODUCTION

Leafy vegetables are good sources of antioxidants, minerals, vitamins and other nutritional contents in human diet (Souri *et al*., 2018b). Coriander (*Coriandrum sativum* L.) is an aromatic herb, which is in demand throughout the year. Therefore, to meet the off season demand, its cultivation under controlled conditions of polyhouse is required. Different cultural practices, such as controlled temperature, light, and mineral nutrient levels, have become common practice in vegetable production systems for the enhancement of these nutritive qualities (Barickman and Kopsell, 2016; Saleh *et al*., 2019). In a polyhouse, the crop is generally grown in soilless media. Many plants such as celery (Saleh *et al*., 2019), chinese cabbage (Song *et al.*, 2011) and cucumber (Roosta and Schjoerring, 2008) prefer higher proportion of nitrate to ammonium in nutrient solution. N metabolism can be regulated by nitrogen supply, plant nitrogen status, and its growth rate (Souri and Hatamian, 2019; Dehnavard *et al.*, 2017). Plant growth and physiological performance can be changed by N more than any other mineral element (Marschner, 2012). Plants can uptake both ammonium and nitrate as well as other forms of N compounds; however, many plants prefer a combined application of nitrate and ammonium with different preferential ratios (Aghaye Noroozlo *et al.*, 2019; Souri *et al.*, 2019). Given that information on the relationship between NO_3^- to NH_4^+ ratios on growth and yield in coriander is non-existent, the objective of the present study was to investigate the effects of different nitrate to ammonium ratios on yield and

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quality in coriander raised as the main season and off season crop.

MATERIALS AND METHODS

The study was conducted during the years 2016-17 and 2017-18 in the polyhouse at the College of Agricultural Engineering and Technology, the Vegetable Research Farm, Department of Vegetable Science, and in the laboratories of the Department of Botany, Punjab Agricultural University, Ludhiana. Ludhiana is situated at 30°-56' North latitude, 75°-52' East longitude and 243 metres above the sea level. The seeds of coriander (cv. Punjab Sugandh) were obtained from Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Three crops were raised per year 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 (in soil conditions, control) and another under polyhouse. Under the open field conditions, the crop was raised in 2 m^2 plot as per recommended practices (Anonymous, 2016). The first irrigation was applied about 2 weeks after sowing and subsequent irrigations were given as and when required, taking care that there was no moisture stress in the field. In polyhouse, seeds were directly sown in plastic pots 30 cm (diameter)×30 cm (height) in a coco peat: perlite: vermiculite (3:1:1) medium (Table 1). After emergence, the seedlings were thinned to retain six per pot**.** 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 sowing. The data represented for various parameters is a mean value of 2 years, i.e. a data from six harvestings.

Polyhouse Conditions and Nutrient Solution

Optimum temperature inside the polyhouse was maintained by using heaters and coolers in cool and hot months, respectively. Additionally, green net sheets were used to shade the plants during summer months. The mean maximum and minimum temperatures were 25.2 and 11.4°C during main season (October-January), 28.1 and 16.7°C during the off season crops (February-May) and 28.3 and 16.9°C (July-October), respectively. The basal nutrient solution was a modified Hoagland's nutrient solution (Cao *et al.*, 2010) 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 at 8 mmol L^{-1} (Table, 2). NO_3^- was supplied as $Ca(NO_3)_2$ and NH_4^+ as $(NH_4)_2SO_4$, respectively. The pH of the solution was adjusted to 6.0±0.2 with dilute sodium hydroxide (NaOH) or hydrochloric acid (HCl). Nutrient solution was made fresh every 10 days. Nutrient solution was added manually to each pot as per requirement (at one-day interval in seasonal and two-day interval during off-seasons). Approximately 2 (main season) and 3 L (off season crop) solution was supplied to each pot for the entire growing season (3 months).

Growth Parameters

The growth parameters were recorded at the time of each harvesting. The values presented are mean of data obtained from six harvestings (three harvestings per year). Leaf area of three leaves 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 water potential meter (PSYPRO, ELI Tech Group WESCOR ®). Plant height was measured from the base to the tip of the topmost leaf using a measuring tape. Fresh weight (g) was recorded immediately upon harvest and dry weight (g) was recorded after drying the sample in oven at 60°C for 48 hours.

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Table 1. Sowing and harvesting schedule of polyhouse and open field crops.

	Main season	Off season	
	October	February	July
Month of sowing	3rd Week of October (2016 and 2017)	2nd Week of February (2017 and 2018)	2nd Week of July (2017 and 2018)
Month of harvesting	3rd wWeek 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. [a]

Treatments	NO_3^-/NH_4^+ ratio	Source of nutrients (mmol L^{-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

[a] N concentration was kept constant at 8 mmol L^{-1}.

Quality Attributes

Total chlorophyll and carotenoids (Anderson and Boardma, 1964), Proline (Bates *et al.,* 1973), Ascorbic acid (Ranganna, 1986), and α- tocopherol (Jayarama, 1981) were estimated from the freshly harvested leaves. Total soluble sugars and total starch (Dubois *et al.*, 1956), total soluble proteins (Lowry *et al.*, 1951) and free amino acids (Lee and Takahashi, 1956) were estimated from oven dried leaf samples. Activities of enzymes viz; SOD (Marklund and Marklund, 1974), POD (Shannon *et al.*, 1966), CAT (Chance and Maehly, 1955) were determined from freshly harvested leaves.

Statistical Analysis

Data obtained from this experiment were analyzed by factorial design using SPSS (Version16.0 SPSS) software. Significant differences (P≤ 0.05) were determined by Tukey's test, and were marked with lower-case letters in figures.

RESULTS AND DISCUSSIONS

Growth Parameters

Maximum leaf area, plant height, and fresh and dry weight of shoot (Figures 1-a, -b, -c, and d) were recorded for the crops sown in the main season and within each season, polyhouse grown crop showed higher values as compared to the soil grown crop. Among the nutrient solutions, the plants provided with nutrient solution T2 (NO_3^-/NH_4^+= 75:25) had the maximum leaf area, plant height, and fresh and dry weight of shoot, while the least values for these parameters were observed in the plants supplied with nutrient solution T5 (NO_3^-/NH_4^+= 0:100). This decrease in growth at higher supply of NH_4^+ (50% or above) (viz; treatments T4 and T5) in nutrient solution may be because the carbohydrates are diverted for detoxification of ammonium and hence are not available for plant growth (Britto and Kronzucker, 2013). Higher yield in plants supplied with higher proportion of NO_3^- with respect to NH_4^+ have also been reported in

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strawberry (Tabatabaei *et al.*, 2006), rocket salad (Kim *et al.*, 2006), soyabean (Chen *et al.*, 2011), spinach (Zhang *et al.*, 2005), ryegrass (Cao *et al.*, 2010), and taro (Osorio *et al.*, 2003), and celery (Saleh *et al.*, 2019). Increased plant growth under polyhouse can be related to controlled environmental conditions along with optimum supply of nutrients and water to the plants that led to more vegetative growth.

Proline and Leaf Water Potential

Among the treatments, maximum proline content in all the seasons was recorded in

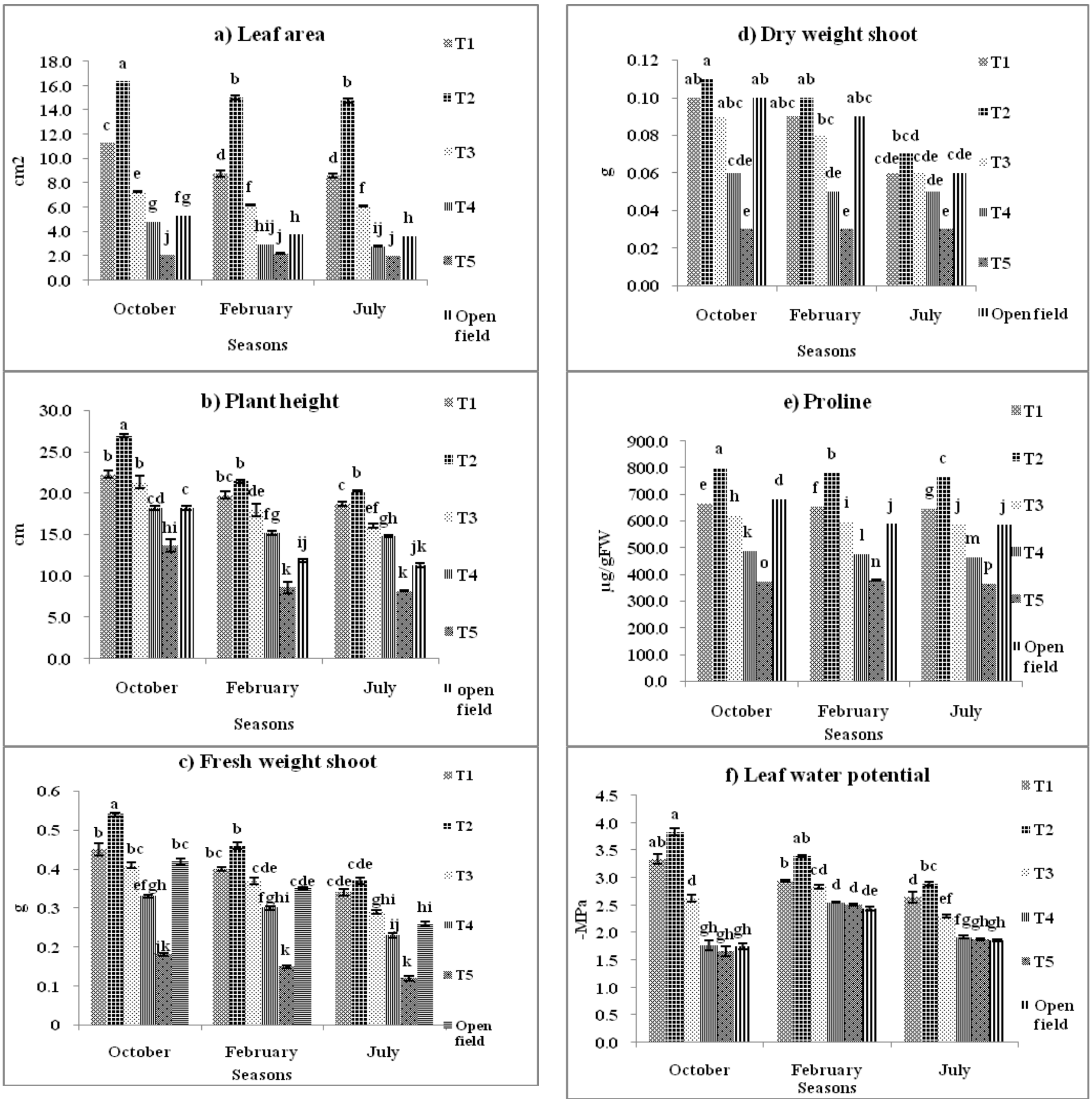

Figure 1. Effects of different $NO_3^-:NH_4^+$ on: (**a**) Leaf area, (**b**) Plant height, (**c**) Fresh weight shoot, (**d**) Dry weight shoot, (**e**) Proline content, and (**f**) Leaf water potential. T1 ($NO_3^-:NH_4^+$= 100:0), T2 ($NO_3^-:NH_4^+$= 75:25), T3 ($NO_3^-:NH_4^+$= 50:50, T4 ($NO_3^-:NH_4^+$= 25:75), and T5 ($NO_3^-:NH_4^+$= 0:100).

the plants supplied with nutrient solution containing NO_3^-/NH_4^+ in the ratios of 75:25 (Figure 1-e). Sun *et al.* (2014) have also observed maximum accumulation of proline in Chinese chive seedlings supplied with NO_3^- and NH_4^+ in the ratio of 75:25. Also, lower values (more negative) of water potential were recorded in leaves obtained from crop supplied with nutrient solution having NO_3^- to NH_4^+ ratio of 75:25 (T2) (Figure 1-f). This explains accumulation of proline, which is an osmolyte that helps in maintaining osmoticum. It has been shown that proline gets accumulated under a broad range of stress conditions, including low water potential in white clover (Kim *et al.*, 2004) and in rice under drought stress (Dien *et al.*, 2019). Further, in the present study, polyhouse grown crop accumulated more proline than soil grown crop.

Total Chlorophyll and Carotenoids

Chlorophylls contribute to leaf colour and have a direct effect on the consumer preference (Wu and Kubota, 2008; Merajipoor *et al*., 2020). Carotenoids play an important role in human nutrition, because of their antioxidative properties (Maiani *et al.*, 2009). In October (main season) and February (off season) sown crop, plants that were supplied with nutrient solution T2 and T1 ($NO_3^-:NH_4^+$= 75:25 and 100:0, respectively) had maximum total chlorophyll content, while in July sown crop maximum chlorophyll content was observed in plants supplied with nutrient solution T2 (Figure 2-a). Carotenoids content in October (main season) sown crop was significantly higher in the plants provided with nutrient solution T2, whereas in off seasons sown crop (February and July), maximum carotenoid content was observed in plants supplied with nutrient solution T1 and T2 (Figure 2-b). Carotenoids content in the plants supplied with treatments T3, T4, and T5 did not differ significantly during all the growing seasons. It means that increasing the proportion of NH_4^+ in the nutrient solution from 50 to 100% did not lead to accumulation of carotenoids. Overall, nutrient solution T2 resulted in maximum chlorophyll and carotenoid content in both the main season and off-season crops. Previous studies have also reported maximum chlorophyll and carotenoids content in crop supplied with NO_3^-/NH_4^+ in the ratio of 75:25 e.g. Kale (Kopsell *et al.*, 2007) and Swiss chard (Barickman and Kopsell, 2016).

Total Soluble Sugars and Starch

Among the seasons, in February sown crop, plants supplied with nutrient solution T2 had significantly higher total soluble sugar. However, in October and July sown crop, total soluble sugar content was higher in the plants supplied with nutrient solutions T1 and T2 (Figure 2-c). The total starch content was maximum in the plants supplied with nutrient solution T2 at all dates of sowing (Figure 2-d). It means that nutrient solution with lower proportion of NH_4^+ (i.e. upto 25%) resulted in maximum accumulation of sugars and starch. Likewise, maximum accumulation of total soluble sugars and starch in the crop supplied with nutrient solution having NO_3^- and NH_4^+ in the ratio of 75:25 was recorded in spinach (Zhang *et al.*, 1990), tomato (Yang *et al.*, 2003) and Chinese chive seedlings (Sun *et al.*, 2014). A NO_3^-/NH_4^+ ratio of 80:20 increased the total soluble sugars content in Chinese kale (Zhu *et al.*, 2018) and celery (Saleh *et al*., 2019).

Total Soluble Proteins and Free Amino Acids Content

In October sown crop, maximum mean total soluble proteins content was recorded for the plants that were supplied with treatment T3 (NO_3^-/NH_4^+= 50:50), whereas in off season crop, plants provided with nutrient solutions T2 resulted in maximum mean total soluble proteins content (Figure 2-e). During all growing seasons, plants

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provided with nutrient solution T2 had the maximum total free amino acids content (Figure 2-f). Likewise, ratios of 80:20 and 75:25 (NO_3^-/NH_4^+) in the nutrient solution increased the total soluble proteins and amino acids in Chinese kale (Zhu *et al.*, 2018) and in chives (Sun *et al.,* 2014), respectively. Free amino acids content in off-season sown crop was significantly more than main season crop. More accumulation of free amino acids content in off-season crop can be related with higher temperature (acting as a stress signal) during off-season sown crop than main season crop. Total soluble proteins and free amino

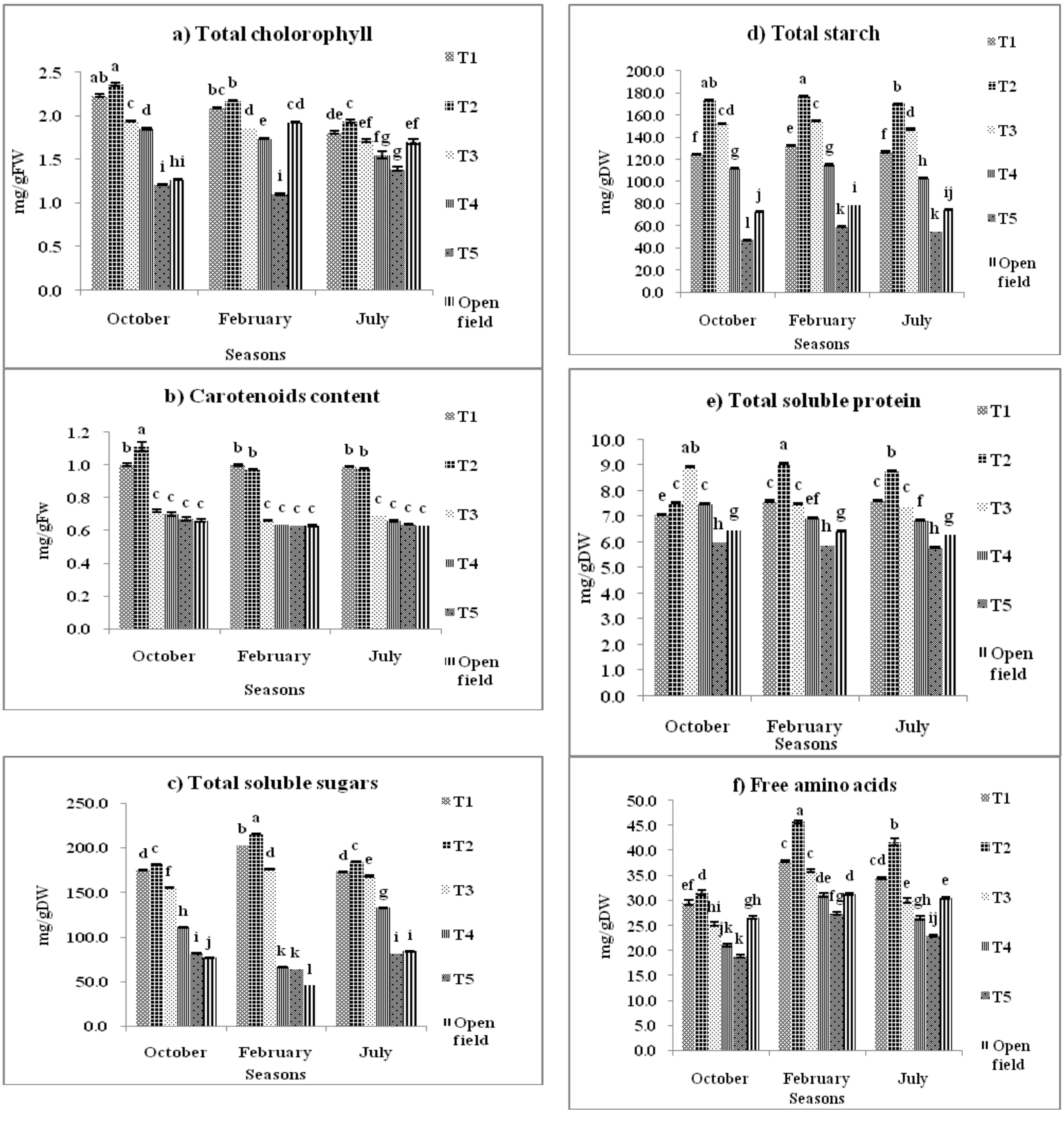

Figure 2. Effects of different NO_3^-:NH_4^+ on: (**a**) Total chlorophyll content, (**b**) Total Carotenoids content, (**c**) Total soluble sugars, (**d**) Total starch, (**e**) Total soluble proteins, and (**f**) Free amino acids content. T1 to T5 as defined previously.

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acids content has been reported more in wheat under heat stress (Kaur *et al.,* 2018).

Ascorbic Acid and α- Tocopherol Content

In the main season as well as in off-season and among different nutrient solutions, the maximum ascorbic content was recorded in the plants supplied with nutrient solution T2 (Figure 3-a). It means that a nutrient solution with lower proportion of NH_4^+ resulted in higher ascorbic acid content. Similarly, a higher ascorbic acid content was obtained with the supply of nutrient solution

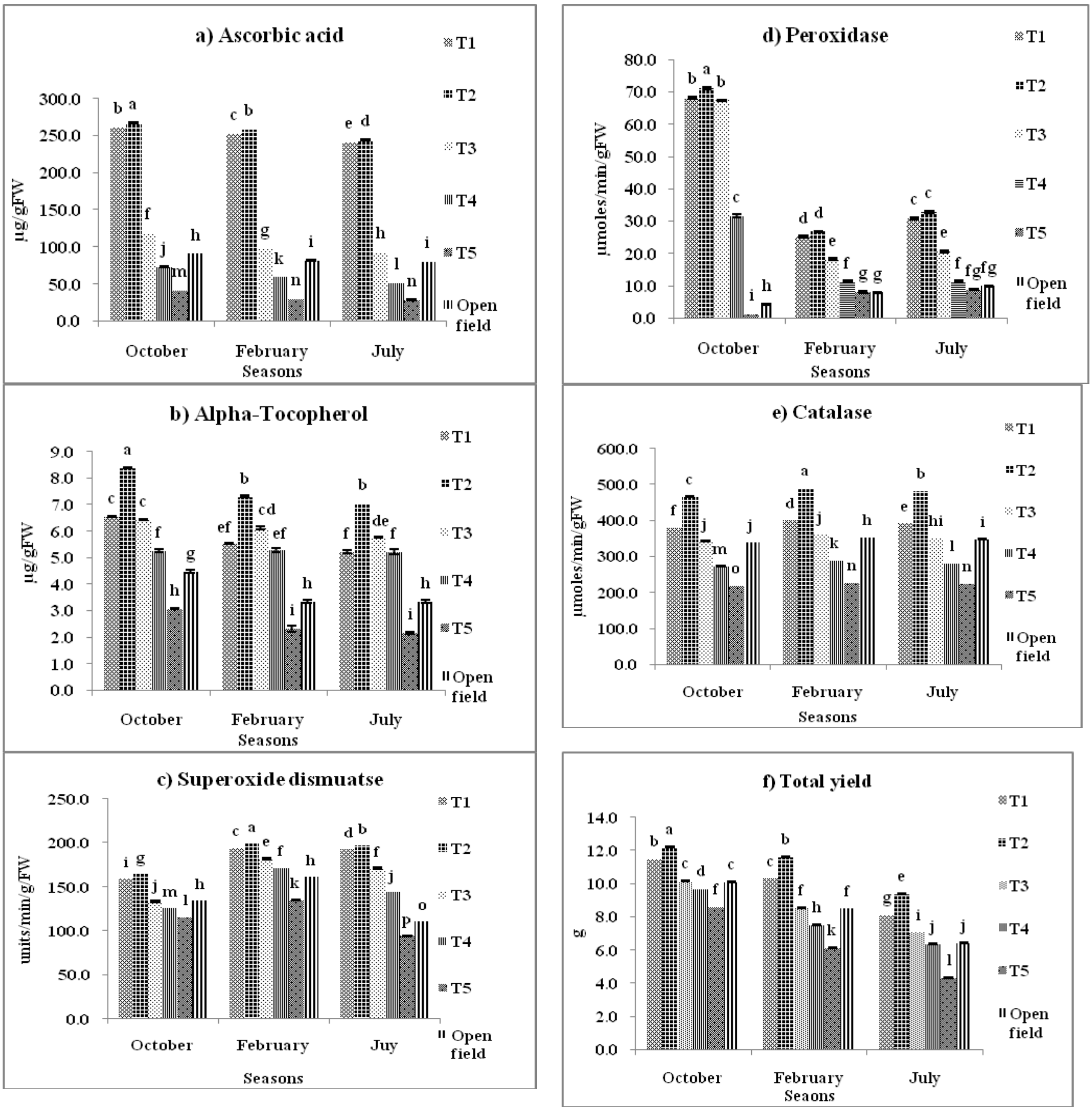

Figure 3. Effects of different NO_3^-:NH_4^+ on: (**a**) Ascorbic acid content, (**b**) Alpha-tocopherol content, (**c**) SOD activity, (**d**) POD activity, (**e**) CAT activity, and (f) Total yield/plant. T1 to T5 as defined previously.

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containing NO_3^- and NH_4^+ in the ratio of 80:20 in Chinese kale (Zhu *et al.,* 2018) and celery (Saleh *et al.*,2019) and a ratio of 3.5:1.5 (NO_3^-:NH_4^+) in basil (Saadatian *et al.,* 2014). α-tocopherol content followed the same trend as ascorbic acid content i.e. it reached the maximum in the plants that were supplied with nutrient solution T2 (Figure 3-b). In other studies, it was also shown that application of ammonium or amino acids, particularly in foliar applications or higher levels, significantly reduce ascorbic acid content of plants (Souri *et al.,* 2018a; Mohammadipour and Souri, 2019; Aghayie Noroozlo *et al.,* 2018; Kaniszewski *et al.,* 2019).

Ascorbic acid and α- tocopherol content in the leaves were higher in main season crop raised under polyhouse. Phillip *et al.* (2018) have also reported significantly more ascorbic acid content in winter (main season) as compared to summer and spring cultivated spinach, potatoes, and oranges. In all the seasons, polyhouse-grown crop had higher ascorbic acid and α-tocopherol content than the soil-grown crops. Likewise, hydroponically grown lettuce recorded more ascorbic and α- tocopherol content than soil-grown lettuce (Buchanan and Omaye 2013).

Antioxidants Activity

During all the three growing seasons, maximum SOD, POD and CAT activities were recorded in plants provided with nutrient solution T2 (Figures 3-c, -d, and e). Our results are in agreement with the findings of Sun *et al.* (2014) who observed higher activities of SOD and CAT in Chinese chive supplied with NO_3^- to NH_4^+ in the ratios of 50:50 and 75:25. Likewise, higher activities of anti-oxidant enzymes was observed in *Polygonatum odoratum*, which were grown aeroponically with low and moderate proportion of NH_4^+ mixed solutions (NO_3^-:NH_4^+= 90:10) treatment compared with treatments in which nutrient solutions contained all nitrate. NH_4^+ is an important material to synthesize amino acids, nevertheless, its superfluous usage would be toxic to plant (Zou *et al.,* 2017). Consequently, T2 nutrient solution, which had a low or moderate NH_4^+ proportion, helped in promoting the activities of anti-oxidant enzymes in the present investigation. Both SOD and CAT activities were significantly more in off-season (February and July) crop than the main season crop (October) because the plants were stressed in off-season than the main season sown crop. However, POD activity was more in main season than off-season sown crop and that can be explained by the fact that POD activity was compensated by the activity of SOD and CAT during the same seasons (Gill and Tuteja, 2010).

Total Yield

Irrespective of the season, yield in polyhouse grown plants supplied with nutrient solution T2 was more than soil-grown crop. The yield enhancement of 21.74, 21.11 and 46.19% was recorded in October, February, and July sown crops, respectively (Figure 3-f). Similar biomass enhancement with nutrient solution having NO_3^- to NH_4^+ in the ratio of 75:25 has been observed in pepper (Bar-Tal *et al.,* 2001), French beans (Guo *et al.,* 2002), taro (Osorio *et al.,* 2003), and lettuce (Wang and Shen, 2011).

CONCLUSIONS

In conclusion, in the main season as well as off-season sown coriander crop, the yield was significantly higher in polyhouse as compared to the open field sown crop. Further, the crop provided with nutrient solutions having higher proportion of NO_3^- had higher values of quality parameters and the maximum antioxidant activity compared to the open field crop. Hence, providing nutrient solutions with appropriate ratios of NO_3^-:NH_4^+ improves the yield and quality traits in polyhouse-grown coriander. In this

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study, nutrient solution having NO_3^-/NH_4^+ ratios of 75:25 promoted growth and yield and improved the nutritional quality of coriander raised under polyhouse conditions.

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اثر نسبت NO_3^- به NH_4^+ در محلول غذایی و فصل رشد بر عملکرد و کیفیت گشنیز

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چکیده

در این پژوهش، اثر محلول های غذایی دارای شکل های شیمیایی مختلف نیتروژن بر عملکرد گشنیز (کولتیوار Punjab Sugandh) در یک محیط بدون خاک در شرایط گلخانه پلاستیکی بررسی شد. کشت گیاه در سه تاریخ انجام شد: به ترتیب در اکتبر(فصل اصلی)، فوریه و ژوئیه (خارج از فصل). در گلخانه پلاستیکی، نیتروژن با نسبت های متغیر NO_3^- **به** NH_4^+ شامل ۱۰۰:۰(T1)، ۷۵:۲۵ (T2)، ۵۰:۵۰ (T3)، ۲۵:۷۵ (T4)، و ۰:۱۰۰(T5) به گونه ای افزوده شد که کل نیتروژن افزوده شده ثابت ماند. برداشت گشنیز در هر فصل در سه نوبت با فواصل ۳۰ روزه از تاریخ کشت انجام شد. در هر برداشت، پارامترهای کیفیت رشد گیاه شامل فعالیت آنتی اکسیدانی آنزیم ها اندازه گیری شد. نتایج نشان داد که فارغ از فصل رشد، عملکرد گیاه در کشت گلخانه ای به طور معناداری از کشت مزرعه

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(داخل خاک) بیشتر بود و در میان محلول های غذایی، تیمار (T2) در طول فصل شد، به طور معناداری دارای عملکرد و رشد بیشتر و پارامترهای کیفیتی بالاتری بود. از این رو، می توان نتیجه گرفت که نسبت NO_3^- **به** NH_4^+ در محلول غذایی معادل نسبت ۷۵:۲۵ موجب بهبود عملکرد و کیفیت گیاه شده و درنتیجه برای گشنیز مناسبترین تیمار است.

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