

**EFFECT OF NITRATE AND AMMONIUM RATIOS
ON PHYSIOLOGICAL AND QUALITY ATTRIBUTES
OF HYDROPONICALLY GROWN LEAFY
VEGETABLES**

Dissertation

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in partial fulfillment of the requirements
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**DOCTOR OF PHILOSOPHY
in
BOTANY
(Minor Subject: Biochemistry)**

By

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CERTIFICATE I

This is to certify that the dissertation entitled, “**Effect of nitrate and ammonium ratios on physiological and quality attributes of hydroponically grown leafy vegetables**” submitted for the degree of Ph.D., in the subject of **Botany** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ratnesh Kumari (L-2015-BS-67-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

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ABSTRACT

The effect of variable NO_3^- to NH_4^+ ratios in the nutrient solution was evaluated on growth, nutritional quality and yield attributes of hydroponically grown leafy vegetables. Spinach (cv. Punjab Green), Coriander (cv. Punjab Sugandh) and Lettuce (cv. Iceberg) grown under substrate hydroponics with intermittent supply of water and nutrients. Simultaneously crop was raised in open field by conventional practices. Crop was sown on three dates viz., October (main season) and February and July (off-season) respectively. N 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 from each respective treatment at 30 days interval starting from date of sowing. At each harvesting, growth (fresh and dry biomass of shoot and root, plant height, leaf area and total yield per plant), quality parameters (total chlorophyll, carotenoids, soluble sugars, starch, soluble proteins, free amino acids and proline) and antioxidant enzyme activities (SOD, CAT, and POD) were determined. During all the three seasons, the yield was significantly more in hydroponically than open field sown crop. Under substrate hydroponics, maximum yield was obtained in the plants provided with nutrient solution T2 (NO_3^- : NH_4^+ : 75:25) and also had higher values of quality parameters and highest antioxidants activities than the open field crop. It is suggested that NO_3^- to NH_4^+ ratio of 75:25 improve the growth, antioxidants activity, quality attributes and is thus most suitable for leafy vegetables growth under substrate hydroponics.

Key words: Ammonium, Coriander, Lettuce, Nitrate, Proline, Quality, Spinach.

Signature of Major Advisor

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ਹਾਇਡਰੋਪੋਨਿਕਲੀ ਉਗਾਈਆਂ ਸਬਜ਼ੀਆਂ ਪਾਲਕ (ਕਿਸਮ ਪੰਜਾਬ ਗ੍ਰੀਨ), ਧਨੀਆ (ਪੰਜਾਬ ਸੁਗੰਧ), ਲੈਟਸ (ਆਇਸਬਰਗ) ਦੇ ਵਿਕਾਸ, ਪੋਸਟਿਕਤਾ, ਗੁਣਵੱਤਾ ਅਤੇ ਝਾੜ ਉੱਪਰ ਤੱਤਾਂ ਦੇ ਘੋਲ ਵਿੱਚ NO_3^- ਅਤੇ NH_4^+ ਬਦਲਦੇ ਅਨੁਪਾਤ ਦਾ ਅਸਰ ਦੀ ਜਾਂਚ ਕੀਤੀ ਗਈ। ਪਾਲਕ, ਧਨੀਆ, ਲੈਟਸ ਨੂੰ ਪਾਣੀ ਅਤੇ ਤੱਤਾਂ ਦੀ ਸਪਲਾਈ ਵਿੱਚ ਉਗਾਇਆ ਗਿਆ। ਫਸਲ ਤਿੰਨ ਤਰੀਕਾਂ, ਅਕਤੂਬਰ (ਮੁੱਖ ਸੀਜਨ), ਫਰਵਰੀ ਅਤੇ ਜੁਲਾਈ (ਆਫ ਸੀਜਨ) ਵਿੱਚ ਉਗਾਇਆ ਗਿਆ। N ਦੀ ਮਾਤਰਾ ਨੂੰ ਇਕੋ ਜਿਹੀ ਰੱਖਦੇ ਹੋਏ NO_3^- ਅਤੇ NH_4^+ ਦੇ ਵੱਖ-ਵੱਖ ਅਨੁਪਾਤ ਜਿਵੇਂ 100:0 (T1), 75:25 (T2), 50:50 (T3), 25:75 (T4), 0:100 (T5) ਰਾਹੀਂ ਨਾਇਟ੍ਰੋਜਨ ਦਿੱਤੀ ਗਈ। ਹਰ ਸੀਜਨ ਵਿੱਚੋਂ ਤਾਜ਼ਾ ਪੱਤਿਆਂ ਦੀ ਤਿੰਨ ਵਾਰ ਕਟਾਈ ਕੀਤੀ ਗਈ। ਹਰ ਤੁੜਾਈ ਤੋਂ ਵਿਕਾਸ (ਤਾਜ਼ਾ ਅਤੇ ਸੁੱਕਾ ਬਾਇਓਮਾਸ, ਪੌਦਾ ਲੰਬਾਈ, ਪੱਤਾ ਖੇਤਰਫਲ, ਪ੍ਰਤੀ ਪੌਦਾ ਝਾੜ), ਗੁਣਵੱਤਾ ਮਾਪਦੰਡ (ਕੁੱਲ ਕਲੋਰੋਫਿਲ, ਕੈਰੋਟੀਨਾਇਡ, ਘੁਲਣਸ਼ੀਲ ਸ਼ੂਗਰ, ਸਟਾਰਚ, ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਟੀਨ, ਫਰੀ ਅਮੀਨੋ ਏਸਿਡ ਅਤੇ ਪ੍ਰੋਲਾਇਨ) ਅਤੇ ਐਂਟੀਆਕਸੀਡੇਂਟ ਇੰਜਾਇਮ ਕਿਰਿਆ (SOD, CAT ਅਤੇ POD) ਨਿਰਧਾਰਿਤ ਕੀਤੇ ਗਏ। ਤਿੰਨੋਂ ਸੀਜਨਾਂ ਦੌਰਾਨ ਹਾਇਡਰੋਪੋਨਿਕ ਵਿੱਚ ਝਾੜ ਖੁੱਲ੍ਹੇ ਖੇਤ ਦੇ ਮੁਕਾਬਲੇ ਵੱਧ ਸੀ। ਸਬਸਟਰੇਟ ਹਾਇਡਰੋਪੋਨਿਕ ਵਿੱਚ ਸਭ ਤੋਂ ਵੱਧ ਝਾੜ T2 ($\text{NO}_3^- : \text{NH}_4^+ :: 75:25$) ਤੱਤਾਂ ਦਾ ਘੋਲ ਪਾਇਆ ਗਿਆ ਅਤੇ ਇਸਦੀ ਗੁਣਵੱਤਾ ਅਤੇ ਐਂਟੀਆਕਸੀਡੇਂਟ ਕਿਰਿਆ ਖੁੱਲ੍ਹੇ ਖੇਤ ਵਿੱਚ ਲੱਗੀ ਫਸਲ ਤੋਂ ਵੱਧ ਸੀ। ਇਹ ਸੁਝਾਅ ਦਿੱਤਾ ਜਾਂਦਾ ਹੈ ਕਿ ਤੱਤਾਂ ਦੇ ਘੋਲ ਵਿੱਚ $\text{NO}_3^- : \text{NH}_4^+$ ਅਨੁਪਾਤ 75:25 ਵਿਕਾਸ, ਐਂਟੀਆਕਸੀਡੇਂਟ ਕਿਰਿਆ, ਗੁਣਵੱਤਾ ਮਾਪਦੰਡ ਨੂੰ ਬਿਹਤਰ ਕਰ ਸਕਦਾ ਹੈ। ਇਸ ਲਈ $\text{NO}_3^- : \text{NH}_4^+$ (75:25) ਅਨੁਪਾਤ ਵਾਲਾ ਤੱਤਾਂ ਦਾ ਘੋਲ ਸਬਸਟਰੇਟ ਹਾਇਡਰੋਪੋਨਿਕ ਤਹਿਤ ਹਰੇ ਪੱਤੇਦਾਰ ਸਬਜ਼ੀਆਂ ਪੈਦਾ ਕਰਨ ਲਈ ਢੁਕਵਾਂ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਅਮੋਨੀਅਮ, ਨਾਇਟ੍ਰੇਟ, ਧਨੀਆਂ, ਪ੍ਰੋਲੀਨ, ਗੁਣਵੱਤਾ, ਪਾਲਕ

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CHAPTER I

INTRODUCTION

Vegetables play an important role in food and nutritional security of ever growing population of our country. Leafy vegetables contain vitamins (A, B, C, E and K), minerals (Ca, Mg, Fe, and K), fibre are low in fats and carbohydrates and provide an excellent source of proteins (Singh *et al* 2006).

Nowadays there is an increase in demand of fresh vegetables in the market as the consumers are becoming aware of their health benefits. External features like dark green colour, firmness, appropriate textures and fibre content contribute towards consumers' preferences. Sugars, aminoacids, organic acids, vitamins and minerals determine quality attributes of vegetables (Weerakkody 2003). Leafy vegetables are described as an excellent source of chlorophyll and carotenoids. Carotenoids are regarded as highly nutritious for human diet as they help in protection from free radical (Gonzalez *et al* 2016).Vegetables like lettuce, coriander and spinach can provide all the nutrients which are important for human health. Moreover, an increased consumption and interest in vegetable products has been associated with the reduced risk of some types of cancer.

Lettuce (*Lactuca sativa*) is the well liked vegetable as per its consumption rate and commercial value the world over (Coelho *et al* 2005). It is grown as a leaf vegetable, but sometimes for its stem and seeds. Likewise spinach (*Spinacia oleracea*) is an important green leafy vegetable in temperate climates presenting high dietary value being rich in minerals and vitamins. Coriander (*Coriandrum sativum* L.) is a popular herb and it has high antioxidant activity due to the presence of polyphenols and phyto-chemicals (Bhat *et al* 2014). Presence of high amount of carotenoids is also responsible for its antioxidant content (Peethambaran *et al* 2012).

Demands for the fresh vegetables and fruits are increasing day by day as consumers are progressively becoming conscious of beneficial and nutritional aspects of foods. There is a huge demand for off season vegetables in local as well as international market. This demand can be met by growing vegetables in off season under protected conditions like hydroponics. This results in maximum production using less agricultural area, improves quality and also fetches huge profits to farmers.

Although vegetables production under green house is increasing but the basic knowledge regarding the optimum concentration of nutrient solution is still there to figure out. Also we need to find out the effects of various other environment factors for the production of vegetables. For achieving the maximal productivity and commercial viability, greenhouse agricultural needs to be altered (Ferguson *et al* 2014). In hydroponics system different nutrients along with water are supplied continuously to growing plants and at high

relative water potentials (Wheeler 2010, Ferguson *et al* 2014). Nutrients levels and their composition need to be optimized for efficiency and reduction of waste. Hydroponics structure can be altered to evaluate the effect of growing media on vegetable production.

In hydroponics plants are grown in a soilless medium which is having all the essential elements required by the plant for its growth, nutrition and development. Both components i.e. inorganic and organic need to be broken down into inorganic elements so they become available for plant uptake (Haddadi 2016). Hydroponics is a useful, money-making and unpolluted technology for growing plants. This system is helpful in facing the challenges of climate change and also helping in production system management for efficient utilization of natural resources and reducing dietary deficiency (Maharana *et al* 2011). Vegetables which are grown under hydroponics have a threefold increase in vitamins and minerals as compared to the plants grown in soil.

Nitrogen (N) is a particular nutrient because, unlike the other essential nutrient elements, plants can use it in two forms. Plant can use it either in the cation form i.e. ammonium (NH_4^+), or the anion form i.e. nitrate (NO_3^-) (Wang *et al* 2009). The NH_4^+ is the inorganic form of N assimilation, since nitrates and nitrites must be first reduced to NH_4^+ (Ruckert and Giani 2004) and generally recommended for application in small amounts after transplantation. According to Britto and Kronzucker (2002), if the NH_4^+ is the only N source, it generally has harmful effects on the growth of plants and may result in symptoms of toxicity in many of them. According to Tabatabaei *et al* (2008) most of the plants show stunted growth, decreased leaf size, decreased root systems, and chlorosis in severe cases when plants have high rate of NH_4^+ -N as N source. According to Simonne *et al* (2001), this is due to irreversible alteration of the structure of the thylakoid membrane. Furthermore, the plant response to continuous nutrition with NH_4^+ depends on the species. For example, spinach is sensitive. NO_3^- affects the growth of tea plant roots when it is the only source of N (Ruan *et al* 2007). A very high concentration of nitrate has been found to be associated with respiratory problems and to the formation of the carcinogenic compound in human digestive track (Taiz and Zeiger 1998).

The relative uptake of anions and cations can be changed in an efficient way by controlling the N supply and that can be done by varying the ratio of NO_3^- and NH_4^+ (Sonneveld 2002). Some NH_4^+ -N is mostly good to leafy vegetables, although they prefer NO_3^- to NH_4^+ (Wu *et al* 2003). Previous studies from greenhouse laboratory (Chen *et al* 2005) have found that different NO_3^- : NH_4^+ ratios had significantly different effects on the growth and NO_3^- contents of Chinese cabbage (*Brassica chinensis*L.) and partial replacement of NO_3^- by NH_4^+ usually resulted in a higher biomass and a lower NO_3^- content. Combined supply of both NH_4^+ and NO_3^- can result in maximum growth rates and higher plant yields (Marschner 1995). However, the optimal ratio of NO_3^- -N to NH_4^+ -N varies according to the plant species,

stage of development, environmental conditions, and concentrations supplied (Guo *et al* 2012). The $\text{NO}_3^-:\text{NH}_4^+$ ratios in the root zone offer a significant means for regulating the relative uptake of nutrients (Azarmi and Esmaeilpour 2010) and fruit quality (Clay *et al* 1984). However, $\text{NO}_3^-:\text{NH}_4^+$ uptake ratio may be considerably influenced by the actual supply ratio between these two N-forms (Sonneveld 2002). Many researchers have indicated that N form and their varying ratios can ultimately increase the chlorophyll and carotenoid contents in leafy vegetables (Kopsell *et al* 2007, Barickman and Kopsell 2016).

Leafy vegetables are the sources of antioxidants, minerals, vitamins and other nutritional contents. 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). According to Marschner (2012) N metabolism can be regulated by supply, demand for growth, and amino acid content in plant. Therefore all these mechanisms are responsible for yield and quality of crops. Plant composition can be changed by N more than any other mineral element (Marschner 2012).

Application of N with different $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio will influence different nutritional and quality parameters of vegetables. Therefore, practically controllable nitrification and understanding the relationship between $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio in soilless medium could be the main thing to boost the characteristics of leafy vegetables.

The present study was conducted with the following objectives:

1. To standardize a suitable $\text{NO}_3^-:\text{NH}_4^+$ ratio in nutrient formulation for optimum growth and yield of leafy vegetables under substrate hydroponics system with intermittent water and nutrient supply.
2. To compare the growth, physiological and biochemical attributes, yield and quality traits of leafy vegetables under different seasons and growing conditions (substrate hydroponics and open field).

CHAPTER II

REVIEW OF LITERATURE

Soil is an essential growing element of plants which provides minerals, nutrients and anchorage for plant growth. Emergences of civilization, urbanization have caused several adverse effects on soil based cultivation. Nowadays, cultivation of crops in soil is becoming more challenging because of the poor structure or texture of soil, infertility, presence of disease causing agents. Also it requires more space, more labour and also needs more volume of water in soil based cultivation (Barman *et al* 2016). In many parts of the world like urban areas, sufficient fertile arable lands are lacking because of their unfavorable geographical or topographical conditions with over population density. So soil-less cultivation might be commenced successfully and considered as alternative option for growing healthy food plants, crops or vegetables (Butler and Oebker 2006).

Soil-less cultivation mainly refers to the technique of hydroponics. Hydroponics means a technique of growing of plants in soil-less condition with their roots immersed in nutrient solution (Maharana and Koul 2011). Hydroponics crop production is increasing worldwide because it has several advantages such as; water conservation, year-round production, increased yields (Rouphael *et al* 2004). There is no need for the application of pesticides under hydroponics system. Productivity in hydroponics is high (with reduced pest problems and constant feeding of nutrients to the roots), despite limited plant growth by the low levels of CO₂ in the atmosphere, or limited light (Singh and Singh, 2012). Some sealed greenhouses inject CO₂ into their environment to help growth (CO₂ enrichment), add lights to lengthen the day and control vegetative growth and thus it lead to increase in yield. Additionally, hydroponically grown fruits and vegetables have higher nutritional value and sensory attributes as compared to soil grown produce. This has been demonstrated in carrots (Gichuhi *et al* 2009), in basil (Sgherri *et al* 2010), in lettuce (Selma *et al* 2012). It is because plants grown in soilless culture have a higher uptake of N, Mg, Na, Fe, Cu, Mn, Zn than those grown in soil (Rouphael *et al* 2004).

2.1 Techniques of Hydroponics (Sardare and Admane 2013)

A) Liquid Hydroponics method: In this method plant roots are directly suspended into the nutrient solution. It can further be classified into-

i) Circulating methods (closed system)/ Continuous flow solution culture

a) Nutrient film technique (NFT) b) Deep flow technique (DFT)

A consistent nutrient environment for the growth of plant roots is provided in flowing solution culture systems and they are automatically controlled but if the flow of solution stops for any reason then rapid plant desiccation can be experienced. Thus regular care is needed.

ii) Non-circulating method (open systems)/ Static solution culture

a) Root dipping technique b) Floating technique c) Capillary action technique

Suitable vessels for static systems include polythene beakers, pots, glass jar and containers lined with black polythene film.

B) Media culture

There is a solid medium for the roots and is named for the type of inert medium, e.g. gravel culture, sand culture, rock wool culture. There are two main variations for each medium, sub-irrigation and top-irrigation. It is further classified as: 1. Grow bag technique 2. Hanging bag technique 3. Pot technique 4. Trench or trough technique

B) Aeroponics

It is a method of growing plants where they are anchored in holes in styrofoam panels and their roots are suspended in air beneath the panel. It is usually practiced in protected structures. This method is best for growing low leafy vegetables like lettuce, spinach, etc. Fog feed technique and root mist technique are the two techniques under this group.

Shoot: root ratio, number of leaves, leaf area and stomatal conductance were higher in hydroponically grown lettuce than that of soil grown lettuce whereas dry matter partitioning of roots was higher in soil grown lettuce. As a result photosynthetic rates and partitioning of photosynthates to above ground parts (leaves) were higher in hydroponically grown lettuce (Anver *et al* 2005). A comparative study was carried out to determine ascorbic acid and tocopherol concentrations in hydroponic and soil-grown lettuces. The tocopherol and ascorbic acid content of hydroponically-grown crop was higher when compared to their soil-grown counterpart (Buchanan and Omaye 2013).

For growth and development of plants, mineral nutrients are required in optimum concentrations. N is a mineral nutrient which is required in large quantities and its availability is an important factor for normal growth and development of plants (Kraiser *et al* 2011). Vegetable production requires large quantities of this element because it is a major constituent of protein, many other related metabolites synthesis and nucleic acids, therefore plays an important role in the quality of crops (Ehaliotis *et al* 2010, Corespe- Leon *et al* 2009). Nitrogen may be supplied as nitrate (NO_3^- and/ or ammonium (NH_4^+) salts.

The use of ammonium as the sole or principal N source resulted in impaired growth and yield reductions in French bean (Guo *et al* 2002). This is ascribed to the high toxicity of ammonia at an intracellular level in lettuce (Savvas *et al* 2006). Several studies have been carried out to explain the toxic effects of ammonium (NH_4^+) in plants. For example, NH_4^+ reduced cell division and elongation, resulting in smaller plants in tobacco (Walch *et al* 2001).

Nutrient deficiency induced by NH_4^+ is caused by impaired uptake of ions, , alteration in the osmotic balance, modification of phytohormones, acidification of the root zone and

impairment of N enzyme metabolism. This has been demonstrated in blueberry, raspberry and strawberry by Claussen and Lenz (1999). Sonneveld (2002) has recommended that for the soilless culture $\text{NH}_4^+\text{-N}$ should not exceed 25% of the total- N supply for hydroponically grown vegetables and ornamentals. Many investigators have reported a stimulatory effect of $\text{NH}_4^+\text{-N}$ on plant growth and development when ammonium is not the major source of N (Sonneveld 2002 and Savvas *et al* 2003). However, the growth of many pepper plant species may be restricted when the $\text{NH}_4^+\text{-N}/\text{total-N}$ ratio exceeds a threshold value (Bar – Tal *et al* 2001). Conversely in strawberry yield and plant growth have been reported to increase when treated with $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ in the ratio of 25:75, respectively (Tabatabaei *et al* 2006).

With increasing concentration of NH_4 ratio in the solution the leaf water content in cucumber leaves decreased. Lasa *et al* (2001) reported that in NH_4^+ fed spinach plant, shoot water content was reduced. Lower water use efficiencies, reduced water uptake rates, and susceptibility to water stress could be the reasons for the reduction of water content in plants supplied with NH_4^+ (Azarmi and Esmailpour 2010). Stomatal conductance was significantly increased in cabbage (Blanke *et al* 1996) and in cucumber leaves (Azarmi and Esmailpour 2010) when the proportion of NH_4 in the nutrient medium enhanced from 0 to 75%.

In plants, NO_3^- has the advantage of being a storage form with no requirement to get accumulated in the roots as compared to NH_4^+ . There is no risk of toxicity at alkaline pH because NO_3^- nutrition results in an increase rather than the decrease in rhizosphere pH. Hence maximum growth rates and plant yields could be obtained by combined supply of both NH_4^+ and NO_3^- . When both NH_4^+ and NO_3^- are supplied, pH balance may be achieved by similar rates of H^+ production (NH_4^+ assimilation) and H^+ consumption (NO_3^- assimilation) and thus has a very low energy requirement (Wang *et al* 2009).

Effect of NO_3^- and NH_4^+ ratios on growth

A hydroponic study was conducted to see the effects of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratios on growth, nitrate uptake and organic acid levels of spinach (*Spinacia oleracea* L.) (Wang *et al* 2009). Total N concentration was 12 mmol L^{-1} in all treatments. With the $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratios being decreased from 100: 0 to 0: 100. Highest fresh and dry weight in spinach plants were recorded which were supplied with $\text{NO}_3^-\text{-N}$ as its sole nitrogen (N) source and was 6 and 5 fold higher respectively of the treatment supplied with sole $\text{NH}_4^+\text{-N}$ treatment.

For chives, the tallest plant was obtained with the 20/80 $\text{NH}_4^+/\text{NO}_3^-$ nutrient solution (Garcia *et al* 2009). Kim *et al* (2006) in rocket salad has reported that ratio of 75:25 and 50:50 ($\text{NO}_3^-/\text{NH}_4^+$) resulted in maximum plant height. The maximum plant height was observed in 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in soyabean (Chen *et al* 2009a, 2011b). For chives maximum leaf area was obtained with the 20/80 $\text{NH}_4^+/\text{NO}_3^-$ nutrient solution (González-García *et al* 2009). In petunia, ratio of 25/75 ($\text{NH}_4^+/\text{NO}_3^-$) produced greater leaf area and biomass (Young and Ryong 2001).

Likewise taro plants grown in a solution rich in NO_3^- (100:0 and 75:25) showed significantly greater growth and leaf nutrient accumulation than those grown in a solution rich in NH_4^+ (25:75 and 0:100). Root growth was inhibited when taro plants were supplied with the NH_4^+ rich solutions and plants had significantly decreased leaf area with increased proportion of NH_4^+ . Maximum leaf area was obtained at $\text{NO}_3^-:\text{NH}_4^+$ ratios of 75:25 or 100:0. (Osorio *et al* 2003).

A green house experiment was carried out to see the effect of NO_3^- to NH_4^+ ratio on growth, yield and elemental composition of hydroponically raised cucumber (Azarmi and Esmailpour (2010). Total nitrogen concentration in the leaves was increased with increased supply of NH_4^+ to the cucumber plants. Chlorophyll content in leaves of cucumber was significantly increased as the proportion of NH_4^+ in the nutrient solution was increased from 0 to 75%. The plants supplied with 75:25 ($\text{NO}_3^- : \text{NH}_4^+$) treatment had the highest total leaf area, while those plants which were supplied with 100:0 ($\text{NO}_3^- : \text{NH}_4^+$) treatment had the lowest total leaf area. The highest and lowest leaf water content were recorded in plants which were supplied with 100:0 ($\text{NO}_3^- : \text{NH}_4^+$) nutrient medium and 25:75($\text{NO}_3^- : \text{NH}_4^+$), respectively.

Biomass of roots and shoots of lettuce was found to increase with the increasing percentage of NH_4^+ -N in the nutrient solution i.e. NH_4^+ -N at 25% of the total N resulted in the highest biomass of roots and shoots. Lowest biomass of both shoots and roots was recorded in the plants which were supplied with NH_4^+ -N at 50% of the total N (Wang and Shen 2011)

Likewise the growth and major nutrient concentrations in cabbage supplied with different $\text{NH}_4^+/\text{NO}_3^-$ ratios was studied (Zhang *et al* 2007). Cabbage seedlings were grown in a greenhouse in nutrient solutions with five $\text{NH}_4^+/\text{NO}_3^-$ ratios (1:0; 0.75:0.25; 0.5:0.5; 0.25:0.75; and 0:1). It was observed that in plants where NH_4^+ was supplied as the sole source of nitrogen, both shoot and root growth was significantly decreased. Plants which were nourished with NH_4^+ as a sole source of N had a lower root length than plants in the other treatment groups. Plants supplied with nutrient solution $\text{NH}_4^+/\text{NO}_3^-$ ratio of 0.5:0.5 produced the highest shoot growth and root length.

A hydroponic study was carried out to study the effects of different $\text{NO}_3^-/\text{NH}_4^+$ ratios in nutrient solution on the yield and growth of gerbera (*Gerbera jamesonii*) cut flowers (Khalaj *et al* 2017). It was reported that different ratios of $\text{NO}_3^-/\text{NH}_4^+$ led to significance differences in inflorescence fresh weight (IFW) and inflorescence dry weight (IDW). 80:20 ($\text{NO}_3^-:\text{NH}_4^+$) and 40:60 ($\text{NO}_3^-:\text{NH}_4^+$) treatment, resulted in the highest IFW and the lowest IFW, respectively. Gerbera plants supplied with nutrient solution containing $\text{NO}_3^-:\text{NH}_4^+$ (80:20) had the highest inflorescence fresh weight (IFW) and inflorescence dry weight (IDW). Cut flowers which were provided with provided with NO_3^- as the sole nitrogen source in nutrient solution had the highest flower stalk length (FSL) and shortest FSL were obtained

from plants nourished with nutrient solution containing $\text{NO}_3^-:\text{NH}_4^+$ (40:60).

The effects of N- NO_3^- : N- NH_4^+ ratios on fruit dry matter and stem were studied in pepper plants grown in aero- hydroponic system (Bar- Tal *et al* 2001). It was observed that the maximum of fruit and stem dry matter were obtained at N- NO_3^- : N- NH_4^+ ratio of 3.7 and 3.5. However, they found that extending the N- NO_3^- : N- NH_4^+ ratio to 9:1 did not reduce fruit yield.

A green house experiment was conducted to study growth and N uptake by barley (*Hordeum vulgare* L.) supplied with five different NH_4^+ -N/ NO_3^- -N ratios at electrical conductivity of 0 and 8 dS/m. The five NH_4^+ -N/ NO_3^- -N ratios were 0/100, 25/75, 50/50, 75/25 and 100/0. Mixed supply of NH_4^+ -N and NO_3^- -N resulted in significantly more production of root and shoot dry matter than NH_4^+ or NO_3^- supplied alone. Plants provided with nutrient combination viz; NH_4^+ -N/ NO_3^- -N in the ratios, of 50/50 and 25/75 produced maximum yields. Significantly lower root and shoot dry matter yields were observed when plants were grown in 100% NH_4^+ than plants grown in 100% NO_3^- . In barley, detrimental effect of high NH_4^+ was more profound when no NO_3^- was supplied (Ali *et al* 2001).

Effects of supplied nitrogen form on growth and water uptake of French bean (*Phaseolus vulgaris* L.) plants were grown with a split-root system were compared (Guo *et al* 2002). Three nutrient combinations were applied, viz; sole nitrate (NO_3^-), sole ammonium (NH_4^+) and spatially separated supply of NO_3^- and NH_4^+ . In split-root experiment, sole nitrate supply resulted in higher root, shoot and total DM compared to sole NH_4^+ supply. Spatially separated supply resulted in higher total root DM compared to sole NH_4^+ supply. NO_3^- supplied tubes of treatment NA was more than two times higher than in NH_4^+ supplied tubes. Further, when plants having the same leaf area were transferred plants from NO_3^- to NH_4^+ supply, the water uptake rate decreased under NH_4^+ supply within 1 day.

Likewise in swiss chard plants highest accumulations for fresh mass was found at 25:75 and lowest at 100:0 ratios of NH_4^+ -N/ NO_3^- -N. FM increased 60.5% as the ratio of $\text{NH}_4^+:\text{NO}_3^-$ decreased from 100% to 0% (Barickman and Kopsell 2016; Kopsell *et al* 2007).

Watermelon plants were grown hydroponically in nutrient solutions with various $\text{NO}_3^-/\text{NH}_4^+$ ratios keeping total N concentration fixed at 3.0 mM L^{-1} (Na *et al* 2014). Plant growth was significantly influenced by $\text{NO}_3^-/\text{NH}_4^+$ ratios. As the proportion of NH_4^+ in the nutrient solution increased, dry matter (DM) decreased. The highest DM, shoot DM, and root DM were observed for plants which were supplied with 100% NO_3^- treatment in the nutrient solution. For plants with 25/75 and 0/100 $\text{NO}_3^-/\text{NH}_4^+$ ratios, whole plant biomass was reduced by 61% and 65% respectively as compared to those plants with full NO_3^- nutrition. Shoot height of plants decreased with the increasing proportion of NH_4^+ . Root growth was inhibited when the proportion of NH_4^+ in the N source supplied to the plants increased.

Rye grass seedlings were raised on nutrient solutions with different inorganic N

compositions ($\text{NO}_3^-/\text{NH}_4^+$ ratios of 100/0, 75/25, 50/50, 25/75, 0/100) at a constant N concentration of 8 mmol L^{-1} (Cao *et al* 2010). Both shoot and root growth of ryegrass was affected with NH_4^+ to NO_3^- ratio. Ryegrass plants which were supplied with 75/25 and 50/50 ($\text{NO}_3^-/\text{NH}_4^+$) ratios in the nutrient solutions had the maximum shoot dry matter and also had the maximum leaf area. With increasing proportion of NH_4^+ in the nutrient solution root length of plants was found to decrease. When NH_4^+ reached 100%, root length was the lowest compared with plants in other treatment groups containing NO_3^- .

Zou *et al* (2017) in *Polygonatum odoratum* has observed that there was no increase of plant height was compared with 0:100 ($\text{NH}_4^+:\text{NO}_3^-$) treatment, plant height with 20:80 ($\text{NH}_4^+:\text{NO}_3^-$) treatment was the highest among 10:90, 20:80, and 30:70 ($\text{NH}_4^+:\text{NO}_3^-$) treatments. Leaf area of seedling with 20:80 ($\text{NH}_4^+:\text{NO}_3^-$) treatment was the highest among 10:90, 20:80, and 30:70 ($\text{NH}_4^+:\text{NO}_3^-$) treatments, and was significantly higher than that of 0:100 ($\text{NH}_4^+:\text{NO}_3^-$) treatment.

In cucumber plant grown at high ratio of NH_4^+ the solution, concentration of Ca, K and Mg decreased in cucumber fruits (Kotsiras *et al* 2002).

Three aromatic herbs, chives (*Allium schoenoprasum*), basil (*Ocimum basilicum* L.) and dill (*Anethum graveolens* L.) were cultivated in hydroponics system with drip irrigation. A modified Steiner nutrient solution was used and was replaced in every week, pH was adjusted between 5.5 and 6.0. The chive plants which were supplied with 20/80 ($\text{NH}_4^+/\text{NO}_3^-$) treatment in nutrient solution recorded with tallest plants and had maximum leaf area. Whereas, the lowest plant height and minimum leaf area was recorded in chive plants which were supplied with NH_4^+ as sole source of nitrogen. For basil, the response of aromatic plant to the nitrogen sources was different from the response of the chives. Combined supply of both nitrate and ammonium gave the best results in relation with the leaf area of the plants treated with either NH_4^+ or NO_3^- as sole source of nitrogen. The tallest plant height was achieved when the ratio 100/0 ($\text{NH}_4^+ / \text{NO}_3^-$) was used; the results with this ratio were superior to those obtained with ratios in which NO_3^- was used in any proportion (Garcia *et al* 2009).

In lettuce plant fresh weight showed an increase under both i.e. NO_3^- -N as well as NH_4^+ -N solutions with superiority effect for NO_3^- -N solution than NH_4^+ -N solution. The NO_3^- -N solution showed also higher effect on dry weight of plants compared to NH_4^+ -N solution (El-Nemr *et al* 2012).

Goyal *et al* (1982) also reported that plant growth is notably favoured when treated with both forms of nitrogen. Greater leaf area and biomass was produced in petunia plants which were supplied with ratio of 25/75 ($\text{NH}_4^+ / \text{NO}_3^-$) (Young and Ryong 2001). The success of combining the two sources of nitrogen in plant nutrition was confirmed by Kraus *et al* (2002) in rudbeckia (herbaceous plant); with application of different $\text{NH}_4^+ / \text{NO}_3^-$ ratios (75:25,

50:50, 25:75). The authors concluded that the use of both nitrogen sources favors growth and production of herbaceous crops more than when they are used separately.

A green house experiment was conducted where strawberry plants were grown at constant nitrogen concentration of 7 mM/L and constant pH of 5.8. A balanced nutrient solution by drip irrigation system was used for the irrigation of plants. Nitrogen was supplied as $\text{NO}_3^-:\text{NH}_4^+$ ratios in four treatments. Significant effects of $\text{NO}_3^-:\text{NH}_4^+$ ratios were observed on fruit fresh weight (yield) and number of fruits. The highest yield was obtained in the plants which were supplied with the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 5.5:1.5 and other treatments were not different from each other statistically. It means that adding ammonium to the nutrient solution increased the strawberry yield (Neumann and Kafkafi 1983). Changes in fruit number had the same pattern as yield. The better growth of strawberry plants in a mixture of $\text{NO}_3^-:\text{NH}_4^+$ might be because of the neutralization of H^+ and OH^- production (Tuil 1965) in the nitrogen metabolism of NH_4^+ and NO_3^- , respectively. It is probable that minimum stress is inflicted on the cell when minimal pH changes occur (Taghavi *et al* 2004).

An experiment was carried out to study the effects of nitrogen forms on plant growth of vegetable soybean (Chen *et al* 2011). Maximum plant height was observed in plants which were supplied with 75:25 ($\text{NO}_3^-:\text{NH}_4^+$), while the minimum plant height was observed in 25:75 ($\text{NO}_3^-:\text{NH}_4^+$). The numbers of flowers and pods in NH_4^+ (75%) were significantly low compared with other ammonium-nitrate ratios in which the numbers of flowers and pods were not influenced by nitrogen forms. Both fresh and dry weights of 100 seeds and dry matter percentage were significantly lower when a high concentration of either NO_3^- (100%) or NH_4^+ (75%) was the primary nitrogen source in the solution. The fresh and dry weights of vegetable soybean seeds were reduced by 12.9%, 25.5% NO_3^- (100%), and 23.2%, 38.9% in NH_4^+ (75%), respectively, compared with those in NO_3^- (75%) in which the best plant growth vigor was observed. Dry weight of both root and shoot was significantly higher in NO_3^- (75%) than that in other ammonium-nitrate ratios. Excessive ammonium resulted in decreased biomass production in soybean. In NH_4^+ (75%), the root shoot ratio decreased by 11.5%, while the dry matter percentages of root and shoot decreased by 34.6% and 24.0%, respectively, compared with NO_3^- (75%).

Effect of NO_3^- and NH_4^+ ratios on biochemical constituents

Chlorophyll is a class of green pigments which help plants to acquire the light energy. Its content is often used as an indicator of photosynthetic capacity. It is also used as a monitor of the nutritional condition of crops (Bojovic and Stojanovic 2005, Shibghatallah *et al* 2013). Blanke *et al* (1996) in kohlrabi, Everett *et al* (2010) in douglas-fir seedling and Huang *et al* (2013) in duckweed) has demonstrated that high proportions of NH_4^+ suppressed plant growth and decreased chlorophyll content, whereas low proportions of NH_4^+ increased chlorophyll content

The effects of nitrogen (N) form and ratio on the carotenoid and chlorophylls concentrations in swiss chard leaf tissue has been investigated (Barickman and Kopsell 2016). Plants were supplied with different ratios of ammonium and nitrate. Swiss chard carotenoid concentrations were highest at a ratio of 25:75 for $\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$. There was a significant increase in Chl a and Chl b as the ratio of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ decreased. Highest accumulation for Chl a and b occurred at $\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$ (25:75) and lowest at $\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$ (100:0). Kopsell *et al* (2007) has also recorded the same observations in kale when increasing ratios of $\text{NO}_3^- \text{-N}$ were applied to plants.

Carotenoids in *Corchorus olitorius* were higher, with a maximum level of 75 mg 100 g^{-1} during the summer season in nutrient solution 50% conditions ($\text{NO}_3^-/\text{NH}_4^+ = 6:1.9$) (Giro and Ferrante (2016) in *Corchorus olitorius*, Kaulmann *et al* (2014) in *Brassica oleraceae* and plum varieties).

Similar increase in chlorophyll content in NH_4 fed plants was reported by Blanke *et al* (1996) who showed that chlorophyll content and chlorophyll a and b were enhanced in NH_4 fed kohlrabi plants. The increased leaf N concentration was accompanied by enhanced leaf chlorophyll content. The favourable effect of nitrogen on the formation of chloroplast pigments is due to a more intensive protein synthesis (Marschner 1995).

Total chlorophyll contents of *Polygonatum odoratum* seedlings with 10:90 or 20:80 ($\text{NH}_4^+/\text{NO}_3^-$) treatment was significantly higher than that of 0:100 ($\text{NH}_4^+/\text{NO}_3^-$) treatment (Zou *et al* 2017). Chlorophyll content in *Corchorus Olitorius* were higher during the summer season in nutrient solution 50% conditions ($\text{NO}_3^-/\text{NH}_4^+ = 6:1.9$) (Giro and Ferrante 2016).

In a green house experiment, Chlorophyll a, b, total chlorophyll, and carotenoid levels were significantly affected by the form of nitrogen and the cultivar, whereas the interactions of nitrogen form and cultivar were not significant. The concentrations of chlorophyll a, b, total chlorophyll, and carotenoids decreased with increasing proportion of NH_4^+ in the nutrient solution. The highest concentrations of total chlorophyll and carotenoid were found in flowers under 40:60 ($\text{NO}_3^-:\text{NH}_4^+$) treatment (Khalaj *et al* 2017).

Dong *et al* (2004) and Wang *et al* (2009) reported that increasing the ratio of $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ (25, 50 and 75%) in the nutrient solution led to a significant decrease in organic acid levels of spinach. Wang *et al* (2009) observed that NO_3^- acts as the signal to initiate coordinated changes in carbon and nitrogen metabolism and organic acid production. Malate content was highest at $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio of 25:75 and decreased at the ratio of 0:100. However, the change of the oxalate (water soluble) content was different from other organic acids, the oxalate content was highest at $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio of 100:0 and lowest at $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio of 25:75 (Wang *et al* 2009).

The effects of improved Hoagland nutrient solution with different $\text{NO}_3^-\text{-N}$ to $\text{NH}_4^+\text{-N}$ and 100:0) on nutritional quality and physiological characteristics were studied for the three

cultivars of Chinese chive under a total N concentration of 14.3 mmol/L and ratios of 25:75, 50:50, 75:25. The proline content in leaves of Chinese chive were higher when the concentration of $\text{NO}_3^- \text{-N} / \text{NH}_4^+ \text{-N}$ was 50:50 and 75:25 in the nitrogen solution than in the other solutions (Sun *et al* 2014). Proline is the primary substrate for osmotic adjustment in plant cells and plays adaptive role in stress tolerance in crops (Verbruggen and Hermans 2008).

Zhang *et al* (2007) in *Brassica campestris* has reported that there was a large difference in the proline concentration observed between the treatment groups, with proline concentration decreasing in the order of $\text{NH}_4^+ / \text{NO}_3^-$ ratio 1:0, 0.75:0.25, 0.25:0.75, 0:1, and 0.5: 0.5.

In an experiment, spinach seedlings were raised in a greenhouse and were supplied with five combinations of $\text{NH}_4^+ / \text{NO}_3^- \text{-N}$ in ratios of 0:100 (control), 25:75, 50:50, 75:25 and 100:0. Scientist reported that spinach leaf chlorophyll content differed significantly among treatments. Chlorophyll a, chlorophyll b and total chlorophyll in spinach leaves was found to decrease with increasing $\text{NH}_4^+ / \text{NO}_3^- \text{-N}$ ratios in the supplied nutrient solutions. It was reported that the increase in yield and growth were due to the reduced energy requirement. It was due to use of ammonium instead of nitrate in protein synthesis, and from increased photosynthetic capacity. Further Shaviv *et al* (1990) reported that a mixed ammonium and nitrate N source produced larger dry matter yield and protein contents in wheat as compared to ammonium or nitrate alone.

Effect of NO_3^- and NH_4^+ ratios on nutrition and quality

For vegetables, nutritional quality parameters include soluble protein, soluble sugar, ascorbic acid, tocopherol and other factors. The effects of improved Hoagland nutrient solutions with different $\text{NO}_3^- \text{-N}$ to $\text{NH}_4^+ \text{-N}$ ratios on the nutritional quality and physiological characteristics of three Chinese chive cultivars were investigated by modulating the ratio of nitrite nitrogen ($\text{NO}_3^- \text{-N}$) to ammonia nitrogen ($\text{NH}_4^+ \text{-N}$) (Sun *et al* 2014). Different nitrogen forms resulted in accumulation of different levels of soluble protein content. $\text{NO}_3^- \text{-N} : \text{NH}_4^+ \text{-N}$ ratio of 50:50 led to higher soluble protein and sugar contents in the chive leaves whereas, the leaves treated with 100:0 ($\text{NO}_3^- \text{-N} : \text{NH}_4^+ \text{-N}$) nitrogen solution maintained the lowest levels of soluble protein content. Vitamin c contents in the leaves was significantly increased with the higher proportion of NO_3^- in the solution .

Zhu *et al* (2018) who opined that 20% replacement of nitrate with ammonium in the nutrient solution i.e. $\text{NO}_3^- / \text{NH}_4^+ = 80:20$, can increase the total soluble sugars in Chinese kale. Sugars are involved in osmotic regulation activity is light dependent and influenced by season (Fallovo *et al* 2009 and Santamaria, 2006). Sugars in *Corchorus olitorius* were higher in 50% nutrient solution conditions ($\text{NO}_3^- / \text{NH}_4^+ = 6: 1.9$) and 100% nutrient solution conditions ($\text{NO}_3^- / \text{NH}_4^+ = 12: 3.8$) (Giro and Ferrante 2016, Kaulmann *et al* 2014). It has been reported

that contents of starch in the fruits of tomato were increased when NH_4^+ supply was increased in the nutrient solution (Yang *et al* 2003).

Wang *et al* 2018 has reported the same results in lettuce suggested that the soluble protein were highest in the NO_3^- -N: NH_4^+ -N ratio of 75:25. In plants, most soluble proteins are involved in the metabolism of various enzymes. It has been shown that in the supplied nutrient solution with a higher amount of amino acids the soluble protein content increased (Chen *et al* 2010; Chen *et al* 2002; Peng *et al* 2011). Zhu *et al* (2018) has reported the same results in chinese kale.

In an experiment Chen *et al* (2005) reported that from NH_4^+ -N/ NO_3^- -N ratios of 0:100 to the ratio of 25:75 and 50:50, NO_3^- contents in 3 Chinese cabbage cultivars decreased, on average, by 37 and 52%, respectively. Zhang *et al* (1990) has reported that in the leaves of spinach, the contents of soluble sugar and vitamin C were increased when NO_3^- -N to NH_4^+ -N ratio was decreased and a ratio of 50:50 (NO_3^- -N to NH_4^+ -N) provided the maximum amount of soluble sugar and Vitamin C. Vitamin C content of tobacco was found to increased by increasing light intensity but it was decreased by increasing NH_4^+ -N (Zhu *et al* 1998).

Yang *et al* (2003) concluded that the contents of soluble sugar and vitamin C in tomato fruit increased with increased NH_4^+ -N. In an experiment by Osorio *et al* (2003) high levels of NH_4^+ supply i.e. in the ratios of 25:75 and 0:100 (NO_3^- -N to NH_4^+ -N) resulted in drastic decrease in pH even in buffered solution which ultimately resulted in detrimental effect on root membranes and nutrient uptake in Taro plants.

The nitrate content of lettuce decreased with increase in the percentage of NH_4^+ -N of total N supplied, and this resulted in the highest NO_3^- content in every lettuce cultivar. It was observed that with NH_4^+ -N at 0% of the total nitrogen (N), the lowest NO_3^- content was obtained in the treatment with NH_4^+ -N at 50% of total N supplied.

A hydroponic study was carried out to evaluate the growth of two varieties of basil in function of four nutrient solutions containing different NH_4^+ / NO_3^- ratios (Saadatian *et al* 2014). They reported that the highest chlorophyll content was obtained in purple variety with nutrient solution 3 (NH_4^+ :0.5/ NO_3^- :4). Vitamin C content in basil showed that the highest vitamin C was obtained in with nutrient solution 3 (NH_4^+ :0.5/ NO_3^- :4) and 1 (NH_4^+ :1.5/ NO_3^- :3.5), respectively.

Previous studies have shown that partial replacement of nitrate with other nitrogen (N) forms affects nutritional qualities (Chen *et al* 2002, Peng *et al* 2011 and Song *et al* 2012). It has been reported that adding ammonium (in small proportion) in the nutrient solution significantly increased the VC content of Chinese kale (Zhu *et al* 2018).

Free amino acids in the chive leaves were highest in the NO_3^- -N: NH_4^+ -N ratio of 75:25 (Sun *et al* 2014) Wang *et al* (2018) in lettuce has also reported the same results. External nitrogen (N) affects the metabolism of free amino acids and protein. It can reflect the

changes in nitrogen transport, absorption, and metabolism in plants. Different nitrogen (N) forms have been shown to directly affect the content of free amino acids (Chen *et al* 2002, and Mobini *et al* 2014). In the present study, partial replacement of nitrate with other ammonium forms significantly increased the free amino acid content in all the three crops and these results are in agreement with the results obtained by Zhu *et al* (2018) in Chinese kale.

Effect of NO_3^- and NH_4^+ ratios on enzymes activity

Reactive oxygen species (ROS) are very easily formed in plant cells on the onset of osmotic stress and they can easily harm the plant cells if they are not eliminated from the plant cells (Zhang *et al* 2005). An efficient antioxidant system is important to deal with abiotic stress. Under abiotic stress, an increase in the activity of antioxidant enzymes, could be indicative of increased production of reactive oxygen species and thus suggests acceleration of a protective mechanism to reduce oxidative damage triggered by various abiotic and biotic stress. Superoxide dismutase and peroxidase are protective enzymes for the cell membrane and the most important antioxidative enzymes for scavenging reactive oxygen species. Enzyme activity of superoxide dismutase and peroxidase in leaves of chinese chive was both significantly higher in the 50:50 nitrogen solution with higher NO_3^- content. The optimum ratios of NO_3^- -N to NH_4^+ -N for protecting chinese chive against oxidative stress was 50:50 and 75:25.

An experiment was carried out to determine the antioxidant activity in gerbera flower (Khalaj *et al* 2017). Catalase and peroxidase activity varied significantly in response to NO_3^- : NH_4^+ ratios. With increased proportion of ammonium in the nutrient solution to 60%, Catalase and peroxidase enzyme activity increased. The highest and lowest Catalase and peroxidase activities were observed under 40:60 (NO_3^- : NH_4^+) and 80:20 (NO_3^- : NH_4^+) treatments respectively.

A hydroponic study was carried out to determine the most suitable nitrogen (N) source ratio of ammonium (NH_4^+) to nitrate (NO_3^-) for aeroponic culture of Chinese fragrant solomonseal *Polygonatum odoratum* (Mill.) (Zou *et al* 2017). Scientists have reported that superoxide dismutase activity of 20:80 (NH_4^+ : NO_3^-) treatment was higher than that of 0:100 (NH_4^+ : NO_3^-) treatment. The superoxide dismutase activity of 30:70 (NH_4^+ : NO_3^-) treatment showed the lowest value. Peroxidase activities showed a different style. Peroxidase activity of 10:90 (NH_4^+ : NO_3^-) treatment increased and was higher than that of 0:100 (NH_4^+ : NO_3^-) treatment. Catalase activity of 10:90 (NH_4^+ : NO_3^-) treatment was significantly higher than those of 20:80 and 30:70 (NH_4^+ : NO_3^-) treatments and it was higher than that of 0:100 (NH_4^+ : NO_3^-) treatment. Boschiero *et al* (2019) has reported the same results in sugarcane.

The effect of NH_4^+ and NO_3^- on oxidative stress reported in previous studies is heterogeneous: while some NH_4^+ -sensitive plants had a decrease of oxidative stress under NH_4^+ supply, the opposite was observed in other species (Domínguez *et al* 2008, Podgórska *et*

al 2013 and Poleskaya *et al* 2004). Although, it has been stated that NO_3^- reduction can consume the excess reductive equivalents and hence could result in lowering the oxidative stress. It remains unclear to what extent the alleviation of NH_4^+ toxicity in the presence of NO_3^- is affecting the oxidative stress (Bloom *et al* 1992, Escobar *et al* 2006 and Hachiya *et al* 2012).

In purple variety of basil with nutrient solution 2 (NH_4^+ :1/ NO_3^- :4), the highest number of antioxidant capacity was obtained and the lowest value were related to sweet variety of basil with the same nutrient solution. Sweet variety of basil showed the highest antioxidant capacity when fed with nutrient solution 3 (NH_4^+ :0.5/ NO_3^- :4) and 4 (NH_4^+ :0/ NO_3^- :5) (Saadatian *et al* 2014).

Effect of NO_3^- and NH_4^+ ratios on yield

Yield increased by 10% in sweet peppers when they were fertigated with a nutrient solution containing 80/20 (NO_3^- / NH_4^+) compared to the fertigation with NO_3^- as the sole source of nitrogen in the nutrient solution (Zornoza *et al* 1988).

Cucumber seedlings were raised hydroponically on nutrient solutions with four different NO_3^- : NH_4^+ ratios (100:0, 75:25, 50:50, 25: 75) while keeping total nitrogen concentration i.e. 200 mg l⁻¹ constant in all solutions. Plants which were grown in 75:25 (NO_3^- : NH_4^+) nutrient solution produced greater fruit yield as compared to plants grown in 25:75 (NO_3^- : NH_4^+) nutrient solution. (Azarmi and Esmailpour 2010).

It has been found that a reasonable NH_4^+ -N/ NO_3^- -N ratio (25:75) increased crop yield and reduce NO_3^- content of vegetable (Shen *et al* 2003, Dong *et al* 2004 in tomato and Chen *et al* 2005 in chinese cabbage). Moreover, with controlled nitrification, different ratios of NH_4^+ -N/ NO_3^- -N in a nutrient solution not only affected plant growth, but also the organic acid content to a large extent.

0:100 of NH_4^+ -N/ NO_3^- -N ratio resulted in obtaining higher yield (Ge 2002). Plant growth viz; root growth, is poor in NH_4^+ -N- fed plants when NH_4^+ -N concentration is high. By the supply of a combination of NH_4^+ -N and NO_3^- -N, higher growth rate and yields are obtained in many cereal plants (Marschner 1995). Spinach had the highest yield when supplied with NH_4^+ -N and NO_3^- -N in the ratio of 50:50 and 25:75 (Zhang *et al* 1990). Wang *et al* (2005) found that the biomass yield of spinach was the highest when supplied with 100% of NO_3^- -N.

With increased NH_4^+ -N/ NO_3^- -N ratios spinach dry matter yield decreased. However, there was no significant difference in the above ground dry weights between 0:100 and 25:75 treatments. Dry matter yield of spinach had higher correlation with net photosynthetic rate and the initial Rubisco activity (Xing *et al* 2015).

Strawberry raised in semi-hydroponics with a ratio of NH_4^+ : NO_3^- (40:60) had effects on yield, growth and nutrient uptake. Yield and fruit quality in various cultivars of strawberry

increased when supplied with higher proportion of NO_3^- rather than NH_4^+ in the nutrient solution i.e. 25:75 of $\text{NH}_4^+:\text{NO}_3^-$ (Choi *et al* 2001; (Tabatabaei *et al* 2006). The positive result is due to fruit size observed, i.e., length and fresh weight of the fruits. Moreover, the higher net photosynthetic rate obtained could benefit the plant growth, yield and quality of fruit. Many scientists have also showed that the maximum yield of plant was produced at 0: 100 of $\text{NH}_4^+:\text{N}/\text{NO}_3^-:\text{N}$ ratio (Ge, 2002; Wang *et al* 2005). It's appeared that, absolute and at a very higher supply of $\text{NH}_4^+:\text{N}$ tends to inhibit plant growth (Walch *et al* 2001) or to generate toxicity in aerobic crops (Britto and Kronzucker, 2002). In some plant species highest biomass production has been recorded under 100% $\text{NO}_3^-:\text{N}$ (Chance *et al* 1999).

By increasing the NO_3^- and decreasing NH_4^+ concentration in the nutrient solution, the highest total biomass production was found in chives. The 0/100 ($\text{NH}_4^+/\text{NO}_3^-$) ratio resulted in attaining the maximum yield. For basil the largest amount of biomass was produced with the ratios 40/60 ($\text{NH}_4^+/\text{NO}_3^-$) followed by the ratios 10/80, 0/100, and 100/0. Whereas for dill, the among the treatments the $\text{NH}_4^+/\text{NO}_3^-$ ratio, 40/60 yielded higher values but the increase in yield was non -significant that those achieved with the ratios 0/100, 20/80 and 100/0 (Gonzalez-Garcia *et al* 2009).

Greater biomass was produced when the plants received mixtures of $\text{NH}_4^+/\text{NO}_3^-$ (Chen *et al* (1998) in wheat and Osorio *et al* (2003) in taro plants).

Interaction between nutrient solution and variety on yield showed that the highest yield was obtained in purple and sweet variety of basil when they were grown in nutrient solution 4 ($\text{NH}_4^+:0/\text{NO}_3^-:5$) and the lowest value were related to sweet variety with nutrient solution 2 ($\text{NH}_4^+:1/\text{NO}_3^-:4$). For most plant species, an NO_3^- supply combined with low quantities of NH_4^+ results in increase in growth, but this depends on the age of the plant and the species of plant (Saadatian *et al* 2014).

In sweet peppers yield was increased by 10% when provided with a nutrient solution in ratio of 80/20 ($\text{NO}_3^-/\text{NH}_4^+$) compared to the fertigation with NO_3^- as the sole source of nitrogen in the nutrient solution (Zornoza *et al* 1988). Plant growth is notably favored when treated with both forms of nitrogen, but it is needed to find the optimal nitrate/ammonium ratios for each crop (Goyal *et al* (1982).

Yield in *Corchorus olitorius* were higher in 50% nutrient solution conditions ($\text{NO}_3^-/\text{NH}_4^+= 6: 1.9$) and 100% nutrient solution conditions ($\text{NO}_3^-/\text{NH}_4^+= 12: 3.8$) (Giro and Ferrante 2016, Kaulmann *et al* 2014). Wang and Shen (2011) has also reported that in lettuce. When purple and sweet variety of basil were grown in nutrient solution 4 ($\text{NH}_4^+:0/\text{NO}_3^-:5$) then highest yield was obtained and the lowest value were related to sweet variety with nutrient solution 2 ($\text{NH}_4^+:1/\text{NO}_3^-:4$) (Saadatian *et al* 2014).

CHAPTER - III

MATERIAL AND METHODS

The present investigation entitled, “Effect of nitrate and ammonium ratios on physiological and quality attributes of hydroponically grown leafy vegetables.” was conducted in the green houses at the College of Agricultural Engineering and Technology and at the laboratories of the Department of Botany, Punjab Agricultural University, Ludhiana during 2016-17 and 2017-18.

3.1 Location and Climate

Ludhiana is situated at 30°-56' North latitude, 75°-52' east longitude and 243 meters above the sea level. The weather is very hot (45-46°) in the summer during May and June and very cold during the winter from December to February. The minimum temperature during winter season falls down to 1°C. Total rainfall during the year is 600-700 mm. About 70% of total rainfall occurs during July to September.

3.2 Crop husbandry

The experiments were conducted on three leafy crops viz; spinach (cultivar. Punjab Green), lettuce (cultivar. Iceberg) and coriander (cultivar. Punjab Sugandh). The seeds of all the three crops were obtained from department of Vegetable Science, Punjab Agricultural University, Ludhiana. Two crops were raised simultaneously. One in open field conditions (control) and another under controlled conditions in a green house using substrate hydroponics system with intermittent supply of water and nutrients. Three crops were raised per year in the month of October, February and July respectively. Under the open field conditions all the three crops viz; spinach, lettuce and coriander were planted in 2m² plot with 3 replications during all the three seasons. For the first crop of lettuce (Oct 2016-17) the nursery was raised at field area of department of Vegetable Science during the 2nd week of September and transplanted during last week of October. Similarly for the 2nd crop of lettuce (Feb 2017-18) the nursery was raised during the 1st week of January and transplanted during mid of February. For the third crop of lettuce (July 2017-18) nursery was raised during the 2nd week of June and transplanted during last week of July. For July crop, the nursery was provided with shade by using green nets. For spinach and coriander crops, the seeds were directly sown during the 3rd week of October (2016-17), 2nd week of February (2017-18) and 2nd week of July (2017-18), respectively. For hydroponics seeds of spinach and coriander were directly sown in plastic pots (30 cm (diameter) × 30 cm (height)) on a coco peat: perlite: vermiculite (3:1:1) medium. Lettuce plantlets were transplanted in similar pots. The potting mixture for the experiments was obtained from the local suppliers. After emergence, the seedlings were thinned to six per pot for coriander, four per pot for spinach and one per pot for lettuce. All the required field management practices were followed according to the

specifications given in the Package of Practices for cultivation of Vegetables, Punjab Agricultural University, Ludhiana (Anonymous 2016).

Table 1. Sowing and harvesting schedule of hydroponically and open field raised crops

	Season		Off season
	October	February	July
Month of sowing (Spinach & Coriander)	3 rd week of October (2016 and 2017)	2 nd week of February (2017 and 2018)	2 nd week of July (2017 and 2018)
Raising of nursery (lettuce)	2 nd week of September	1 st week of January	2 nd week of June
Transplantation of plantlets (lettuce)	4 th week of October	2 nd week of february	4 th week of July
Month of harvesting	3 rd week of January (2017 and 2018)	1 st week of May (2017 and 2018)	1 st week of October (2017 and 2018)

3.2.1 Hydroponics system

All the three crops were raised under substrate hydroponics system with intermittent supply of water and nutrients under green house. Environmental data was recorded weekly from 10 am to 5 pm, inside the greenhouse for the entire duration of crop. Temperature measurements were done using a mercury thermometer. The temperature inside the polyhouse was maintained by using heaters in winters and coolers and water sprinklers in summers. Moreover, the polyhouse was shaded using green net to block sun rays during months of July till end of September. The average day maximum temperatures were 25.2°C during season crop (October- January), 28.1°C during the off season crops (February-May) and 28.3°C (July-October). The average day minimum temperatures were 11.4°C during October-January, 16.7 °C during February-May) and 16.9°C (July-October). The range of temperature for season crop was between 18.2°C and 25.2°C and for off- season crop, it was between 16.2°C and 28.3°C.

3.2.2 Nutrient solution

The basal nutrient solution was a modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) that contained all the macronutrients viz; potassium nitrate, calcium nitrate, calcium chloride, ammonium sulphate, potassium dihydrogen sulphate, magnesium sulphate and potassium chloride. Nutrients were provided by using substrate hydroponics system with intermittent supply of water and nutrients. For micro-nutrients, a commercially available micronutrients formulation was supplied. There were five treatments with varying ratios of NO₃⁻-N and NH₄⁺-N while keeping the total N content constant (Table.2) NO₃⁻ was supplied as Ca (NO₃)₂ and NH₄⁺ as (NH₄)₂ SO₄, respectively. The pH of the solution was adjusted to 6.0 ± 0.2 with dilute sodium hydroxide (NaOH) or hydrochloric acid (HCl). The nutrient solutions were contained in plastics drums and supplied to respective pots (as per treatment) with the

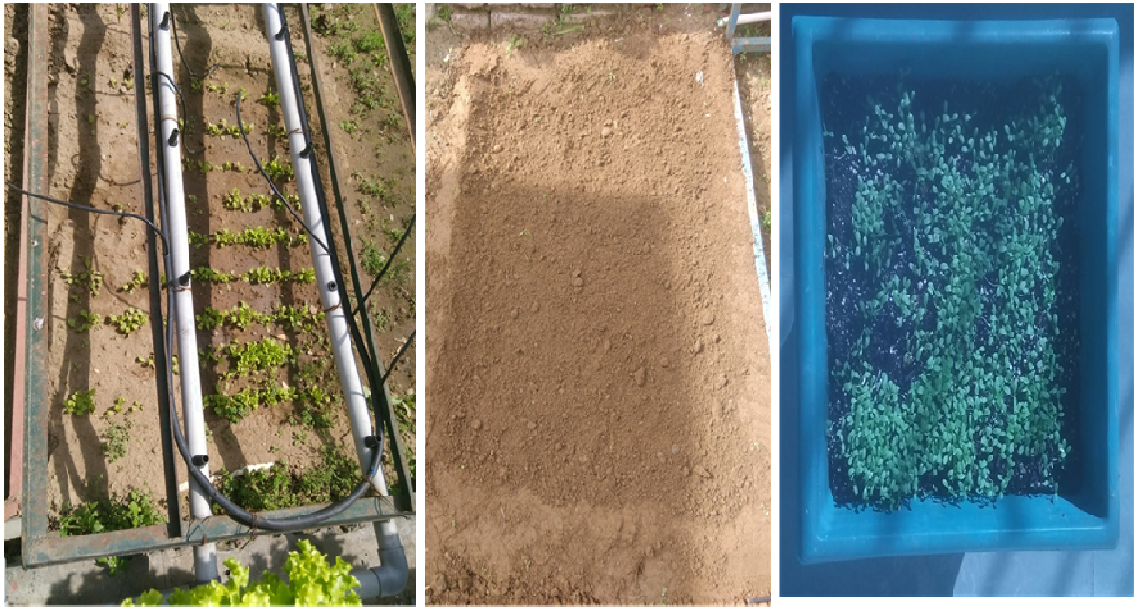


Plate 1 : Raising of nursery in the field (Lettuce)



Plate 2 : Crops raised in the field (Spinach, Coriander & Lettuce)



Plate 3 : Crops raised in a green house using substrate hydroponics system with intermittent supply of water and nutrients

help of plastic pipes. Nutrient solutions were pumped into the pipes with the help of electric motor and the surplus was collected in a return tank. Approximately 2L nutrient solution was added to each pot during the experiment. Nutrient solution was made fresh every 10 days.

3.2.3 Experiment layout and statistical analysis:

Main plot (nutrient solution treatments) :	5
Sub plots (hydroponic systems) :	1
Sub- sub plots (growing seasons) :	3
Replicates :	3
Total number of pots :	45

The data on morpho-physiological, biochemical, nutritional quality and antioxidant activity parameters of cultivars of spinach, coriander and lettuce was analyzed using Tukey's range test. Critical differences at 5% level of probability was computed to compare the statistical significance among treatments and three crop seasons. Analysis of variance was conducted for various parameters using SPSS (Version 16.0 SPSS) software and were marked with lower-case letters in tables and figures.

Table 2. Compositions (mmol L⁻¹) of nutrient solutions with different NO₃⁻/NH₄⁺ ratios but the same N concentration (8 mmol L⁻¹)

Treatments	NO ₃ ⁻ :NH ₄ ⁺ ratio	Source of nutrients (mmolL ⁻¹) (mgL ⁻¹)						
		KNO ₃	Ca(NO ₃) ₂ ·4H ₂ O	CaCl ₂ ·2H ₂ O	(NH ₄) ₂ SO ₄	KH ₂ PO ₄	MgSO ₄ ·7H ₂ O	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

3.3 Observations

For each season, crop was harvested one month after sowing. At harvesting, foliage was cut close to the base of the plant with a pair of scissors. Thereafter, two more harvestings were done at an interval of one month each. Data represented for various parameters is a mean value of 2 years.

3.3.1: Morpho-physiological parameters:

The morpho-physiological observations were recorded from one plant from each replication. The data were expressed as a mean value.

- i. **Plant height (cm):** The length of the main stem was measured in cm from the ground level to the tip of the top most leaf using a cm scale.
- ii. **Leaf water potential:** Leaf water potential is the thermodynamic expression of the water status of the leaves and was measured with a water potential measuring

instrument (PSYPRO, ELI Tech Group WESCOR ®). Leaf discs were cut with a cork borer of 6mm diameter from leaves and placed in the disc chamber of the instrument for 30 seconds to obtain the stable readings.

- iii. **Leaf area (cm²):** For measuring leaf area, all leaves were collected and passed through LI-COR(LI-3100) leaf area measuring machine.
- iv. **Fresh weight of shoot and root (one, two and three months, respectively after sowing) (g):** After 30-35 days of growth, leaves of all the three crops viz; spinach, coriander and lettuce were harvested and divided into shoots and roots and their fresh weight was measured.
- v. **Dry weight of shoot and root (g):** Plant shoots and roots were dried in air oven at 60°C for 48 hours and their weight was measured.

3.3.2: Biochemical observations

Total chlorophyll and carotenoid content, total soluble sugars, total starch, total soluble proteins, amino acids and proline content were estimated from leaves (at one, two and three months after sowing).

i. **Total Chlorophyll and Carotenoid content:** (Anderson and Boardman 1964).

Reagents:

A: 80% acetone

Fresh leaves (100 mg) were taken and homogenised thoroughly in pestle mortar with 3.0 ml of 80% acetone and transferred to centrifuge tubes. These were then centrifuged at 3000 rpm for 10 minutes and a clear green coloured supernatant was collected in the test tube. Pellet was extracted again with 2.0 ml of 80% acetone and re-centrifuged. The two supernatants were pooled and final volume was adjusted to 10 ml with 80% acetone. The absorbance of this extract was then read at 645, 665 and 480 nm, respectively. 80% acetone was used as blank. The amount of chlorophyll was calculated by following formula:-

$$\text{Total Chlorophyll} = 20.2 (A_{645}) + 8.02 (A_{665}) \times \frac{V}{1000 \times W}$$

The carotenoid content was determined using following formula:-

$$\text{Carotenoid} = A_{480} + 0.114 A_{665} - 0.638 A_{645} \times \frac{V}{1000 \times W}$$

A_{480} = Absorbance at 480 nm; A_{665} = Absorbance at 665 nm; A_{645} = Absorbance at 645 nm; V = Total volume of the extract (ml); W = Weight of the sample (g)

The value of chlorophyll content and carotenoid content were expressed as mg g⁻¹ fresh weight.

ii. **Extraction and estimation of total soluble sugars (Dubois *et al* 1956)**

Principle: Sugars react with concentrated sulphuric acid to form a dehydration product i.e. furfural or 5-hydroxymethyl furfural. This dehydration product then reacts with phenol which

acts as a chromophore and gives orange yellow colour.

Reagents: A: 80% ethanol

B: 5% phenol

C: Chilled concentrated sulphuric acid

Extraction: Dry leaf tissue (100mg) was homogenized in pestle and mortar and extracted with 80% ethanol and centrifuged at 3000 rpm for 15 minutes. The extraction procedure was repeated twice. The supernatants were pooled and then ethanol was evaporated. The final volume was adjusted to 50 ml with distilled water. This extract was used for estimation of total soluble sugars.

Estimation: To 0.2 mL of extract, 1.0 mL of 5% phenol was added. The solution was kept for 10 minutes followed by addition of 5.0 mL of chilled concentrated H_2SO_4 with constant shaking. After 10 minutes, the tubes were cooled to room temperature under running tap water. The absorbance was recorded at 490 nm in spectrophotometer against 80% ethanol as blank. The concentration of total soluble sugars (as glucose) was calculated from glucose standards run simultaneously and the total soluble sugar content was expressed as $mg\ g^{-1}\ dw$.

iii. Extraction and estimation of total starch (Dubois *et al* 1956)

Principle: Starch is hydrolyzed with the help of HCl and free sugars are released which then form dehydration product with concentrated sulphuric acid. This dehydration product then reacts with phenol which acts as a chromophore and gives orange yellow colour.

Reagents: A: 80% ethanol

B: 5N HCl

C: 5% phenol

D: Chilled concentrated sulphuric acid

Extraction: The pellet left after the extraction of sugars was retained and washed with 80% ethanol to remove all traces of total soluble sugars. The pellet was then extracted for starch by boiling with 5 ml of 5N HCl. The extraction was repeated twice and supernatants were pooled to be used for starch estimation.

Estimation: To 0.1 ml of starch extract, 1.0 ml of 5% phenol was added. The solution was kept for 10 minutes followed by addition of 5.0 ml of concentrated H_2SO_4 . The sulphuric acid was poured directly in the middle of the test tube to ensure proper mixing of the solutions. After 10 minutes, the tubes were cooled to room temperature under running tap water. After another 20 minutes the absorbance was measured at 490 nm in spectrophotometer against blank. The concentration of total starch was calculated from starch standards (10-60 μg) run simultaneously and expressed as $mg\ g^{-1}\ DW$.

iv. Extraction and estimation of total soluble proteins (Lowry *et al* 1951)

Principle: Proteins (peptide bonds) in the sample react with copper tartarate complex in alkaline solution. The protein-copper complex then reduces phospho- molybdate of folin

reagent to a blue-coloured complex having maximum absorbance at 520 nm.

Reagents A: 2% sodium carbonate in 0.1 N NaOH

B: 0.5% copper sulphate in 0.1% sodium potassium tartarate

C: 50 ml of reagent A mixed with 1 ml of reagent B

D: Folin and Ciocalteu's reagent (2N) diluted with water in 1:1 ratio

Extraction: The oven-dried leaf (0.1 g) sample was ground in 5 ml of 0.1 N NaOH followed by centrifugation at 5000 rpm for 10 min. The extraction procedure was repeated twice and total volume was made to 10 ml. To 1 ml aliquot of protein extract, 1 ml of 15 % trichloroacetic acid (TCA) was added and kept at 4 °C for 24 hours. This was centrifuged for 20 min at 5000 rpm and precipitates so obtained were dissolved in 0.1 NaOH.

Estimation: To 0.2 ml of protein extract, 5 ml of reagent C was added and the mixture was constantly shaken and kept at room temperature for 10 min. To this 0.5 ml of reagent D was added and kept at 37 °C for 30 min. The blue colour thus developed was measured at 570 nm against a blank. Protein content was estimated by comparing with a standard curve prepared by using bovine serum albumin (BSA) and expressed as mg protein g⁻¹ DW.

v. Extraction and estimation of free amino acids (Lee and Takahashi 1966)

Principle: The amino-group in the amino-acid reduces ninhydrin, thus giving rise to ammonia, carbon-dioxide and an aldehyde. This reduced ninhydrin further reacts with the oxidized ninhydrin which produces purple-violet coloured complex having maximum absorbance at 570 nm.

Reagents: Ninhydrin reagent: Reagent was prepared by mixing the following constituents (A, B and C) in the ratio of 5:12:2

A: 1% ninhydrin in 0.5 M citrate buffer (pH 5.5)

B: Pure glycerol

C: 0.5 M citrate buffer (pH 5.5)

Extraction: Dry leaf tissue (100mg) was crushed in 3 ml of 80 % ethanol followed by centrifugation. The extraction was repeated with 2 ml of 80% ethanol and total volume of pooled supernatant was adjusted to 10 ml.

Estimation: Ninhydrin reagent (5.0 ml) was added to 0.1 ml of ethanol extract. The contents were shaken vigorously and the reaction mixture was boiled for 12 minutes in boiling water bath and then cooled at room temperature. Absorbance was recorded at 570 nm with spectrophotometer against a blank of 80% ethanol replacing the extract. The standard curve was prepared using glycine (10-100 µg) as a standard amino acid. The amino acid content was expressed as mg amino acid g⁻¹ dw.

vi. Extraction and estimation of proline (Bates *et al* 1973)

Reagents:

A: Acid ninhydrin: For preparing acid ninhydrin reagent, 1.25 g of ninhydrin was dissolved

in 30 ml of glacial acetic acid by warming gently followed by addition of 20 ml of 6 M phosphoric acid with constant stirring. This reagent was kept cool at 4°C and it remained stable for 24 hour.

B: Toluene

C: Glacial acetic acid

Extraction: Fresh leaves (300mg each) were extracted with 4 ml of 3% aqueous sulfosalicylic acid (Bates *et al* 1973). The supernatant was collected.

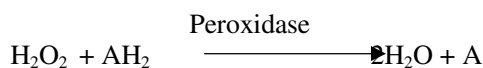
Estimation: To 2ml of supernatant, acidic ninhydrin (2ml) solution and glacial acetic acid (2ml) were added. The tubes were kept in a water bath at 100°C for 1 hour. Thereafter, termination of reaction was done in an ice bath. After this, in the reaction mixture 4 ml toluene was added and mixed properly with a test tube stirrer for 15-20 s. The OD value of upper toluene layer was measured at 520 nm against the reagent blank which is toluene. Using proline standards (0.02 to 0.1 µmoles), the proline content was determined as µg g⁻¹ F W.

3.3.3: Enzyme activity: The activities of antioxidants enzymes viz., peroxidase, catalase and superoxide dismutase were estimated from leaves (at one, two and three months after sowing). For determination of enzymatic antioxidants, the enzymes were extracted with extraction buffers at 4°C to lower the denaturation of the enzymes and assay was done at 30°C. Tissue sample (0.1 g) were extracted with 2000 µl of chilled 0.1M potassium phosphate buffer (pH 7.5) consisting 1mM EDTA, 0.01 M β-mercaptoethanol and 1% PVP using pre-chilled pestle and mortar. The extracted sample was centrifuged at 10,000×g for 25 minutes at 4°C. The pellet was discarded and the remaining supernatant was used for further estimation of enzymes such as SOD, CAT and POD.

i. Peroxidase (Shannon *et al* 1966)

Reaction

Peroxidase catalyses the breakdown of H₂O₂ at the expense of electron acceptors, such as ascorbate, quinones and cytochrome C.



Reagents:

A: Guaiacol (0.05 M)

B: H₂O₂ (0.8 M)

Assays

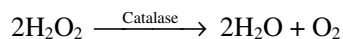
The reaction mixture contained 3000 µl of 0.05 M guaiacol, 200 µl of enzymes extract and 100 µl of 0.8 M H₂O₂. The reaction was started by adding 1000 µl of H₂O₂ and rate of change in optical density was measured at 470 nm using spectrophotometer at an interval of 30 seconds for 3 min. Peroxidase activity has been defined as increase in

absorbance/min/mg protein.

ii. Catalase (CAT) (Chance and Maehly 1955)

Reaction

Catalase catalyses the conversion of H₂O₂ to H₂O using H₂O₂ as electron donor.



Reagents

A: Sodium phosphate buffer (0.05 M, pH 7.5)

B: H₂O₂ solution: 200 µl of 30% H₂O₂ was diluted to 50 ml in 0.05 M sodium phosphate buffer (pH 7.5)

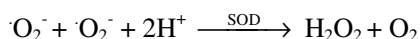
Assay

The reaction mixture contained 1800 µl of 0.05 M sodium phosphate buffer having pH 7.5 and 200 µl of enzyme extract were added in spectrophotometric cuvette. The reaction was started with the addition of H₂O₂. The enzyme activity was measured at an intervals of 30 seconds upto 3 minutes by decrease in optical density at 240 nm. The activity was expressed as nmoles of H₂O₂ decomposed/min/mg protein and molar extinction coefficient is 0.0394 mM⁻¹/cm.

iii. Superoxide dismutase (SOD) (Marklund and Marklund 1974)

Reaction

Superoxide anion free radicals ·O₂⁻ were generated by the transfer of a single electron to O₂, which resulted in the formation of free radical chain reaction.”



Reagents

A: Pyrogallol (6 mM)

B: EDTA-disodium salt (6 mM)

C: Tris-HCl buffer (0.1 M, pH 8.2)

Assay

The reaction mixture contained 1.4 ml of Tris-HCl buffer, 500 µl of 6m M of EDTA and 1000 µl of 6 mM pyrogallol were added in the spectrophotometric cuvette. A unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank and measured at 420 nm.

3.3.4: Yield and quality traits:

Total yield, ascorbic acid and α -tocopherol were measured and calculated.

i. Total yield (Kg/plant)

Leaves from each plant were collected separately at maturity and weighted. Yield was expressed in g per plant.

ii. Ascorbic acid content (Ranganna, 1986)

Reagents:

- A: Trichloroacetic acid (TCA) solution: 0.5mM of Disodium EDTA in 3% TCA.
- B: Dinitrophenyl hydrazine sol. (DNPH): 2.0 g of 2, 4 DNPH + 100ml of 9N H₂SO₄
- C: Thiourea solution: 10% thiourea in 70% ethanol
- D: Sulphuric acid (80%)

Extraction: Fresh leaves (0.1g) were homogenized in 0.5 mM Na₂-EDTA in 3% TCA followed by centrifugation at 5000 rpm. Extraction procedure was repeated twice and pooled supernatant was used for the assay.

Estimation: To 0.2 ml extract added 1.0 ml of 2% DNPH solution, followed by the addition of one drop of thiourea reagent. The glass tubes were boiled in water bath for 15 minutes and then left to cool at room temperature followed by cooling at 0°C. To the reaction mixture, 2.5 ml of 80% (v/v) H₂SO₄ was added drop wise. The absorbance was read at 540 nm. The ascorbic acid content (μg ascorbic acid g⁻¹ fresh weight) was calculated as:

$$\text{Ascorbic acid} = \frac{(10 \times \text{O.D.} \times \text{Total Centrifuge Volume})}{(0.300 \times \text{Volume of sample taken for estimation} \times \text{Fresh weight})}$$

iii. α -tocopherol content (Jayaraman,1981)

Reagents:

- A: Ethanol
- B: α , α -dipyridyl solution (0.5% in ethanol)
- C: Ferric chloride solution (FeCl₃): 0.2% in Ethanol.

Extraction: Total lipids were extracted by the cold extraction method of Folch *et al* (1957). 100 mg of fresh leaves were homogenized using pestle and mortar in chloroform: methanol (2:1 v/v). Isopropanol (10% in methanol) was added to stop the activity of phospholipases. This mixture was transferred to conical flasks and shaken well for an hour. After filtration through sintered funnel, the residue was again homogenized with chloroform: methanol solvent and filtered. The two extracts were pooled to get the crude lipid fraction. The extracts were then transferred to separating funnel followed by addition of 0.9 N NaCl (1/5th of the total volume) to make the extract free of impurities. The contents were shaken well and allowed to separate over-night. After 24 hours, the lower layer containing chloroform and lipid was collected, while the upper layer with dissolved impurities in it with NaCl was discarded. After giving three washings, each with 10.0 ml chloroform to remove residue lipids, chloroform washings were pooled with earlier lipid extract and final volume was made to 50.0 ml. This extract was used to determine tocopherol content (Jayaraman 1981).

Estimation: To 2.0 ml of the prepared lipid extract, added 1.6 ml of ethanol. To this reaction mixture, 0.2 ml of α , α -dipyridyl solution and 0.2 ml of FeCl₃ reagents were added. Mixed the reaction contents well and kept the tubes in the dark till the development of intense red

colour. After that 4.0 ml of distilled water was added to each test tube. Absorbance of the upper aqueous layer was recorded at 520 nm. The α -tocopherol content was expressed as μg tocopherol g^{-1} fresh weight.

CHAPTER IV

RESULTS AND DISCUSSIONS

Demands for the fresh vegetables and fruits are increasing day by day as consumers are progressively becoming conscious of beneficial and nutritional aspects of foods. There is a huge demand for off season vegetables in local as well as international market. This demand can be met by growing vegetables in off season under protected conditions like hydroponics. This results in maximum production using less agricultural area, improves quality and also fetches huge profits to farmers.

In hydroponics system different nutrients along with water are supplied continuously to growing plants and at high relative water potentials (Wheeler 2010, Ferguson *et al* 2014). N is an essential mineral required in large quantities and their availability is an important factor for normal growth and development of plants (Kraiser *et al*, 2011). Vegetable production requires large quantities of this element (Ehaliotis *et al*, 2010), because it is a constituent of protein, related with synthesis of many metabolites and nucleic acids (Coraspe-León *et al*, 2009 in potato) , therefore plays an important role in the quality of crops. According to Marschner (2012) N metabolism can be regulated by supply, demand for growth, and amino acid content in plant. Therefore all these mechanisms are responsible for yield and quality of crops. Plants can use N in two forms, i.e. cation (NH_4^+), or anion (NO_3^-) (Wang *et al* 2009). Most of plant prefers NO_3^- because high concentration of NH_4^+ is toxic for its metabolism. NO_3^- has the advantage of being a storage form in plants with no necessity to be assimilated in the roots. In addition, NO_3^- nutrition induces an increase rather than the decrease in rhizosphere pH and there is no risk of toxicity at alkaline pH (Dev and Herbert 2002).

When the plants are supplied with higher proportion of ammonium (NH_4^+), most of the plants show decrease in growth, leaf size, root systems, and even loss of chlorophyll in severe cases (Tabatabaei *et al* 2006 in strawberry). Osorio *et al* (2003) has observed that a very high levels of NH_4^+ supply i.e. ratios of 25:75 and 0:100 (NO_3^- -N to NH_4^+ -N) resulted in drastic decrease in pH even in buffered solution which ultimately resulted in detrimental effect on root membranes and nutrient uptake in Taro plants. The negative effect of NH_4^+ on plant growth has been attributed to various factors such as changes in medium pH and toxic effects of free NH_4^+ . Hydrogen (H^+) ions move out from the roots when the plants take NH_4^+ as sole source of N which ultimately results in acidity of the rhizosphere. It could be the possible reasons for the toxicity symptoms and detrimental effects of NH_4^+ as free ammonium irreversibly disrupts the structure of the thylakoid membrane (Simonne *et al*, 2001). Also NH_4^+ ion is a photophosphorylation uncoupler so ammonium ion accumulation in spinach plants can reduce the net photosynthesis and hence plant growth (Lasa *et al*, 2001). Similarly,

uptake of NO_3^- ions by the plants as sole source of N results in an efflux of OH^- ions in the nutrient solution lead to increase in alkalinity of nutrient solution. This can also lead to imbalance in nutrient absorption by the plant and hence has negative effects on the plant growth and metabolism. NO_3^- affects the growth of tea plant roots when it is the only source of N (Ruan *et al* 2007). A very high concentration of nitrate has been found to be associated with respiratory problems and to the formation of the carcinogenic compound in human digestive track (Taiz and Zeiger 1998).

When both NH_4^+ and NO_3^- are supplied, pH stat may be achieved by similar rates of H^+ production (NH_4^+ assimilation) and H^+ consumption (NO_3^- assimilation) and thus has a very low energy requirement (Wang *et al*, 2009). Controlling the N supply by varying the ratio of NO_3^- and NH_4^+ could be an efficient way for changing the relative uptake of anions and cations (Sonneveld 2002). Wang *et al* (2005) found that the biomass yield of spinach was the highest in the treatment of 100% of NO_3^- -N. Zhang *et al* (1990) reported highest yield in spinach plants when were supplied with ratios of 50:50 and 75:25 (NO_3^- -N: NH_4^+ -N). *Fraxinus mandshurica* seedlings supplied with a ratio of 75:25 (NO_3^- -N: NH_4^+ -N) showed higher chlorophyll content and biomass production (Wu *et al* 2003).

Application of N with different NH_4^+ -N/ NO_3^- -N ratio can influence different nutritional and quality parameters of vegetables. Therefore, practically controllable nitrification and understanding the relationship between NH_4^+ -N/ NO_3^- N ratio in soilless medium could be the main thing to boost the characteristics of leafy vegetables.

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 leafy vegetables under substrate hydroponics system with intermittent water and nutrient supply.
2. To compare the growth, physiological and biochemical attributes, yield and quality traits of leafy vegetables under different seasons and growing conditions (substrate hydroponics and open field).

4.1. Morpho-physiological parameters

4.1.1 Plant height

Plant height is a selection criterion for screening vigorous plants, and is an index of growth and development. In spinach, during all the three seasons, (October, February and July) maximum mean plant height was observed in the plants which were provided with treatment T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$), whereas least plant height was recorded for the treatment T5 ($\text{NO}_3^-/\text{NH}_4^+ = 0:100$) (Table 3). Hydroponically grown spinach plants showed significantly increased plant height by 35.80%, 38.19% and 64.54% with treatment T1 in October, February and July respectively as compared to open sown crop. In coriander, during all the three seasons, (October, February and July) maximum mean plant height recorded in

the plants which were provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$), whereas least plant height was recorded for the treatment T5 ($\text{NO}_3^-/\text{NH}_4^+ :: 0:100$) (Table 3). Likewise in lettuce, maximum mean plant height recorded in the plants which were provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$), whereas least plant height was recorded for the treatment T5 ($\text{NO}_3^-/\text{NH}_4^+ = 0:100$) during all the three seasons, (October, February and July). In coriander and lettuce, plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) under hydroponics showed increased plant height by 47.56% and 44.31% in October, 80.76% and 111.00% in February and 78.62% and 88.96% in July respectively, as compared to open field sown crops. In all the three crops (spinach, coriander, lettuce) among the seasons, plant height was significantly more in main season (October) as compared to off seasons (February and July) sown crops. Increased plant height under hydroponics can be related to controlled environmental conditions inside the green house and also better use of nutrients and water by the growing plants which led to more vegetative growth. Shoot height of melon plants was decreased when supplied with NO_3^- and NH_4^+ in the ratio of 0:100 (Na *et al* (2014)). Supply of NO_3^- -N and NH_4^+ -N in the ratio of 75:25, resulted in increased plant height in strawberry (Tabatabaei *et al* 2006), rocket salad (Kim *et al* 2006) and soyabean (Chen *et al* 2011). Gonzalez-Garcia *et al* (2009) in chives Zou *et al* (2017) in *Polygonatum Odoratum* obtained maximum plant height when plants were supplied with NO_3^- and NH_4^+ in the ratio of 80:20.

4.1.2 Leaf water potential

In spinach, October sown crop (main season) lower values (more negative) of water potential were recorded in leaves supplied with treatments T2 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$) as compared to open field conditions and plants supplied with treatments T4 ($\text{NO}_3^-:\text{NH}_4^+::25:75$) and T5($\text{NO}_3^-:\text{NH}_4^+::0:100$) maintained the higher leaf water potentials (less negative values) (Table 4). In off season crops (February and July) lower values (more negative) of water potential were recorded in leaves supplied with treatments T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) followed by T1($\text{NO}_3^-:\text{NH}_4^+::100:0$) as compared to open field conditions and plants supplied with treatments T4 ($\text{NO}_3^-:\text{NH}_4^+::25:75$) and T5($\text{NO}_3^-:\text{NH}_4^+::0:100$) maintained the higher leaf water potentials (less negative values).

In coriander lower values (more negative) of water potential were recorded in leaves supplied with treatments T2 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$) followed by treatment T1 ($\text{NO}_3^-:\text{NH}_4^+:: 100:0$) as compared to open field conditions and plants supplied with treatments T4 ($\text{NO}_3^-:\text{NH}_4^+::25:75$) and T5($\text{NO}_3^-:\text{NH}_4^+::0:100$) maintained the higher leaf water potentials (less negative values) (Table 4). Similarly in lettuce during all the three growing seasons, more negative values (less leaf water potential) were observed in the plants which were provided with nutrient solutions T2 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$) followed by nutrient solution T1 ($\text{NO}_3^-:\text{NH}_4^+:: 100:0$) and less negative leaf water potential values (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 ($\text{NO}_3^-:\text{NH}_4^+:: 25:75$) and T5 ($\text{NO}_3^-:\text{NH}_4^+:: 0:100$) (Table 4). The reduction in water potential is a consequence of low water uptake and hydraulic flow rates within plants, or of high rates of water loss in white clover (Lee *et al* 2009). Lower amount of leaf water potential in the leaves of plants provided with treatment T1, T2 and T3 ($\text{NO}_3^-/\text{NH}_4^+::100:0,75:25, 50:50$) was due to accumulation of cytoplasmic osmolytes like proline and is thought to help in reducing the cellular water potential to a level below the external water potential. It enables the water to move into the cell while at the same time minimizing potentially deleterious increases in ionic strength (Hossain *et al* 2014).

4.1.3 Leaf area

Leaf area for all the three crops has given in Table 5. In spinach, application of treatments T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) and T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) resulted in maximum mean leaf area during October and February grown crop and for July grown crop maximum leaf area was found in plants provided with treatment T2($\text{NO}_3^-/\text{NH}_4^+::75:25$) while the plants provided with treatment T4 ($\text{NO}_3^-/\text{NH}_4^+::25:75$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) recorded with minimum mean leaf area during all the three growing seasons. In coriander and lettuce, during all the three growing seasons, maximum mean leaf area was found in the plants which were provided with the nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and plants had minimum mean leaf area which were supplied with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). However, an overall mean among the seasons showed that the leaf area of main season sown crops (spinach, coriander and lettuce) was significantly maximum than the off season sown crops. Our results are on agreement with Cao *et al* (2010) in ryegrass and Osorio *et al* (2003) in taro who observed that leaf area was higher under 75/25 ($\text{NO}_3^-/\text{NH}_4^+$) ratio. For chives and *Polygonatum Odoratum* maximum leaf area was obtained when plants were supplied with a ratio of 80/20 ($\text{NO}_3^-/\text{NH}_4^+$) in nutrient solution (Gonzalez-Garcia *et al* 2009, Zou *et al* 2017).

4.1.4 Fresh weight of shoots and roots

Fresh biomass of shoot of all the three crops (spinach, coriander and lettuce) recorded during all the three seasons (October, February and July) has given in Table 6. In spinach, during all the three growing seasons, maximum mean fresh weight recorded in the plants which were supplied with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and plants provided with treatment T5 ($0:100::\text{NO}_3^-:\text{NH}_4^+$) recorded with least fresh weight. In coriander, maximum mean fresh weight was observed for the plants which were provided with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (0.54g in October, 0.46g in February and 0.37g in July) and plants provided with treatment T5 ($0:100::\text{NO}_3^-/\text{NH}_4^+$) recorded with least fresh weight i.e. 0.18g, 0.15g, 0.12g in October, February and July respectively. In lettuce, maximum mean fresh weight was observed for the plants which were provided with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (3.00g in October, 2.15g in February and 1.41g in July) and plants provided with treatment T5 ($0:100::\text{NO}_3^-/\text{NH}_4^+$) recorded with least fresh weight i.e. 1.30g,

0.76g, 0.59g in October, February and July respectively. More chlorophyll content and increased photosynthesis could be the reason for increased biomass in the plants provided with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$).

Fresh weight root recorded in all the three crops is given in Table 7. In October, February and July grown spinach crop fresh weight of root was maximum in the plants grown in open field conditions and plants provided with treatment T1 ($100:0::\text{NO}_3^-/\text{NH}_4^+$) and plants provided with a ratio of 0:100 ($\text{NO}_3^-/\text{NH}_4^+$) nutrient solution (T5) had the minimum mean fresh weight of roots. In coriander maximum mean fresh weight of root was recorded in the open field plants and the plants fertigated with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$). In October and February grown lettuce, maximum mean fresh weight of root was recorded in the plants provided with T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) treatment and plants grown in open field condition. In July, maximum fresh weight root was recorded in T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) provided plants. Plants had minimum mean fresh weight root which were supplied with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). Least fresh weight at and above 50% NH_4^+ concentrations may be attributed to the greater supply of carbohydrates to roots for assimilation and detoxification of ammonium (Marschner 2011). The strategy of the plant, namely to lower free ammonium in its tissues by assimilation, requires extra C (carbohydrates), which is then not available for growth (Britto and Kronzucker 2013). Fresh biomass of increased by 60.5% as the ratio of $\text{NH}_4^+:\text{NO}_3^-$ -N decreased from 100% to 0% (Barickman and Kopsell 2016; Kopsell *et al* 2007). Zhang *et al* (2005) in spinach, Chen *et al* (2011) in soyabean and Kim *et al* (2006) in rocket salad has also observed the same results where the maximum fresh biomass was recorded for the treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$). In gerbera plant supply of NO_3^- and NH_4^+ in the ratios of 80:20 and 40:60 in the nutrient solutions resulted in highest and lowest production of fresh biomass, respectively (Khalaj *et al* 2017). Similar results were obtained for Chinese cabbage (Song *et al* 2011) and rose (Bar-Yosef *et al* 2009). Wang and Shen (2011), who reported in spinach that the highest biomass of roots was obtained with NH_4^+ -N at 25% of the total N i.e. $\text{NO}_3^-/\text{NH}_4^+::75:25$.

4.1.5 Dry weight of shoot and roots

During all the three growing seasons, maximum mean dry weight of shoots (Table 8) of spinach, coriander and lettuce were recorded in the plants provided with nutrient solutions T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$), T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) and plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) followed by T4 ($\text{NO}_3^-/\text{NH}_4^+::25:75$) had the minimum mean dry weight of shoots.

During all the three growing seasons, spinach plants treated with nutrient solutions T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) and grown in open field conditions were recorded with maximum mean dry weight of roots and plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) had the minimum mean dry weight. In coriander, during all the three growing seasons maximum

Table 3. Effect of nitrate and ammonium ratios on plant height (cm)

	Plant height (cm) Spinach				Plant height (cm) Coriander				Plant height (cm) Lettuce			
	Seasonal	Off seasonal			Seasonal	Off seasonal			Seasonal	Off seasonal		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	26.78 ^a	23.81 ^{bc}	20.14 ^{dc}	23.58 ^a	22.25 ^b	19.75 ^{bc}	18.69 ^c	20.23 ^b	20.08 ^b	17.28 ^d	11.47 ^{gh}	16.28 ^b
T2=75:25	25.84 ^{ab}	21.85 ^{cd}	18.24 ^{ef}	21.98 ^b	26.87 ^a	21.42 ^b	20.22 ^b	22.84 ^a	22.31 ^a	18.23 ^c	11.98 ^{gh}	17.51 ^a
T3=50:50	20.06 ^{de}	18.13 ^{ef}	16.75 ^{fg}	18.31 ^c	21.36 ^b	17.94 ^{de}	16.05 ^{ef}	18.45 ^c	18.32 ^c	16.26 ^e	11.17 ^h	15.25 ^c
T4=25:75	18.12 ^{ef}	16.11 ^{fg}	15.45 ^{fg}	16.56 ^d	18.19 ^{cd}	15.19 ^{fg}	14.76 ^{gh}	16.05 ^d	15.68 ^e	12.78 ^f	8.56 ⁱ	12.34 ^d
T5=0:100	13.81 ^{hi}	11.87 ^{ij}	11.22 ^k	12.30 ^e	13.66 ^{hi}	8.61 ^k	8.20 ^k	10.16 ^f	12.25 ^{fg}	8.40 ⁱ	6.57 ^j	9.07 ^f
Open	19.72 ^{de}	17.23 ^{fg}	12.24 ^{ij}	16.40 ^d	18.21 ^c	11.85 ^{ij}	11.32 ^{jk}	13.79 ^e	15.46 ^e	8.64 ⁱ	6.34 ^j	10.15 ^e
Mean	20.72 ^a	18.17 ^b	15.67 ^c	18.19	20.09 ^a	15.79 ^b	14.87 ^c	16.92	17.35 ^a	13.60 ^b	9.35 ^c	13.43

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 4. Effect of nitrate and ammonium ratios on leaf water potential (-MPa)

	Leaf water potential (-MPa) Spinach				Leaf water potential (-MPa) Coriander				Leaf water potential (-MPa) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO ₃ ⁻ /NH ₄ ⁺ ratio												
T1=100:0	3.00 ^{cd}	3.42 ^b	3.38 ^b	3.27 ^b	3.34 ^{ab}	2.95 ^b	2.65 ^d	2.98 ^b	2.39 ^{ef}	2.92 ^{abc}	2.82 ^{bcd}	2.71 ^b
T2=75:25	3.42 ^b	3.78 ^a	3.74 ^a	3.65 ^a	3.84 ^a	3.40 ^{ab}	2.89 ^{bc}	3.38 ^a	2.89 ^{abc}	3.26 ^a	3.13 ^{ab}	3.09 ^a
T3=50:50	2.41 ^f	3.13 ^c	3.09 ^{cd}	2.88 ^c	2.63 ^d	2.83 ^{cd}	2.30 ^{ef}	2.59 ^c	2.00 ^{gh}	2.87 ^{abcd}	2.66 ^{cde}	2.51 ^c
T4=25:75	1.40 ^g	2.52 ^{ef}	2.46 ^{ef}	2.13 ^e	1.75 ^{gh}	2.44 ^d	1.86 ^{fg}	2.02 ^d	2.12 ^{fg}	2.57 ^{cde}	2.38 ^{ef}	2.36 ^{cd}
T5=0:100	1.39 ^g	2.62 ^e	2.48 ^{ef}	2.16 ^e	1.66 ^{gh}	2.51 ^d	1.88 ^{gh}	2.02 ^d	1.65 ⁱ	2.65 ^{cde}	2.41 ^{ef}	2.24 ^d
Open	1.47 ^g	2.93 ^d	2.50 ^{ef}	2.30 ^d	1.77 ^{gh}	2.55 ^{de}	1.92 ^{gh}	2.08 ^d	1.75 ^{hi}	2.70 ^{cde}	2.50 ^{def}	2.32 ^d
Mean	2.18 ^c	3.07 ^a	2.94 ^b	2.73	2.50 ^b	2.78 ^a	2.25 ^c	2.51	2.13 ^c	2.83 ^a	2.65 ^b	2.54

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 5. Effect of nitrate and ammonium ratios on leaf area (cm²)

	Leaf area (cm ²) Spinach				Leaf area (cm ²) Coriander				Leaf area (cm ²) Lettuce			
	Main season	Off season		Mean	Main season	Off season		Mean	Main season	Off season		Mean
	October	February	July		October	February	July		October	February	July	
NO₃⁻/NH₄⁺ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	55.81 ^a	55.73 ^a	50.53 ^c	54.02 ^b	11.30 ^c	8.78 ^d	8.58 ^d	9.55 ^b	382.05 ^b	183.42 ^h	107.75 ⁿ	224.41 ^b
T2=75:25	56.17 ^a	55.76 ^a	51.44 ^b	54.46 ^a	16.37 ^a	15.04 ^b	14.79 ^b	15.40 ^a	396.59 ^a	199.54 ^g	117.95 ^m	238.02 ^a
T3=50:50	49.16 ^d	48.73 ^d	44.67 ^{ef}	47.52 ^c	7.30 ^e	6.17 ^f	6.12 ^f	6.53 ^c	364.44 ^c	168.18 ^j	91.70 ^o	208.11 ^c
T4=25:75	44.34 ^f	44.29 ^f	41.26 ^h	43.29 ^d	4.80 ^g	2.94 ^{hij}	2.81 ^{ij}	3.52 ^e	301.84 ^e	153.09 ^k	78.83 ^p	177.92 ^d
T5=0:100	42.77 ^g	42.65 ^g	39.42 ⁱ	41.62 ^e	2.10 ^j	2.20 ^j	2.04 ^j	2.14 ^f	253.03 ^f	135.06 ^l	61.57 ^q	149.89 ^e
Open	45.19 ^e	45.17 ^e	39.41 ⁱ	43.26 ^d	5.33 ^{fg}	3.77 ^h	3.63 ^h	4.24 ^d	393.43 ^a	180.74 ⁱ	45.01 ^r	206.39 ^e
Mean	48.91 ^a	48.72 ^b	44.45 ^c	47.36	7.88 ^a	6.48 ^b	6.34 ^c	6.90	348.49 ^a	170.00 ^b	83.80 ^c	200.79

Means followed by the same letters in each row and column do not differ significantly (P < 0.05)

Table 6. Effect of nitrate and ammonium ratios on fresh weight shoots (g)

	Fresh weight shoot (g) Spinach				Fresh weight shoot (g) Coriander				Fresh weight shoot (g) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	4.96 ^b	4.96 ^b	4.76 ^{bc}	4.89 ^b	0.45 ^b	0.40 ^{bc}	0.34 ^{cde}	0.40 ^b	2.04 ^c	1.49 ^{de}	1.34 ^{gh}	1.62 ^b
T2=75:25	5.82 ^a	5.70 ^a	5.45 ^a	5.66 ^a	0.54 ^a	0.46 ^b	0.37 ^{cde}	0.46 ^a	3.00 ^a	2.15 ^b	1.41 ^{ef}	2.19 ^a
T3=50:50	2.81 ^c	2.65 ^c	2.59 ^c	2.68 ^d	0.41 ^{bc}	0.37 ^{cde}	0.29 ^{fghi}	0.36 ^c	1.47 ^d	1.09 ⁱ	0.82 ^{kl}	1.12 ^d
T4=25:75	2.15 ^f	2.13 ^f	1.90 ^f	2.06 ^e	0.33 ^{efgh}	0.30 ^{fghi}	0.23 ^{ij}	0.29 ^d	1.36 ^{fg}	0.84 ^k	0.71 ^m	0.97 ^c
T5=0:100	2.01 ^f	1.90 ^f	1.73 ^f	1.88 ^f	0.18 ^{jk}	0.15 ^k	0.12 ^k	0.15 ^c	1.30 ^h	0.76 ^{lm}	0.59 ⁿ	0.88 ^f
Open	4.75 ^{bc}	4.43 ^{cd}	4.20 ^d	4.46 ^c	0.42 ^{bc}	0.35 ^{cde}	0.26 ^{hi}	0.35 ^c	2.02 ^c	0.93 ^j	0.74 ^m	1.23 ^c
Mean	3.75 ^a	3.63 ^b	3.44 ^c	3.61	0.39 ^a	0.34 ^b	0.27 ^c	0.33	1.86 ^a	1.21 ^b	0.94 ^c	1.34

Means followed by the same letters in each row and column do not differ significantly (P < 0

Table 7. Effect of nitrate and ammonium ratios on fresh weight roots (g)

	Fresh weight root (g) Spinach				Fresh weight root (g) Coriander				Fresh weight root (g) Lettuce			
	Main season	Off season		Mean	Main season	Off season		Mean	Main season	Off season		Mean
		October	February			July	October			February	July	
NO₃⁻/NH₄⁺ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	0.35 ^{bc}	0.35 ^{bc}	0.35 ^{bc}	0.35 ^b	0.05 ^b	0.05 ^b	0.04 ^b	0.05 ^a	0.13 ^d	0.13 ^d	0.12 ^{def}	0.13 ^c
T2=75:25	0.28 ^d	0.26 ^d	0.27 ^d	0.27 ^c	0.04 ^b	0.03 ^{bcd}	0.04 ^b	0.36 ^b	0.27 ^{ab}	0.26 ^{ab}	0.26 ^{ab}	0.26 ^a
T3=50:50	0.27 ^d	0.26 ^d	0.26 ^d	0.26 ^c	0.03 ^{bcd}	0.02 ^d	0.03 ^{bcd}	0.28 ^c	0.12 ^{def}	0.12 ^{def}	0.12 ^{def}	0.12 ^c
T4=25:75	0.19 ^e	0.18 ^e	0.18 ^e	0.18 ^d	0.03 ^{bcd}	0.02 ^d	0.02 ^d	0.24 ^{cd}	0.11 ^{ef}	0.10 ^f	0.11 ^{ef}	0.11 ^d
T5=0:100	0.10 ^f	0.09 ^f	0.09 ^f	0.10 ^e	0.02 ^d	0.02 ^d	0.02 ^d	0.02 ^d	0.08 ^g	0.07 ^g	0.08 ^g	0.08 ^e
Open	0.39 ^a	0.37 ^{ab}	0.34 ^c	0.37 ^a	0.08 ^b	0.05 ^b	0.02 ^d	0.50 ^a	0.28 ^a	0.26 ^{ab}	0.16 ^c	0.23 ^b
Mean	0.26 ^a	0.25 ^b	0.25 ^b	0.26	0.04 ^a	0.03 ^b	0.03 ^b	0.03	0.16 ^a	0.16 ^a	0.14 ^b	0.15

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 8. Effect of nitrate and ammonium ratios on dry weight shoots (g)

	Dry weight shoot (g) Spinach				Dry weight shoot (g) Coriander				Dry weight shoot (g) Lettuce			
	Main season	Off season		Mean	Main season	Off season		Mean	Main season	Off season		Mean
		October	February			July	October			February	July	
NO₃⁻/NH₄⁺ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	1.09 ^{cd}	0.82 ^d	1.05 ^d	0.99 ^b	0.10 ^{ab}	0.09 ^{abc}	0.06 ^{cde}	0.08 ^{ab}	0.43 ^c	0.52 ^d	0.27 ^{hi}	0.41 ^b
T2=75:25	1.29 ^a	1.20 ^{ab}	1.18 ^{bc}	1.23 ^a	0.11 ^a	0.10 ^{ab}	0.07 ^{bcd}	0.09 ^a	0.68 ^a	0.53 ^b	0.31 ^{efgh}	0.51 ^a
T3=50:50	0.62 ^f	0.82 ^f	0.56 ^{fg}	0.67 ^c	0.09 ^{abc}	0.08 ^{bc}	0.06 ^{cde}	0.08 ^b	0.34 ^{def}	0.32 ^{efg}	0.25 ^{ij}	0.30 ^c
T4=25:75	0.47 ^{gh}	0.52 ^h	0.41 ^h	0.47 ^d	0.06 ^{cde}	0.05 ^{de}	0.05 ^{de}	0.06 ^c	0.32 ^{efg}	0.30 ^{fgh}	0.23 ^{jk}	0.28 ^d
T5=0:100	0.44 ^h	0.44 ^h	0.38 ^h	0.42 ^d	0.03 ^c	0.03 ^c	0.03 ^c	0.03 ^d	0.31 ^{fgh}	0.29 ^{ghi}	0.20 ^l	0.27 ^d
Open	1.05 ^d	0.78 ^c	0.91 ^c	0.91 ^b	0.10 ^{ab}	0.09 ^{cde}	0.06 ^{cde}	0.08 ^b	0.37 ^d	0.35 ^{de}	0.21 ^{jk}	0.31 ^c
Mean	0.83 ^a	0.77 ^a	0.75 ^b	0.78	0.08 ^a	0.07 ^b	0.06 ^c	0.07	0.41 ^a	0.38 ^b	0.25 ^c	0.35

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 9. Effect of nitrate and ammonium ratios on dry weight roots (g)

	Dry weight root (g) Spinach				Dry weight root (g) Coriander				Dry weight root (g) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	0.09 ^{ab}	0.09 ^{ab}	0.09 ^{ab}	0.09 ^c	0.04 ^a	0.01 ^d	0.02 ^{cd}	0.02 ^a	0.03 ^{cd}	0.03 ^{cd}	0.02 ^{def}	0.03 ^c
T2=75:25	0.07 ^c	0.05 ^d	0.05 ^d	0.06 ^c	0.03 ^{ab}	0.01 ^d	0.03 ^{ab}	0.03 ^a	0.07 ^a	0.05 ^b	0.06 ^a	0.06 ^a
T3=50:50	0.07 ^c	0.04 ^{deg}	0.04 ^{deg}	0.05 ^d	0.03 ^{cd}	0.01 ^d	0.01 ^d	0.02 ^b	0.03 ^{cd}	0.02 ^{de}	0.02 ^{de}	0.02 ^d
T4=25:75	0.05 ^{de}	0.03 ^{efgh}	0.03 ^{efgh}	0.04 ^e	0.02 ^{cd}	0.01 ^d	0.01 ^d	0.01 ^b	0.03 ^{cd}	0.01 ^{ef}	0.01 ^{ef}	0.01 ^e
T5=0:100	0.03 ^{efgh}	0.01 ⁱ	0.02 ^{hi}	0.02 ^f	0.02 ^{cd}	0.01 ^d	0.01 ^d	0.02 ^b	0.02 ^{de}	0.01 ^{ef}	0.01 ^{ef}	0.01 ^e
Open	0.10 ^a	0.09 ^{ab}	0.09 ^{ab}	0.09 ^a	0.02 ^{cd}	0.01 ^d	0.02 ^d	0.02 ^b	0.07 ^a	0.05 ^b	0.02 ^{de}	0.05 ^b
Mean	0.07 ^a	0.05 ^b	0.05 ^b	0.06	0.03 ^a	0.01 ^c	0.02 ^b	0.02	0.04 ^a	0.03 ^b	0.03 ^b	0.03

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

mean dry weight of root was recorded for the plants provided treatment T1($\text{NO}_3^-/\text{NH}_4^+::100:0$), T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$). Plants had the minimum dry weight provided treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). In lettuce maximum mean dry weight of roots was found in plants provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by dry weight of plants in open field conditions and plants provided with treatments T4 ($\text{NO}_3^-/\text{NH}_4^+::25:75$) and T5($\text{NO}_3^-/\text{NH}_4^+::0:100$) had the minimum mean dry weight of roots. Wang and Shen (2011) observed that the biomass of roots increased with the increasing percentage of NH_4^+ -N. The highest biomass of roots and shoots was obtained with NH_4^+ -N at 25% of the total N. Dry matter (DM) decreased when the proportion of NH_4^+ in the nutrient solution increased upto 25%. Chen *et al* (2011) has reported the same results in soyabean where both in shoots and in roots, the dry weight was significantly higher in NO_3^- (75%) than that in other ammonium-nitrate ratios. Our results are also in agreement with Zhu *et al* (2018) who opined that 20% replacement of nitrate with ammonium in the nutrient solution i.e. $\text{NO}_3^-/\text{NH}_4^+ =80:20$, can increase the dry biomass in Chinese kale. Significantly lower root and shoot dry matter yields were observed when plants were grown in 100% NH_4^+ than plants grown in 100% NO_3^- (Ali *et al* 2001).

4.2 Biochemical parameters

4.2.1 Total chlorophyll

The crops (spinach, coriander and lettuce) supplied with different nutrient solutions showed significant variation in total chlorophyll content (Table 10) during all the three growing seasons (October, February and July). In spinach maximum mean total chlorophyll content was recorded for the plants supplied with nutrient solution T2 ($\text{NO}_3^- : \text{NH}_4^+::75:25$) and plants provided with nutrient solution T5 ($\text{NO}_3^- : \text{NH}_4^+:: 0:100$) had minimum mean total chlorophyll content during all the three seasons. In coriander plants which were supplied with nutrient solution T2 ($\text{NO}_3^- : \text{NH}_4^+::75:25$) had maximum mean total chlorophyll content i.e. 2.36, 2.17 and 1.94 mg/gFW in October, February and July respectively as compared to open field conditions i.e. 1.27, 1.93 and 1.70 mg/gFW in October, February and July respectively. Plants supplied with treatment T5 ($\text{NO}_3^- : \text{NH}_4^+:: 0:100$) had minimum mean total chlorophyll content i.e. 1.21, 1.10 and 1.39 mg/gFW in October, February and July respectively. Likewise in lettuce plants which were supplied with nutrient solution T2 ($\text{NO}_3^- : \text{NH}_4^+::75:25$) had maximum mean total chlorophyll content i.e. 1.55, 1.40 and 1.35 mg/gFW in October, February and July respectively as compared to open field conditions i.e. 0.93, 1.20 and 1.23 mg/gFW in October, February and July respectively. Plants supplied with treatment T5 ($\text{NO}_3^- : \text{NH}_4^+:: 0:100$) had minimum mean total chlorophyll content but more than open field grown plants i.e. 1.18, 1.05 and 1.06 mg/gFW in October, February and July respectively. Chlorophylls are an important quality parameter because they contribute to leaf colour and have a direct effect on the consumer preference. Early investigations demonstrated that high

proportions of NH_4^+ suppressed plant growth and decreased chlorophyll content, whereas low proportions of NH_4^+ increased chlorophyll content (Blanke *et al* 1996; Everett *et al* 2010; Huang *et al* 2013). In the present study, an increase in total chlorophyll content was observed with ratios 75:25($\text{NO}_3^-/\text{NH}_4^+$) and 100:0 ($\text{NO}_3^-/\text{NH}_4^+$), indicating that the supplies of N forms in the solutions were related to the chlorophyll contents, and low and moderate amount of NH_4^+ favored the chlorophyll accumulation in spinach, coriander and lettuce leaves. Decreased chlorophyll content was observed at a high NH_4^+ concentration ($\text{NO}_3^-/\text{NH}_4^+::0:100$) in the solution. It might be probably due to NH_4^+ toxicity. Similar observations of increased chlorophyll content in crop supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratio of 75:25 have been reported in swiss chard (Barickman and Kopsell 2016) and in kale (Kopsell *et al* 2007). It has been observed that total chlorophyll contents of *Polygonatum odoratum* seedlings supplied with $\text{NO}_3^-: \text{NH}_4^+$ in the ratios of 90:10 or 80:20 was significantly higher than the seedlings which were provided with NH_4^+ as sole source of N (i.e. $\text{NO}_3^-/\text{NH}_4^+=0:100$) (Zou *et al* 2017).

4.2.2 Carotenoid content

Carotenoids content (Table 11) in spianch during the main season (October) and off seasons (February and July) was maximum in the plants provided with T1 ($\text{NO}_3^-: \text{NH}_4^+:: 100:0$), T2 ($\text{NO}_3^-: \text{NH}_4^+:: 75:25$) and T3 ($\text{NO}_3^-: \text{NH}_4^+:: 50:50$). No significant differences were observed between the carotenoids content of the plants supplied with nutrient solutions T1 to T3 and open field sown crop during all the three seasons. Plants had minimum mean carotenoids content which were nourished with nutrient solution T5 ($\text{NO}_3^-: \text{NH}_4^+:: 0:100$). In coriander, during all the three growing seasons, maximum mean carotenoids content was recorded in the plants provided with nutrient solution T2 ($\text{NO}_3^-: \text{NH}_4^+:: 75:25$) i.e. 1.11, 0.97 and 0.98 mg/gFW in October, February and July, respectively and plants had minimum mean carotenoids content which were nourished with nutrient solution T5 ($\text{NO}_3^-: \text{NH}_4^+:: 0:100$) i.e. 0.67, 0.63 and 0.66 mg/gFW October, February and July, respectively. In lettuce during all the three growing solution, treatment T2 ($\text{NO}_3^-: \text{NH}_4^+:: 75:25$) resulted in maximum accumulation of carotenoids content and plants had minimum mean carotenoids content which were nourished with nutrient solution T5 ($\text{NO}_3^-: \text{NH}_4^+:: 0:100$). Carotenoids have an important role in human nutrition, because they act as antioxidants (Maiani *et al* 2009). Similar observations of increased carotenoids content in crop supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratio of 75:25 have been reported in swiss chard (Barickman and Kopsell 2016) and in kale (Kopsell *et al* 2007). Carotenoids in *Corchorus olitorius* were higher, with a maximum level of 75 mg 100 g⁻¹ during the summer season in nutrient solution 50% conditions ($\text{NO}_3^-/\text{NH}_4^+= 6:1.9$) (Giro and Ferrante 2016, Kaulmann *et al* 2014).

4.2.3 Total soluble sugars

Total soluble sugars (Table 12) content was maximum in the spinach plants supplied with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) while, those plants which were supplied with T5 ($0:100::\text{NO}_3^-/\text{NH}_4^+$) treatment had minimum total soluble sugars accumulation during all the three seasons. It showed that total soluble sugars (TSS) content were significantly ($P<0.05$) decreased when concentration of NH_4^+ in the nutrient solution was above 25%. Moreover plants grown under green house were able to accumulate more sugars and starch as compared to open field conditions. Total soluble sugars accumulation in the plants which were supplied with treatment T2 ($75:25::\text{NO}_3^-/\text{NH}_4^+$) was increased by 140.64%, 94.05% and 109.28% in October, February and July sown crops respectively as compared to crop sown in open field conditions in the same seasons. In coriander, total soluble sugars (Table 12) content was maximum in the plants supplied with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) while, those plants which were supplied with T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) treatment had minimum total soluble sugars accumulation during all the three seasons. Nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) supplied plants under hydroponics accumulated 137.34%, 377.45% and 119.95% more sugars than open field sown crop in the same seasons. Likewise lettuce plants had maximum and minimum mean total soluble sugars which were provided with nutrient solutions T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) respectively. Percent increase in total soluble sugars content in hydroponically grown lettuce plants treated with T2 treatment was 80.70%, 84.28% and 86.13% more than open field sown lettuce crop in October, February and July respectively. Likewise in Chinese chive seedlings total soluble sugars were increased when the NO_3^- to NH_4^+ ratio in the nutrient solution was reduced and the ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) (Sun *et al* 2014). Contents of total soluble sugars was increased when the proportion of NH_4^+ in the supplied nutrient solution was 25% in tomato (Yang *et al* 2003). Zhang *et al* (1990) has also reported that contents of soluble sugar were increased when spinach plants were supplied with a ratio of 75:25 (NO_3^- -N to NH_4^+ -N). A $\text{NO}_3^-/\text{NH}_4^+$ ratio of 80:20 increased the total soluble sugars content in Chinese kale (Zhu *et al* 2018) and a ratio of 6: 1.9 ($\text{NO}_3^-/\text{NH}_4^+$) in *Corchorus olitorius* (Giro and Ferrante 2016).

4.2.4 Total starch content

In spinach, during all the three seasons, the plants supplied with ratios of 75: 25 (T2) followed by 100:0 (T1) ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solutions had maximum accumulation of total starch (Table 13). It means starch content was maximum till the proportion of ammonium (NH_4^+) was 0 or 25% in the nutrient solution (i.e. T1 & T2) and plants had the least total starch content which were supplied with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). In coriander, during all the three growing seasons, plants which were supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) had the maximum mean accumulation of starch and plants had minimum mean starch content which were supplied with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$).

Similarly lettuce plants fed with T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) nutrient solutions had maximum and plants treated with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) had minimum content of total starch. It was observed that hydroponically grown crops accumulated more starch content than the open field sown crops in the same seasons. Contents of starch were increased when the proportion of NH_4^+ in the supplied nutrient solution was 25% in tomato (Yang *et al* 2003).

4.2.5 Total soluble proteins

In the present study, different nitrogen forms led to accumulate different levels of total soluble protein (Table 14) in spinach, coriander and lettuce. Total soluble proteins contents generally increased with the increased ratios of NH_4^+ and then was found to decreased when NH_4^+ supply was above 25%. In spinach, among the treatments the highest total soluble protein content in all the three seasons occurred in leaves of plants which were supplied with nutrient solutions containing NO_3^- and NH_4^+ in the ratios of 100:0, 75:25, respectively. The plants supplied with treatment T5=0:100 ($\text{NO}_3^-/\text{NH}_4^+$) nitrogen solution and the plants raised in open field conditions had the lowest levels of soluble protein content. In October sown coriander, maximum mean total soluble proteins content recorded for the plants which were supplied with treatment T3 ($\text{NO}_3^-/\text{NH}_4^+::50:50$), whereas in off seasons grown coriander plants provided with nutrient solutions T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) resulted in maximum mean total soluble proteins content. Plants which were fed with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) had minimum mean total soluble proteins content. In lettuce plants nourished with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) had maximum and minimum mean total soluble proteins content. In all the three crops content of proteins was more in hydroponically grown crops than open field sown crops and also the total soluble proteins content in off seasons was more than that of main season sown crops. Zhu *et al* (2018) observed that a nutrient solution with $\text{NO}_3^-/\text{NH}_4^+$ in the ratio of 80:20 increased the total soluble proteins in Chinese kale. It has been observed in spinach plants, that total soluble protein content were decreased when they were supplied with higher proportion of ammonium ($\text{NO}_3^-\text{-N}:\text{NH}_4^+\text{-N}=1.5:3$) than the nitrate in the nutrient solution (Dominguez- Valdivia *et al* 2008). In the previous study, it has been observed that the content of total soluble protein were highest in the plants which were supplied with $\text{NO}_3^-\text{-N}:\text{NH}_4^+\text{-N}$ in the ratio of 75:25 in the chive (Sun *et al* 2014) and in *Camellia oleifera* (Wang *et al* 2018).

4.2.6 Free amino acids

Free amino acids content recorded in all the three crops (spinach, coriander and lettuce) during the three seasons (October, February and July) is given in Table 15. In February and July sown spinach crop, free amino acids content accumulated maximum in plants provided with treatments T2 and T1($\text{NO}_3^-/\text{NH}_4^+::75:25,100:0$) in the nutrient solution and the plants supplied with treatment T5=0:100 ($\text{NO}_3^-/\text{NH}_4^+$) nitrogen solution maintained the lowest levels of free amino acids content (Table 15). In October sown crop, free amino

Table 10. Effect of nitrate and ammonium ratios on total chlorophyll content (mg/gFW)

	Total chlorophyll (mg/gFW)				Total chlorophyll (mg/gFW)				Total chlorophyll (mg/gFW)			
	Spinach				Coriander				Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	1.97 ^b	1.60 ^{ef}	1.67 ^{de}	1.75 ^b	2.23 ^{ab}	2.09 ^{bc}	1.81 ^{de}	2.04 ^a	1.50 ^{ab}	1.34 ^{cde}	1.28 ^{cdef}	1.37 ^b
T2=75:25	2.08 ^a	1.71 ^{cd}	1.77 ^c	1.85 ^a	2.36 ^a	2.17 ^b	1.94 ^c	2.15 ^b	1.55 ^a	1.40 ^{bc}	1.35 ^{cd}	1.43 ^a
T3=50:50	1.69 ^{cd}	1.47 ^{hi}	1.53 ^{gf}	1.56 ^c	1.94 ^c	1.85 ^d	1.72 ^{ef}	1.84 ^c	1.29 ^{cdef}	1.27 ^{def}	1.21 ^{fg}	1.26 ^c
T4=25:75	1.53 ^{fg}	1.35 ^j	1.39 ^{ij}	1.42 ^d	1.85 ^d	1.74 ^e	1.55 ^{fg}	1.71 ^d	1.20 ^{fg}	1.27 ^{def}	1.13 ^{gh}	1.20 ^d
T5=0:100	1.03 ^k	1.06 ^k	1.09 ^k	1.06 ^f	1.21 ⁱ	1.10 ⁱ	1.39 ^g	1.24 ^f	1.18 ^{fg}	1.05 ^h	1.06 ^h	1.10 ^c
Open	1.39 ^{ij}	1.33 ^j	1.37 ^j	1.36 ^c	1.27 ^{hi}	1.93 ^{cd}	1.70 ^{ef}	1.64 ^c	0.93 ⁱ	1.20 ^{fg}	1.23 ^{efg}	1.12 ^c
Mean	1.62 ^a	1.42 ^c	1.47 ^b	1.50	1.81 ^a	1.82 ^a	1.69 ^b	1.77	1.27 ^a	1.26 ^a	1.21 ^b	1.25

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

Table 11. Effect of nitrate and ammonium ratios on total carotenoids content (mg/gFW)

	Carotenoids (mg/gFW) Spinach				Carotenoids (mg/gFW) Coriander				Carotenoids (mg/gFW) Lettuce			
	Main season	Off season		Mean	Main season	Off season		Mean	Main season	Off season		Mean
		October	February			July	October			February	July	
NO₃⁻/NH₄⁺ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	0.62 ^a	0.58 ^a	0.59 ^b	0.60 ^{ab}	1.00 ^b	1.00 ^b	0.99 ^b	1.00 ^a	1.16 ^b	1.11 ^b	1.12 ^b	1.13 ^b
T2=75:25	0.66 ^a	0.62 ^a	0.63 ^a	0.64 ^a	1.11 ^a	0.97 ^b	0.98 ^b	1.02 ^a	1.29 ^a	1.25 ^a	1.26 ^a	1.27 ^a
T3=50:50	0.56 ^a	0.54 ^a	0.56 ^a	0.55 ^b	0.72 ^c	0.66 ^c	0.69 ^c	0.69 ^b	0.86 ^c	0.78 ^d	0.80 ^{cd}	0.81 ^c
T4=25:75	0.41 ^b	0.38 ^c	0.40 ^b	0.40 ^c	0.70 ^c	0.64 ^c	0.66 ^c	0.67 ^{bc}	0.60 ^{ef}	0.59 ^{ef}	0.59 ^{ef}	0.59 ^c
T5=0:100	0.18 ^c	0.16 ^c	0.16 ^c	0.17 ^d	0.67 ^c	0.63 ^c	0.64 ^c	0.65 ^c	0.55 ^f	0.54 ^f	0.54 ^f	0.55 ^f
Open	0.54 ^a	0.58 ^a	0.61 ^a	0.58 ^b	0.66 ^c	0.63 ^c	0.63 ^c	0.64 ^c	0.65 ^e	0.60 ^{ef}	0.62 ^e	0.62 ^d
Mean	0.50 ^a	0.48 ^a	0.49 ^a	0.49	0.81 ^a	0.76 ^b	0.77 ^b	0.78	0.85 ^a	0.82 ^b	0.81 ^b	0.83

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 12. Effect of nitrate and ammonium ratios on total soluble sugars content (mg/gDW)

	Total soluble sugars (mg/gDW) Spinach				Total soluble sugars (mg/gDW) Coriander				Total soluble sugars (mg/gDW) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	163.51 ^c	164.49 ^d	171.77 ^c	166.59 ^b	174.70 ^d	202.66 ^b	172.86 ^d	183.41 ^b	176.29 ^e	184.26 ^c	175.31 ^e	178.62 ^b
T2=75:25	176.49 ^{ab}	182.62 ^a	176.32 ^{ab}	178.48 ^a	181.45 ^c	215.37 ^a	183.99 ^c	193.60 ^a	202.81 ^b	210.71 ^a	181.82 ^{cd}	198.44 ^a
T3=50:50	144.11 ^g	154.25 ^f	154.82 ^f	151.06 ^c	155.21 ^f	176.28 ^d	168.20 ^e	166.56 ^c	174.87 ^e	177.37 ^{de}	163.08 ^f	171.78 ^c
T4=25:75	137.10 ^h	143.32 ^g	132.83 ⁱ	137.78 ^d	111.11 ^h	66.11 ^k	132.39 ^g	103.20 ^d	118.18 ^{gh}	122.16 ^g	74.19 ^k	104.84 ^e
T5=0:100	64.00 ⁿ	81.80 ^l	73.05 ^m	72.95 ^f	81.47 ⁱ	63.73 ^k	81.42 ⁱ	75.54 ^e	57.84 ^m	62.86 ^l	65.12 ^l	61.94 ^f
Open	73.34 ^m	94.11 ^j	84.25 ^k	83.90 ^e	76.45 ^j	45.78 ^l	83.65 ⁱ	68.63 ^f	112.23 ⁱ	114.34 ^{hi}	97.68 ^j	108.09 ^d
Mean	126.44 ^c	136.77 ^a	132.17 ^b	131.79	130.06 ^b	128.32 ^c	137.08 ^a	131.82	140.37 ^b	145.29 ^a	126.20 ^c	137.29

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 13. Effect of nitrate and ammonium ratios on total starch content (mg/gDW)

	Total starch (mg/gDW) Spinach				Total starch (mg/gDW) Coriander				Total starch (mg/gDW) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	82.17 ^c	96.95 ^b	90.80 ^c	89.97 ^b	124.58 ^f	132.53 ^c	126.73 ^f	127.95 ^c	99.23 ^{bc}	101.37 ^b	94.72 ^d	98.44 ^b
T2=75:25	86.93 ^d	104.22 ^a	96.38 ^b	95.84 ^a	173.46 ^{ab}	177.12 ^a	170.18 ^b	173.59 ^a	98.63 ^{bc}	106.33 ^a	100.45 ^{bc}	101.81 ^a
T3=50:50	64.71 ⁱ	80.69 ^{ef}	72.02 ^g	72.47 ^c	152.2 ^{cd}	154.73 ^c	147.12 ^d	151.35 ^b	93.48 ^d	98.22 ^c	93.01 ^d	94.91 ^c
T4=25:75	40.85 ^l	67.11 ^h	53.89 ^k	53.95 ^e	112.19 ^g	115.47 ^g	103.19 ^h	110.28 ^d	89.57 ^e	94.81 ^d	88.01 ^e	90.80 ^d
T5=0:100	21.13 ^o	33.49 ^m	26.61 ⁿ	27.08 ^f	47.28 ^l	59.19 ^k	54.62 ^k	53.70 ^f	64.88 ⁱ	67.98 ^h	61.47 ^j	64.78 ^f
Open	54.81 ^k	80.20 ^f	60.96 ^j	65.32 ^d	72.71 ^j	78.60 ^l	74.31 ^{ij}	75.21 ^e	78.70 ^f	80.91 ^f	76.13 ^g	78.58 ^c
Mean	58.43 ^c	77.11 ^a	66.78 ^b	67.43	113.74 ^b	119.61 ^a	113.74 ^a	115.35	87.42 ^b	91.60 ^c	85.63 ^c	88.22

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 14. Effect of nitrate and ammonium ratios on total soluble proteins (mg/gDW)

	Total soluble proteins (mg/gDW) Spinach				Total soluble proteins (mg/gDW) Coriander				Total soluble proteins (mg/gDW) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	5.67 ^{ab}	5.83 ^a	5.81 ^a	5.77 ^b	7.08 ^c	7.60 ^c	7.61 ^c	7.43 ^c	6.73 ^b	6.82 ^b	6.82 ^b	6.79 ^b
T2=75:25	5.82 ^a	5.95 ^a	5.81 ^a	5.86 ^a	7.53 ^c	9.07 ^a	8.78 ^b	8.46 ^a	8.69 ^a	8.80 ^a	8.76 ^a	8.75 ^a
T3=50:50	5.35 ^{cd}	5.47 ^{bc}	5.44 ^{cd}	5.42 ^c	8.94 ^{ab}	7.49 ^c	7.39 ^c	7.94 ^b	5.47 ^c	5.46 ^c	5.44 ^c	5.46 ^c
T4=25:75	4.24 ^e	4.24 ^e	4.36 ^e	4.28 ^e	7.50 ^c	6.94 ^{ef}	6.86 ^f	7.10 ^d	4.17 ^{de}	4.12 ^{de}	3.95 ^e	4.08 ^e
T5=0:100	3.20 ^g	3.34 ^f	3.75 ^f	3.43 ^f	5.98 ^h	5.86 ^h	5.78 ^h	5.87 ^f	3.42 ^f	3.48 ^f	3.41 ^f	3.44 ^f
Open	5.12 ^d	5.30 ^{cd}	5.27 ^{cd}	5.23 ^d	6.46 ^g	6.42 ^g	6.28 ^g	6.38 ^c	4.16 ^{de}	4.26 ^d	4.30 ^d	4.24 ^d
Mean	4.90 ^b	5.02 ^a	5.07 ^a	4.50	7.25 ^a	7.23 ^a	7.12 ^b	7.19	5.44 ^a	5.49 ^a	5.44 ^a	5.46

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 15. Effect of nitrate and ammonium ratios on free aminoacids (mg/gDW)

	Free amino acids (mg/gDW) Spinach				Free amino acids (mg/gDW) Coriander				Free amino acids (mg/gDW) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	33.90 ^{defg}	41.40 ^{abcd}	38.40 ^{abcde}	37.90 ^{bc}	29.62 ^{ef}	37.84 ^c	34.49 ^{cd}	33.98 ^b	33.04 ^{de}	37.66 ^{ab}	35.87 ^{bc}	35.52 ^b
T2=75:25	36.81 ^{bcdefg}	45.76 ^a	43.85 ^{ab}	42.14 ^a	31.58 ^d	45.73 ^a	41.70 ^b	39.67 ^a	35.88 ^{bc}	39.58 ^a	39.09 ^a	38.18 ^a
T3=50:50	31.60 ^{efgh}	39.19 ^{abcde}	35.49 ^{cdefg}	35.43 ^c	25.28 ^{hi}	35.94 ^c	29.96 ^e	30.39 ^c	27.49 ^{hi}	32.12 ^{def}	30.13 ^{fgh}	29.92 ^d
T4=25:75	27.48 ^{hi}	33.74 ^{defgh}	31.23 ^{fgh}	30.82 ^d	21.09 ^{jk}	31.04 ^{de}	26.55 ^{gh}	26.23 ^d	21.49 ^j	29.37 ^{gh}	26.20 ⁱ	25.68 ^e
T5=0:100	20.03 ⁱ	29.29 ^{gh}	21.44 ⁱ	25.59 ^e	18.89 ^k	27.44 ^{fg}	22.94 ^{ij}	23.09 ^e	19.21 ^j	20.55 ^j	19.75 ^j	19.84 ^f
Open	36.54 ^{bcdefg}	42.22 ^{abc}	39.95 ^{abcd}	39.57 ^{ab}	26.62 ^{gh}	31.32 ^d	30.50 ^{efg}	29.48 ^c	31.90 ^{efg}	34.58 ^{cd}	30.85 ^{efg}	32.44 ^c
Mean	31.06 ^c	38.60 ^a	35.06 ^b	35.24	25.51 ^c	34.89 ^a	31.02 ^b	30.47	28.17 ^c	32.31 ^a	30.31 ^b	30.27

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

acids content was found maximum in the plants provided with nutrient solution T2($\text{NO}_3^-/\text{NH}_4^+::75:25$). In October sown coriander crop, maximum content of free amino acids recorded in plants which were supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and the plants supplied with treatment T5=0:100 ($\text{NO}_3^-/\text{NH}_4^+$) nitrogen solution maintained the lowest levels of free amino acids content. It was observed that during off season, maximum mean free amino acids content recorded in the plants which were provided treatment T2($\text{NO}_3^-/\text{NH}_4^+::75:25$) and was also more than main season sown crop. It was observed that main seasons and off seasons grown lettuce crop provided with nutrient solutions T2($\text{NO}_3^-/\text{NH}_4^+::75:25$) accumulated maximum mean free amino acids content while plants had minimum mean free amino acids content which were provided with T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). Zhu *et al* (2018) observed that a nutrient solution with $\text{NO}_3^-/\text{NH}_4^+$ in the ratio of 80:20 increased the amino acids in Chinese kale. It has been observed in spinach plants, that amino acids content were decreased when they were supplied with higher proportion of ammonium ($\text{NO}_3^-\text{-N}: \text{NH}_4^+\text{-N}= 1.5:3$) than the nitrate in the nutrient solution (Dominguez- Valdivia *et al* 2008). In the previous study, it has been observed that the content of free amino acids were highest in the plants which were supplied with $\text{NO}_3^-\text{-N}: \text{NH}_4^+\text{-N}$ in the ratio of 75:25 in the chive (Sun *et al* 2014) and in *Camellia oleifera* (Wang *et al* 2018).

4.2.7 Proline content

Proline content (Table 16) in spinach was found to be significantly increased when concentration of NH_4^+ in the nutrient solution was upto 25% (T1 and T2) and further there was a decline in proline accumulation at a very higher proportion of ammonium (50 to 100%) in the nutrient solution. Among the treatments maximum mean proline content in October and February sown crop was recorded for the plant supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratios of 75:25 followed by 100:0 whereas in July grown crop maximum mean proline content was recorded for the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$). Plants supplied with nutrient solution in the ratio of 0:100 ($\text{NO}_3^-/\text{NH}_4^+$) maintained the lowest level of proline during all the three growing seasons. Plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated 156.72%, 152.78% and 154.46% more proline than soil grown spinach in October, February and July seasons, respectively. In coriander, among the treatments maximum mean proline content (Table 16) in October, February and July growing crop was recorded for the plant supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratios of 75:25. Plants supplied with nutrient solution in the ratio of 0:100 ($\text{NO}_3^-/\text{NH}_4^+$) maintained the lowest level of proline during all the three growing seasons. Plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated 17.52%, 32.59% and 30.20% more proline than soil grown coriander in October, February and July seasons, respectively. Similarly in lettuce, among the treatments maximum mean proline content (Table 16) in October, February and July growing crop was

recorded for the plant supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratios of 75:25. Plants supplied with nutrient solution in the ratio of 0:100 ($\text{NO}_3^-/\text{NH}_4^+$) maintained the lowest level of proline during all the three growing seasons. Plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated 187.42%, 22.18% and 120.43% more proline than soil grown coriander in October, February and July seasons, respectively. Sun *et al* (2014) has reported the same results that ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) led to maximum accumulation of proline in Chinese chive seedlings.

4.3. Yield and quality traits

4.3.1 Ascorbic acid

During main season (October) maximum mean ascorbic acid content in spinach was found in the plants provided with treatment T1 and T2 ($\text{NO}_3^-/\text{NH}_4^+::100:0, 75:25$). During off season (February and July) sown crop, maximum mean ascorbic acid content was recorded in the plants supplied with treatments T2($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Table 17). Spinach plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated 61.30%, 49.34% and 57.42% more ascorbic acid content than soil grown coriander in October, February and July seasons, respectively. In coriander during all the three seasons, maximum and minimum mean ascorbic acid content was observed in the plants which were provided with nutrient solutions T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) respectively (Table 17). Coriander plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated 193.47%, 216.88% and 206.63% more ascorbic acid content than soil grown coriander in October, February and July seasons, respectively. Similarly in lettuce during all the three seasons, maximum and minimum mean ascorbic acid content was observed in the plants which were provided with nutrient solutions T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) respectively (Table 17). Lettuce plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated 123.77%, 759.56% and 841.43% more ascorbic acid content than soil grown coriander in October, February and July seasons, respectively. Hydroponically grown crops accumulated more ascorbic acid content than open field sown crop in the same seasons and also the ascorbic acid content found in all the three crops was more in main season than off seasons grown crops. Buchanan and Omaye (2013) have observed the same results in lettuce where hydroponically grown lettuce were recorded with maximum content of ascorbic acid than soil grown lettuce. Increased ascorbic acid content was obtained with the supply of NO_3^- and NH_4^+ in the ratio of 80:20 in Chinese kale (Zhu *et al* 2018). Highest ascorbic acid content was obtained in basil with nutrient solution 3 ($\text{NO}_3^-:4/ \text{NH}_4^+:0.5$) and 1 ($\text{NO}_3^-:3.5/ \text{NH}_4^+:1.5$), respectively (Saadatian *et al* 2014).

Table 16. Effect of nitrate and ammonium ratios on proline ($\mu\text{g/gFW}$)

	Proline ($\mu\text{g/gFW}$) Spinach				Proline ($\mu\text{g/gFW}$) Coriander				Proline ($\mu\text{g/gFW}$) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	1209.00 ^d	1221.00 ^c	600.46 ^{ij}	1010.10 ^b	667.88 ^c	654.89 ^f	645.45 ^g	656.07 ^b	879.81 ^d	874.28 ^e	862.73 ^f	872.27 ^b
T2=75:25	1611.00 ^b	1622.00 ^a	1613.00 ^b	1615.20 ^a	800.76 ^a	783.15 ^b	766.63 ^c	783.51 ^a	975.09 ^a	962.91 ^b	941.89 ^c	959.96 ^a
T3=50:50	789.78 ^e	793.13 ^e	792.24 ^e	791.17 ^c	621.99 ^h	598.46 ⁱ	590.88 ^j	603.78 ^d	681.42 ^h	672.72 ⁱ	623.96 ^j	659.37 ^c
T4=25:75	596.74 ^k	602.35 ⁱ	598.13 ^{jk}	599.07 ^e	489.99 ^k	476.45 ^l	464.72 ^m	477.05 ^e	550.23 ^k	557.93 ^l	522.54 ^m	543.57 ^d
T5=0:100	511.69 ^m	520.35 ^l	511.95 ^m	514.66 ^f	375.36 ^o	378.80 ⁿ	366.33 ^p	373.50 ^f	471.54 ⁿ	467.79 ^o	430.16 ^p	456.50 ^f
Open	627.54 ^h	641.66 ^f	633.69 ^g	634.30 ^d	681.36 ^d	590.67 ^j	588.82 ^j	620.28 ^c	339.26 ^q	788.13 ^g	427.30 ^p	518.23 ^e
Mean	890.96 ^b	899.96 ^a	791.61 ^c	860.84	606.22 ^a	580.41 ^b	570.47 ^c	585.70	649.56 ^b	720.63 ^a	634.76 ^c	668.32

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

Table 17. Effect of nitrate and ammonium ratios on ascorbic acid ($\mu\text{g/gFW}$)

	Ascorbic acid ($\mu\text{g/gFW}$) Spinach				Ascorbic acid ($\mu\text{g/gFW}$) Coriander				Ascorbic acid ($\mu\text{g/gFW}$) Lettuce			
	Main Season		Off season		Main Season		Off season		Main Season		Off season	
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	29.39 ^a	23.81 ^c	19.92 ^d	24.37 ^b	260.73 ^b	252.40 ^c	240.64 ^c	251.26 ^b	50.67 ^c	44.52 ^f	43.04 ^f	46.08 ^b
T2=75:25	30.05 ^a	25.91 ^b	23.03 ^c	26.33 ^a	267.03 ^a	259.02 ^b	244.48 ^d	256.84 ^a	141.60 ^a	136.24 ^b	124.74 ^c	134.19 ^a
T3=50:50	22.54 ^c	15.78 ^{fg}	16.35 ^{fg}	18.22 ^c	119.59 ^f	97.20 ^g	92.66 ^h	103.15 ^c	21.17 ^g	18.87 ^h	15.27 ^{ij}	18.44 ^d
T4=25:75	8.81 ^h	6.46 ^{ij}	6.65 ^{ij}	7.31 ^e	73.52 ^j	59.38 ^k	51.83 ^l	61.58 ^d	14.24 ^{ij}	9.69 ^k	8.30 ^{kl}	10.74 ^e
T5=0:100	7.80 ^{hi}	5.22 ^j	5.53 ^j	6.18 ^f	41.04 ^m	30.47 ⁿ	28.66 ⁿ	33.89 ^e	10.27 ^k	8.18 ^{kl}	7.36 ⁱ	8.60 ^f
Open	18.63 ^{de}	17.35 ^e	14.63 ^g	16.87 ^d	90.99 ^h	81.74 ⁱ	79.68 ⁱ	84.14 ^f	63.28 ^d	15.85 ⁱ	13.25 ^j	30.79 ^c
Mean	19.54 ^a	15.76 ^b	14.35 ^c	16.55	142.15 ^a	130.04 ^b	122.99 ^c	131.73	50.21 ^a	38.89 ^b	35.33 ^c	41.47

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

Table 18. Effect of nitrate and ammonium ratios on α -tocopherol content ($\mu\text{g/gFW}$)

	α -Tocopherol ($\mu\text{g/gFW}$) Spinach				α -Tocopherol ($\mu\text{g/gFW}$) Coriander				α -Tocopherol ($\mu\text{g/gFW}$) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	$\text{NO}_3^-/\text{NH}_4^+$ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July
T1=100:0	7.60 ^c	6.62 ^d	6.34 ^{de}	6.86 ^b	6.54 ^c	5.54 ^{ef}	5.22 ^f	5.77 ^c	10.30 ^b	7.37 ^d	7.25 ^d	8.30 ^b
T2=75:25	8.71 ^a	8.08 ^b	7.80 ^{bc}	8.22 ^a	8.38 ^a	7.30 ^b	7.04 ^b	7.57 ^a	12.51 ^a	9.11 ^c	8.70 ^c	10.11 ^a
T3=50:50	7.50 ^c	6.31 ^{de}	6.11 ^e	6.64 ^c	6.43 ^c	6.11 ^{cd}	5.76 ^{de}	6.10 ^b	10.24 ^b	7.17 ^d	6.97 ^d	8.13 ^b
T4=25:75	6.01 ^e	5.07 ^f	4.92 ^{fg}	5.33 ^d	5.25 ^f	5.29 ^{ef}	5.21 ^f	5.25 ^d	4.53 ^e	3.06 ^{fg}	2.79 ^g	3.46 ^d
T5=0:100	4.03 ⁱ	3.05 ^j	2.61 ^k	3.23 ^f	3.06 ^h	2.32 ⁱ	2.17 ⁱ	2.52 ^f	3.43 ^{fg}	1.28 ^h	1.13 ^h	1.95 ^f
Open	6.17 ^e	4.58 ^{gh}	4.22 ^{hi}	4.97 ^e	4.49 ^g	3.34 ^h	3.35 ^h	3.73 ^e	4.53 ^e	3.48 ^f	3.20 ^{fg}	3.74 ^c
Mean	6.67 ^a	5.62 ^b	5.34 ^c	5.88	5.69 ^a	4.98 ^b	5.69 ^c	5.16	7.59 ^a	5.25 ^b	5.01 ^c	5.95

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

4.3.2 α -tocopherol content

α - tocopherol content in October, February and July sown spinach crop was significantly higher in plants supplied with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and the plants which were provided with treatment T5 ($(\text{NO}_3^-/\text{NH}_4^+::0:100)$ had the least mean α -tocopherol content. 41.17% (October), 76.42% (February) and 84.83% (July) percent increase in α -tocopherol content was recorded in hydroponically grown spinach provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) as compared to soil grown spinach (Table 18). Similarly in coriander, during all the three growing seasons maximum content of α -tocopherol recorded in the plants which were supplied with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and the plants which were provided with treatment T5 ($(\text{NO}_3^-/\text{NH}_4^+::0:100)$ had the least mean α - tocopherol content. In coriander with the application of treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$), 86.64%, 11.86% and 11.81% more α -tocopherol content was found in hydroponically grown crop as compared to soil grown crop in October, February and July sown seasons, respectively (Table 18). Likewise in lettuce, during all the three growing seasons maximum content of α -tocopherol recorded in the plants which were supplied with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and the plants which were provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) had the least mean α - tocopherol content (Table 18). With the application of treatment T2 ($\text{NO}_3^-/\text{NH}_4^+ :: 75:25$) hydroponically grown lettuce accumulated significantly more α -tocopherol content than soil grown crop and it was 176.16% (October), 161.78% (February) and 171.88% (July) more than soil grown lettuce. Buchanan and Omaye (2013) has obtained the same results where they found that hydroponically grown lettuce plants accumulated more α -tocopherol than soil grown lettuce.

4.3.3 Total yield per plant

Total plant yield per plant given in Table 19 differed significantly with the different treatments provided. During all the three growing seasons (October, February and July) maximum yield per plant was recorded in the spinach plants which were provided with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Table 19). Hydroponically grown spinach, provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) reported 14.40%, 33.72% and 60.87% more yield than soil grown spinach in October, February and July, respectively. Similarly in coriander during all the three seasons maximum and minimum yield was recorded in the plants which were supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$), respectively. Yield of hydroponically grown coriander plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) was more than soil grown coriander in the same seasons (i.e. 21.74% in October, 21.11% in February and 46.19% in July). In lettuce during all the three growing seasons (October, February and July) maximum yield per plant was recorded in the plants which were provided with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Table 19). Hydroponically grown lettuce, provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) reported 1.75%, 46.88% and

800.00% more yield than soil grown ettuce in October, February and July, respectively. Likewise in taro plants greater biomass was obtained when supplied with nutrient solutions NO_3^- and NH_4^+ in the ratio of 75:25 (Osorio *et al* 2003), in lettuce (Wang and Shen 2011), in pepper (Bar-Tal *et al* 2001) and in French beans (Guo *et al* 2002).

4.4 Antioxidants enzymes activities

4.4.1 Superoxide dismutase activity

In spinach SOD (superoxide dismutase) activity was significantly more in off seasons (February and July) crop than the main season crop (October) (Table 20). During February and July sown crop, maximum SOD activity was seen in the plants which were provided with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and T3 ($\text{NO}_3^-/\text{NH}_4^+::50:50$) and plants had least SOD activity provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). In main season (October) sown crop maximum mean SOD activity was reported in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and plants had the least SOD activity provided with T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) nutrient solution. Similarly in coriander, SOD (superoxide dismutase) activity was significantly more in off seasons (February and July) crop than the main season crop (October) (Table 20). During February and July sown crop, maximum SOD activity was seen in the plants which were provided with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) followed by T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) and plants had least SOD activity provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). In main season (October) sown crop maximum mean SOD activity was reported in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and plants had the least SOD activity provided with T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) nutrient solution. In lettuce, during all the three growing seasons, maximum SOD activity was seen in the plants which were provided with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and plants had least SOD activity provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). In lettuce different style was observed than observed in spinach and coriander i.e. SOD activity in the main season was significantly more than off season grown lettuce crop. Reactive oxygen species (ROS) which cause the lipid peroxidation of cell membranes are detoxified by the plant antioxidant enzymes e.g. SOD, POD, and CAT (Gulen *et al* 2008). Activities of anti-oxidant enzymes were influenced significantly with N forms, and their activities of plants treated with NH_4^+ mixed nutrient solution were significantly higher than those with the sole NO_3^- source of N treatment (Polesskaya *et al* 2004). An increase in activities of anti-oxidant enzymes was observed in *Polygonatum odoratum* which were grown aeroponically with low and moderate proportion of NH_4^+ mixed solutions (90:10= $\text{NO}_3^-: \text{NH}_4^+$) treatment compared with all nitrate N. NH_4^+ is an important material to synthesize amino acids, nevertheless, its superfluous usage would be toxic to plant (Zou *et al* 2017). Consequently, ($\text{NO}_3^-/\text{NH}_4^+ =75:25$) treatment, which had a low or moderate NH_4^+ proportion, helped in promoting the activities of anti-oxidant enzymes

Table 19. Effect of nitrate and ammonium ratios on total yield per plant (g)

NO ₃ ⁻ /NH ₄ ⁺ ratio	Total yield (g) Spinach				Total yield (g) Coriander				Total yield (g) Lettuce			
	Main season	Off season		Mean	Main season	Off season		Mean	Main season	Off season		Mean
	October	February	July		October	February	July		October	February	July	
T1=100:0	41.11 ^d	37.89 ^{efg}	34.50 ^h	37.83 ^c	11.46 ^b	10.31 ^c	8.09 ^g	9.95 ^b	560.00 ^c	430.00 ^h	70.00 ⁿ	353.33 ^b
T2=75:25	57.45 ^a	52.62 ^{bc}	48.97 ^c	48.11 ^a	12.22 ^a	11.62 ^b	9.40 ^e	11.08 ^a	580.00 ^a	470.00 ^g	90.00 ^m	380.00 ^a
T3=50:50	38.71 ^{def}	36.30 ^{fgh}	30.68 ⁱ	35.76 ^d	10.16 ^c	8.55 ^f	7.06 ⁱ	8.59 ^{cd}	530.00 ^d	404.00 ⁱ	60.00 ^o	331.33 ^c
T4=25:75	35.83 ^{gh}	30.20 ⁱ	25.21 ^j	32.98 ^e	9.66 ^d	7.50 ^h	6.38 ^j	7.85 ^d	460.00 ^e	370.00 ^j	35.00 ^p	288.33 ^e
T5=0:100	30.58 ⁱ	22.18 ^k	17.46 ⁱ	28.64 ^f	8.58 ^f	6.12 ^k	4.34 ^l	6.35 ^e	380.00 ^f	300.00 ^l	10.00 ^q	230.00 ^f
Open	50.22 ^{bc}	39.35 ^d	30.44 ⁱ	39.52 ^b	10.09 ^c	8.50 ^f	6.43 ^j	8.34 ^{cd}	570.00 ^b	320.00 ^k	10.00 ^q	300.00 ^d
Mean	42.32 ^a	37.89 ^b	31.21 ^c	37.14	10.36 ^a	8.77 ^b	6.95 ^c	8.69 ^c	513.33 ^a	382.33 ^b	45.83 ^c	313.83

Means followed by the same letters in each row and column do not differ significantly (P< 0.05)

Table 20. Effect of nitrate and ammonium ratios on Superoxide dismutase activity (units/min/g/FW)

	Superoxide dismutase (units/min/g/FW)				Superoxide dismutase (units/min/g/FW)				Superoxide dismutase (units/min/g/FW)			
	Spinach				Coriander				Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	108.16 ⁱ	267.99 ^d	282.26 ^b	219.47 ^b	159.00 ⁱ	194.18 ^c	192.52 ^d	181.91 ^b	342.23 ^b	229.29 ^h	212.31 ^j	261.28 ^b
T2=75:25	127.59 ^g	290.74 ^a	289.26 ^a	235.86 ^a	164.45 ^g	198.92 ^a	196.83 ^b	186.73 ^a	343.40 ^a	232.29 ^g	221.30 ⁱ	265.66 ^a
T3=50:50	102.75 ^j	274.56 ^c	277.63 ^c	218.31 ^b	133.09 ^j	182.19 ^e	170.96 ^f	162.08 ^c	332.53 ^c	167.36 ^k	154.31 ^l	218.07 ^c
T4=25:75	92.99 ^j	265.31 ^d	260.36 ^e	206.22 ^d	125.61 ^m	171.22 ^f	143.90 ^j	146.91 ^d	331.59 ^d	150.92 ^m	137.36 ^o	206.62 ^d
T5=0:100	88.70 ^l	243.22 ^f	244.26 ^f	192.06 ^e	115.38 ^l	135.17 ^k	94.23 ^p	114.93 ^f	287.10 ^f	136.38 ^p	115.25 ^r	179.18 ^f
Open	122.16 ^h	260.12 ^e	265.22 ^d	215.84 ^e	134.25 ^h	161.13 ^h	110.38 ^o	135.25 ^e	308.21 ^e	146.55 ⁿ	120.81 ^q	191.85 ^c
Mean	107.06 ^c	266.99 ^b	269.83 ^a	214.63	138.63 ^c	173.80 ^a	151.47 ^b	154.63	324.17 ^a	177.13 ^b	160.22 ^c	220.51

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

Table 21. Effect of nitrate and ammonium ratios on peroxidase activity ($\mu\text{moles}/\text{min}/\text{gFW}$)

	Peroxidase ($\mu\text{moles}/\text{min}/\text{gFW}$) Spinach				Peroxidase ($\mu\text{moles}/\text{min}/\text{gFW}$) Coriander				Peroxidase ($\mu\text{moles}/\text{min}/\text{gFW}$) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
T1=100:0	44.75 ^b	14.68 ^f	11.89 ^g	23.78 ^b	68.14 ^b	25.18 ^d	30.91 ^c	41.41 ^b	19.79 ⁱ	50.63 ^{bc}	44.23 ^c	38.22 ^b
T2=75:25	50.50 ^a	19.13 ^d	16.57 ^e	28.73 ^a	71.22 ^a	26.70 ^d	32.86 ^c	43.59 ^a	26.37 ^{fg}	54.37 ^a	51.45 ^{ab}	44.07 ^a
T3=50:50	23.89 ^c	11.84 ^g	8.35 ^h	14.69 ^c	67.41 ^b	18.24 ^e	20.61 ^e	35.42 ^c	12.14 ^j	47.90 ^{cd}	46.56 ^{de}	35.53 ^c
T4=25:75	7.60 ^{hi}	8.46 ^h	6.38 ^{jk}	7.48 ^d	31.53 ^c	11.18 ^f	11.21 ^f	17.98 ^d	8.92 ^{kl}	27.92 ^f	26.84 ^{fg}	21.23 ^d
T5=0:100	1.56 ^m	7.27 ^{hi}	4.67 ^l	4.50 ^f	1.19 ⁱ	8.12 ^g	8.76 ^{fg}	6.02 ^f	5.90 ^l	23.94 ^{gh}	22.10 ^{hi}	17.31 ^f
Open	5.51 ^{kl}	7.86 ^{hi}	6.81 ^{ij}	6.73 ^e	4.34 ^h	7.93 ^g	9.88 ^{fg}	7.38 ^e	9.45 ^{jk}	24.45 ^{gh}	23.93 ^{gh}	19.28 ^e
Mean	22.30 ^a	11.54 ^b	9.11 ^c	14.32	68.14 ^c	25.18 ^b	30.91 ^c	41.41 ^b	13.76 ^c	38.20 ^a	35.85 ^b	29.27

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

Table 22. Effect of nitrate and ammonium ratios on catalase activity ($\mu\text{moles}/\text{min}/\text{gFW}$)

	Catalase ($\mu\text{moles}/\text{min}/\text{gFW}$) Spinach				Catalase ($\mu\text{moles}/\text{min}/\text{gFW}$) Coriander				Catalase ($\mu\text{moles}/\text{min}/\text{gFW}$) Lettuce			
	Main season	Off season			Main season	Off season			Main season	Off season		
	October	February	July	Mean	October	February	July	Mean	October	February	July	Mean
NO₃⁻/NH₄⁺ ratio												
T1=100:0	341.31 ^f	372.72 ^d	358.05 ^c	357.36 ^b	380.85 ^f	400.17 ^d	392.12 ^c	391.05 ^b	90.84 ^f	104.16 ^d	97.38 ^c	97.46 ^b
T2=75:25	402.63 ^c	436.69 ^a	425.74 ^b	421.69 ^a	466.55 ^c	489.57 ^a	481.34 ^b	479.15 ^a	107.4 ^c	121.87 ^a	114.71 ^b	114.67 ^a
T3=50:50	266.59 ^{lm}	283.83 ^h	274.53 ^j	274.98 ^d	342.13 ^j	360.17 ^g	350.22 ^{hi}	350.84 ^d	41.10 ^j	53.52 ^g	46.49 ⁱ	47.04 ^c
T4=25:75	260.06 ⁿ	279.60 ⁱ	270.62 ^{kl}	270.09 ^e	272.83 ^m	287.61 ^k	279.84 ^l	280.09 ^e	19.27 ^m	29.31 ^k	23.86 ^l	24.15 ^d
T5=0:100	220.26 ^q	246.85 ^o	238.00 ^p	235.04 ^f	218.46 ^o	226.30 ⁿ	223.77 ⁿ	222.84 ^f	8.47 ^o	13.80 ⁿ	12.38 ⁿ	11.55 ^c
Open	266.29 ^m	291.14 ^g	284.26 ^p	280.56 ^c	340.56 ^j	352.77 ^h	348.24 ⁱ	347.19 ^d	42.75 ^j	50.20 ^h	48.72 ^{hi}	47.22 ^c
Mean	292.86 ^c	308.53 ^b	318.47 ^a	306.62	336.90 ^c	352.76 ^a	345.92 ^b	345.20	51.64 ^c	114.67 ^a	57.26 ^b	57.01

Means followed by the same letters in each row and column do not differ significantly ($P < 0.05$)

in the present investigation. Our results are also on agreement with the findings of Sun *et al* (2014) in Chinese chive where the optimum ratios of NO_3^- -N to NH_4^+ -N for protecting Chinese chive against oxidative stress are 50:50 and 75:25. Boschiero *et al* (2019) has reported the same results in sugarcane.

4.4.2 Peroxidase activity

POD (Peroxidase) activity in spianch during main season (October) was significantly maximum in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+$:: 75:25). During off seasons (February and July) grown crop maximum POD activity was found in plants provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+$:: 75:25) (Table 21) plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+$::0:100) and grown in open field conditions recorded with minimum level of POD activity. In coriander main season sown crop showed maximum mean POD activity than off season sown crop. POD (Peroxidase) activity in coriander during main season (October) was significantly maximum in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+$:: 75:25) i.e. 71.22 $\mu\text{moles}/\text{min}/\text{gFW}$. During off seasons i.e. February and July grown crop maximum POX activity was found in plants provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+$:: 75:25) i.e. 26.70 and 32.86 $\mu\text{moles}/\text{min}/\text{gFW}$, respectively (Table 21) and plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+$ =0:100) and grown in open field conditions recorded with minimum level of POX activity. Similarly in lettuce, POX (Peroxidase) activity during main season (October) and off seasons was significantly maximum in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+$:: 75:25) i.e. 26.37, 54.37 and 51.45 $\mu\text{moles}/\text{min}/\text{gFW}$ in October, February and July, respectively. Plants had minimum POD activity which were provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+$::0:100). Unlike spinach and coriander POD activity in lettuce was significantly more in off seasons than main season sown crop. Activities of anti-oxidant enzymes were influenced significantly with N forms, and their activities of plants treated with NH_4^+ mixed nutrient solution were significantly higher than those with the sole NO_3^- source of N treatment (Polesskaya *et al* 2004). An increase in activities of anti-oxidant enzymes was observed in *Polygonatum odoratum* which were grown aeroponically with low and moderate proportion of NH_4^+ mixed solutions (90:10= NO_3^- : NH_4^+) treatment compared with all nitrate N. NH_4^+ is an important material to synthesize amino acids, nevertheless, its superfluous usage would be toxic to plant (Zou *et al* 2017). Consequently, ($\text{NO}_3^-/\text{NH}_4^+$ =75:25) treatment, which had a low or moderate NH_4^+ proportion, helped in promoting the activities of anti-oxidant enzymes in the present investigation. Our results are also on agreement with the findings of Sun *et al* (2014) in Chinese chive where the optimum ratios of NO_3^- -N to NH_4^+ -N for protecting Chinese chive against oxidative stress are 50:50 and 75:25. Boschiero *et al* (2019) has reported the same results in sugarcane.

4.4.3 Catalase activity

CAT activity (Table 22) was recorded maximum in the spinach plants which were

supplied with T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) treatments and plants provided T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) maintained the lowest level of CAT activity, during all the three seasons. CAT activity during off seasons was significantly maximum than main season sown crop. In coriander, CAT activity was recorded maximum in the plants which were supplied with T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) treatments and plants provided with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) maintained the lowest level of CAT activity, during all the three seasons. CAT activity during off seasons was significantly maximum than main season sown crop. CAT activity was recorded maximum in the lettuce plants which were supplied with T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) treatments and plants provided T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) maintained the lowest level of CAT activity, during all the three seasons. Like spinach and coriander, lettuce plants grown in off seasons showed maximum CAT activity than main seasons sown crop. More temperature during off season than main season could be the reason for increased activity of this enzyme. Reactive oxygen species (ROS) which cause the lipid peroxidation of cell membranes are detoxified by the plant antioxidant enzymes e.g. SOD, POD, and CAT (Gulen *et al* 2008). Activities of anti-oxidant enzymes were influenced significantly with N forms, and their activities of plants treated with NH_4^+ mixed nutrient solution were significantly higher than those with the sole NO_3^- source of N treatment (Polesskaya *et al* 2004). An increase in activities of anti-oxidant enzymes was observed in *Polygonatum odoratum* which were grown aeroponically with low and moderate proportion of NH_4^+ mixed solutions (90:10= $\text{NO}_3^-:\text{NH}_4^+$) treatment compared with all nitrate N. NH_4^+ is an important material to synthesize amino acids, nevertheless, its superfluous usage would be toxic to plant (Zou *et al* 2017). Consequently, ($\text{NO}_3^-/\text{NH}_4^+ =75:25$) treatment, which had a low or moderate NH_4^+ proportion, helped in promoting the activities of anti-oxidant enzymes in the present investigation. Our results are also on agreement with the findings of Sun *et al* (2014) in Chinese chive where the optimum ratios of NO_3^- -N to NH_4^+ -N for protecting Chinese chive against oxidative stress are 50:50 and 75:25. Boschiero *et al* (2019) has reported the same results in sugarcane.

CHAPTER- V

SUMMARY

The present investigation entitled, “Effect of nitrate and ammonium ratios on physiological and quality attributes of hydroponically grown leafy vegetables.” was undertaken with objectives to standardize a suitable $\text{NO}_3^-:\text{NH}_4^+$ ratio in nutrient formulation for optimum growth and yield of leafy vegetables under substrate hydroponics system with intermittent water and nutrient supply and to compare the growth, physiological and biochemical attributes, yield and quality traits of leafy vegetables under different seasons and growing conditions (substrate hydroponics and open field).

The experiments were conducted on three leafy crops viz; spinach (cultivar. Punjab Green), lettuce (cultivar. Iceberg) and coriander (cultivar. Punjab Sugandh). Two crops were raised simultaneously i.e. one in open field conditions (control) and another under controlled conditions in a green house using substrate hydroponics system with intermittent supply of water and nutrients. Three crops were raised per year in the month of October, February and July respectively. Under the open field conditions all the three crops viz; spinach, lettuce and coriander were planted in 2m^2 plot with 3 replications during all the three seasons. For the first crop of lettuce (Oct 2016-17) the nursery was raised at field area of department of Vegetable Science during the 2nd week of September and transplanted during last week of October. Similarly for the 2nd crop of lettuce (Feb 2017-18) the nursery was raised during the 1st week of January and transplanted during mid of February. For the third crop of lettuce (July 2017-18) nursery was raised during the 2nd week of June and transplanted during last week of July. For July crop, the nursery was provided with shade by using green nets. For spinach and coriander crops, the seeds were directly sown during the 3rd week of October (2016-17), 2nd week of February (2017-18) and 2nd week of July (2017-18), respectively. For hydroponics seeds of spinach and coriander were directly sown in plastic pots (30 cm (diameter) \times 30 cm (height)) on a coco peat: perlite: vermiculite (3:1:1) medium. Lettuce plantlets were transplanted in similar pots. The potting mixture for the experiments was obtained from the local suppliers. After emergence, the seedlings were thinned to six per pot for coriander, four per pot for spinach and one per pot for lettuce. All the required field management practices were followed according to the specifications given in the Package of Practices for cultivation of Vegetables, Punjab Agricultural University, Ludhiana (Anonymous 2016). There were five treatments with varying ratios of NO_3^- -N and NH_4^+ -N while keeping the total N content constant (Cao *et al* 2010).

The various morpho-physiological observations viz., plant height, leaf area, leaf water potential, fresh and dry weights of both shoots and roots were recorded. The leaf samples were collected at vegetative stage of plant development and were analysed for the

estimation of total chlorophyll and carotenoids content, total soluble sugars, total starch, total soluble proteins, free amino acids and proline content. Yield and quality contributing parameters viz., ascorbic acid, α -tocopherol content were also determined. Enzymatic activities of superoxide dismutase, peroxidase and catalase were also observed.

The salient findings of the study are:

- For the October, February and July sown spinach crop, among the treatments, maximum mean plant height was recorded for the treatment T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) followed by treatment T2 (75:25) whereas, in coriander and lettuce maximum mean plant height was recorded in plants which were provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by treatment T1.
- Lower values (more negative) of water potential in leaves with the application of T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) in all the three crops i.e. spinach, coriander and lettuce in all the three growing seasons. Less negative leaf water potential in all the three crops was recorded when ratio of NH_4^+ in the nutrient solution was increased from 50% to 100% i.e. plants provided with treatment T5 ($\text{NO}_3^-:\text{NH}_4^+::0:100$) were recorded with lower negative value (more leaf water potential).
- The plants grown in ratios of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) and 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient medium had the significantly maximum mean leaf area in all the three growing seasons.
- In all the three crops maximum mean fresh weight of shoots and roots and also the dry weight of both shoots and roots was recorded for the plants provided with a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$).
- Significantly higher total chlorophyll content was recorded for the plants which were supplied with ratios of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) followed by 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solutions.
- Significantly higher carotenoids content was recorded for the plants which were supplied with ratios of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) followed by 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solutions.
- In all the three growing seasons (October, February and July) the plants grown with ratios of 75:25 and 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) had the maximum total soluble sugars content in spinach, coriander and lettuce, while those grown in 0:100 ($\text{NO}_3^-/\text{NH}_4^+$) treatment had the minimum total soluble sugars content.
- In all the three growing seasons (October, February and July) the plants grown with ratios 75:25 and 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) treatments had the maximum starch content in spinach, coriander and lettuce, while those grown in 0:100 ($\text{NO}_3^-/\text{NH}_4^+$) treatment had the minimum total starch content.
- Yield was maximum in the plants (spinach, coriander and lettuce) provided treatment T2

$\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by treatment T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) in the nutrient solution.

- In spinach, coriander and lettuce content of total soluble proteins accumulated maximum in plants provided with a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) followed by 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) whereas plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) in the nutrient solution had the minimum mean total soluble proteins content.
- In spinach, coriander and lettuce content of free aminoacids accumulated maximum in plants provided with a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) followed by 100:0 ($\text{NO}_3^-/\text{NH}_4^+$) whereas plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) in the nutrient solution had the minimum mean free aminoacids content.
- Leaf proline content was maximum with the supply of nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) during all the three growing seasons.
- Treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by treatment T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$) resulted in maximum mean accumulation of ascorbic acid in hydroponically sown crops as compared to open field conditions.
- In all the three seasons maximum mean σ -tocopherol content recorded in the plants provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) followed by treatment T1 ($\text{NO}_3^-/\text{NH}_4^+::100:0$).
- SOD, POX and CAT (superoxide dismutase, peroxidase, catalase) activity was significantly more in the leaves of spinach, coriander and lettuce plants provided with nutrient solution in the ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in all the three seasons (October, February and July).

Conclusion: In conclusion, overall, for each date of sowing, maximum yield was obtained in plants provided with nutrient solution T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$). In main season as well as off season sown crops (spinach, coriander and lettuce) the yield was significantly more in hydroponically than open field sown crop. Further, the crop provided with nutrient solutions having higher proportion of NO_3^- had higher values of quality parameters and maximum antioxidant activity than the open field crop. Hence providing nutrient solutions with appropriate ratios of $\text{NO}_3^-:\text{NH}_4^+$ improves the yield and quality traits in hydroponically grown crops. It is therefore suggested that $\text{NO}_3^-/\text{NH}_4^+$ ratios of 75:25 could promote growth, increase yield and improve the nutritional quality of spinach, coriander and lettuce under hydroponics.

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Appendix-I

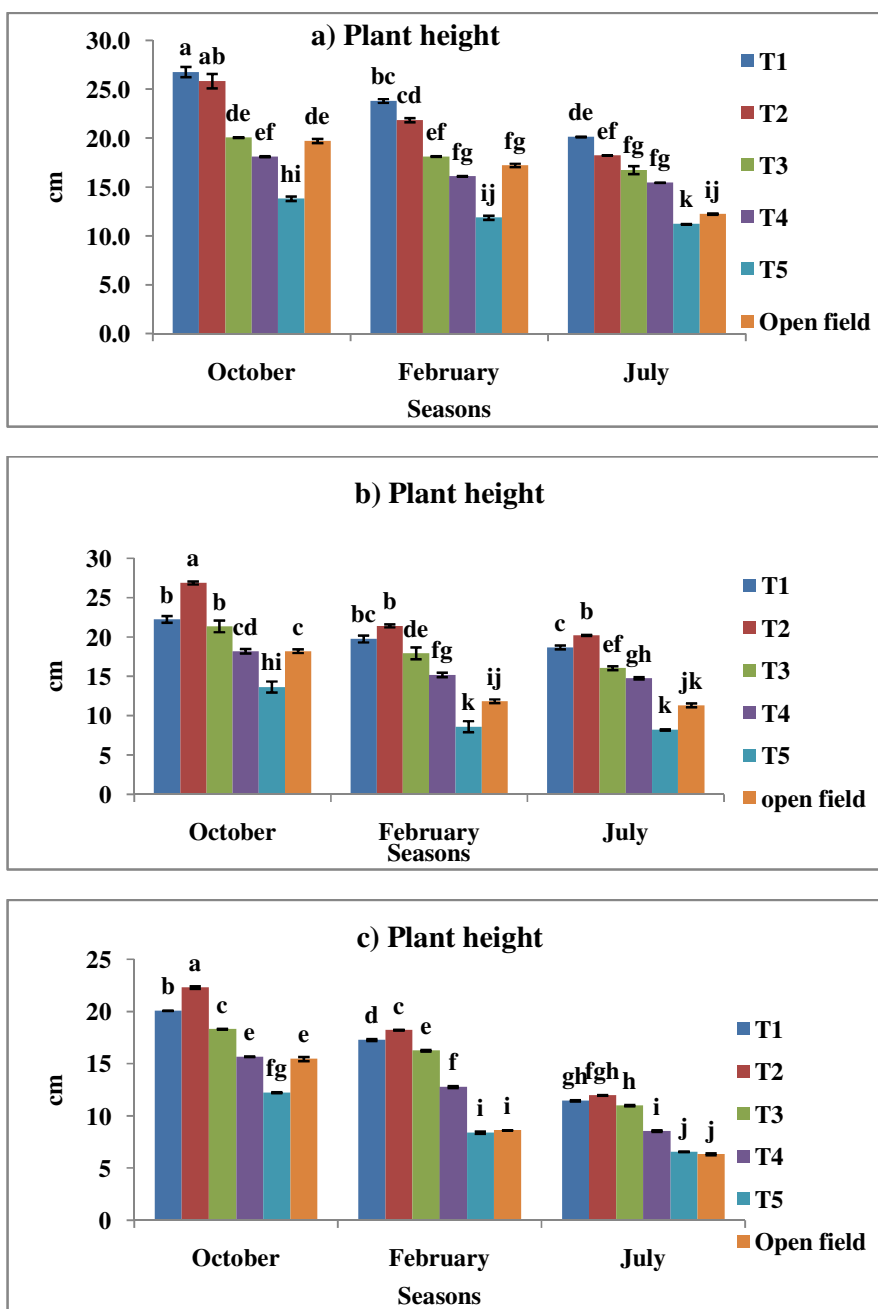


Figure1. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on plant height (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

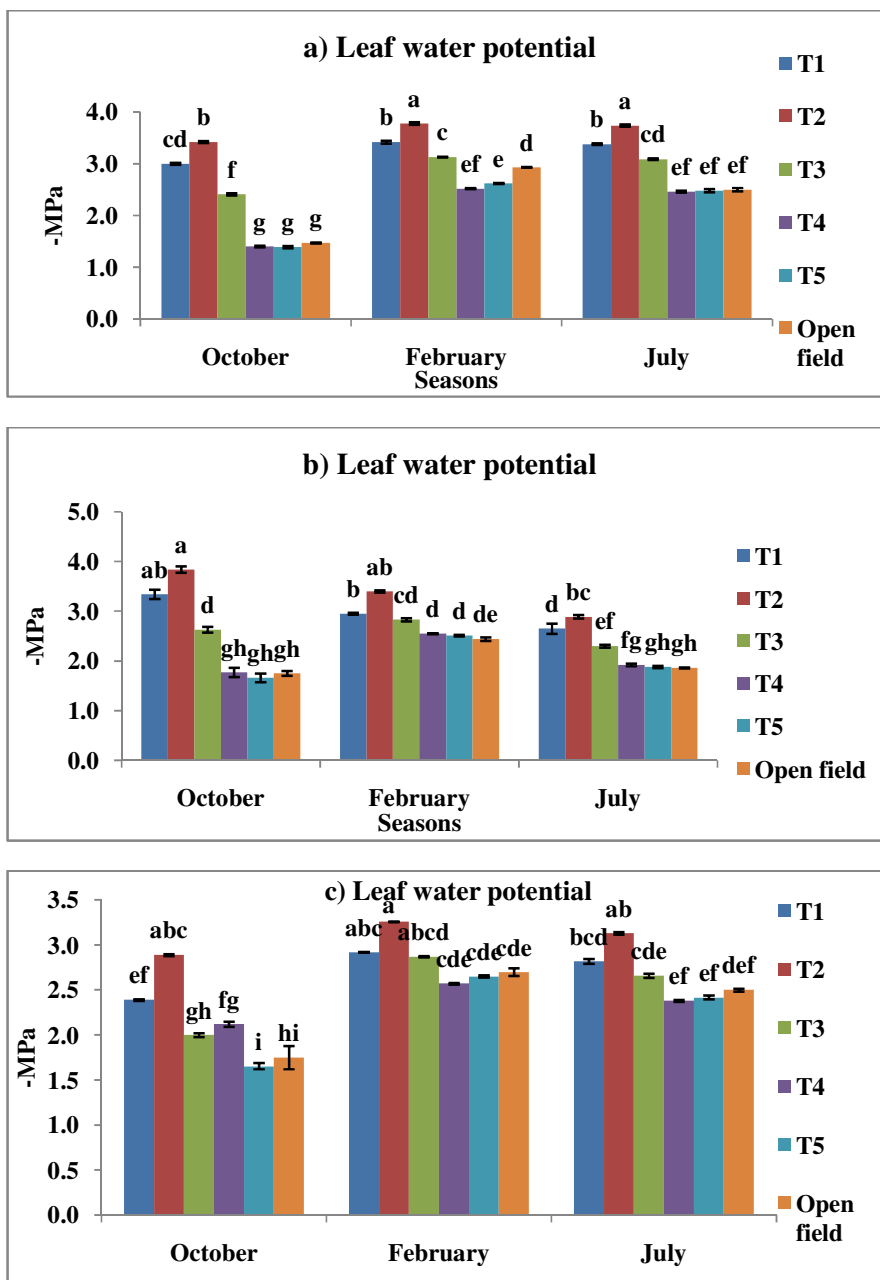


Figure2. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on leaf water potentials in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

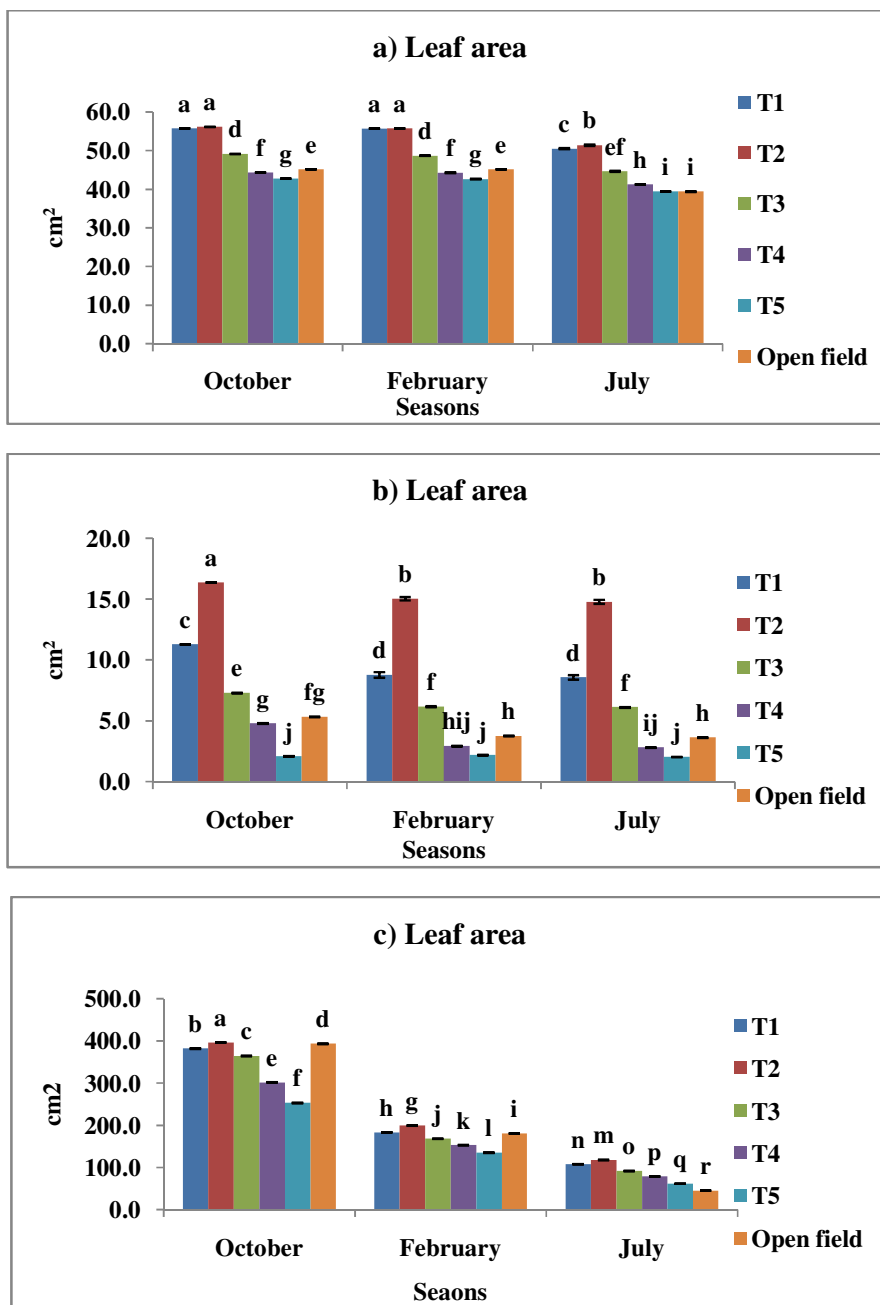


Figure3. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on leaf area in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

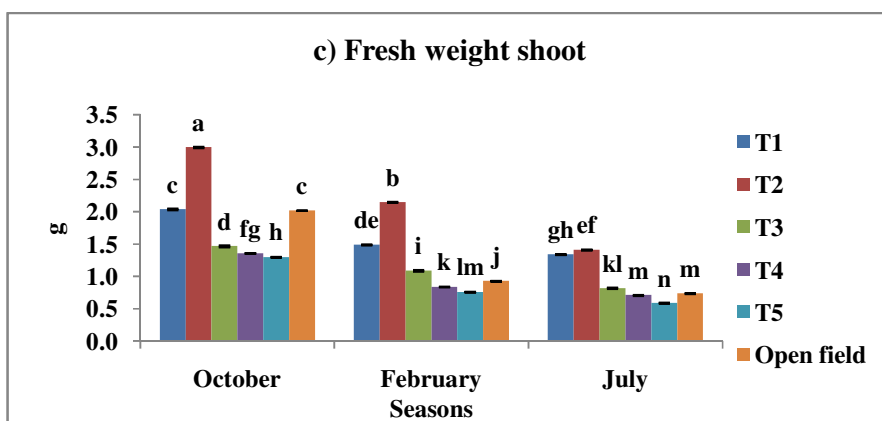
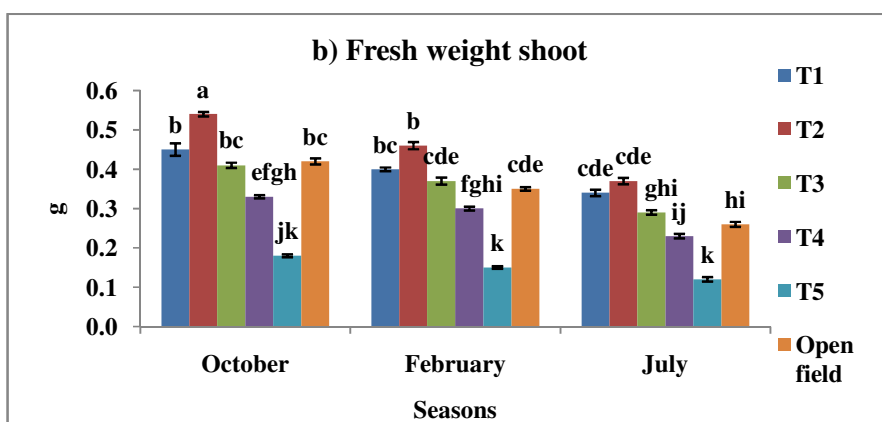
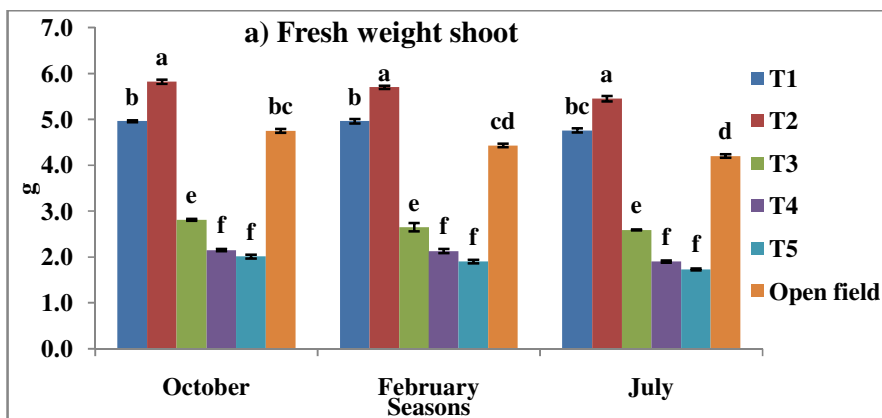


Figure4. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on fresh weight shoot in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

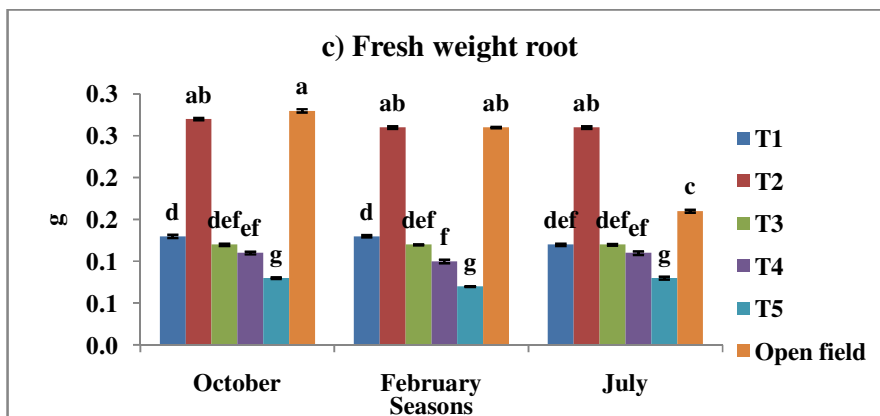
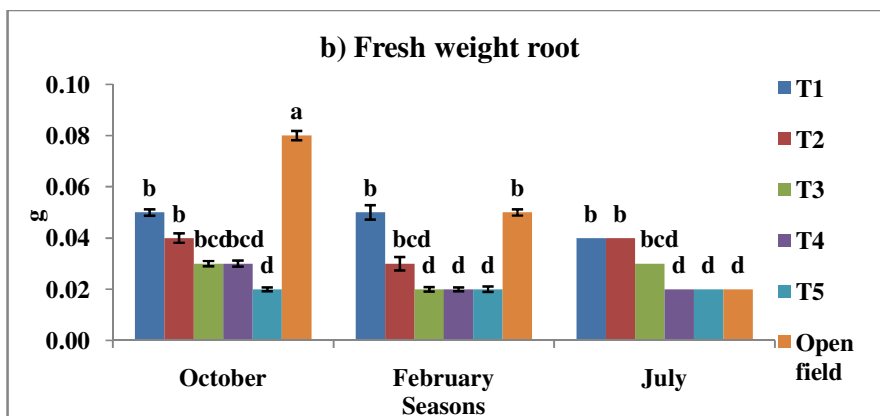
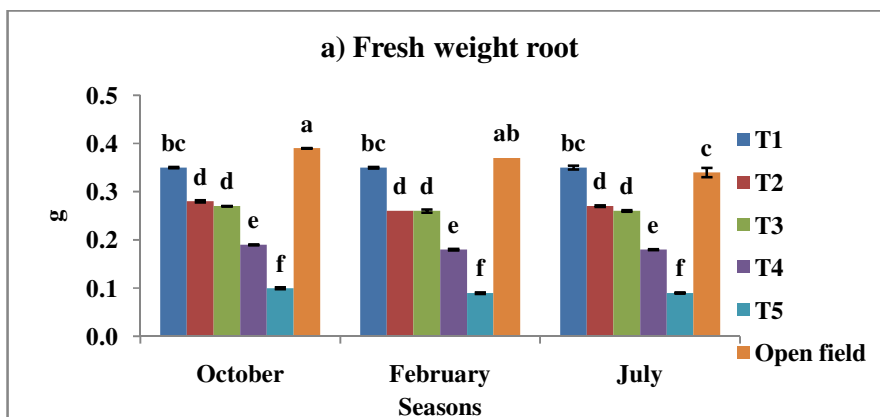


Figure5. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on fresh weight root in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

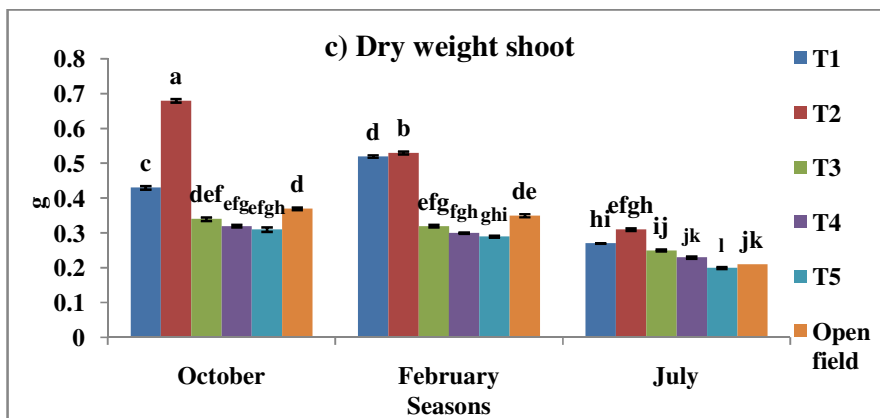
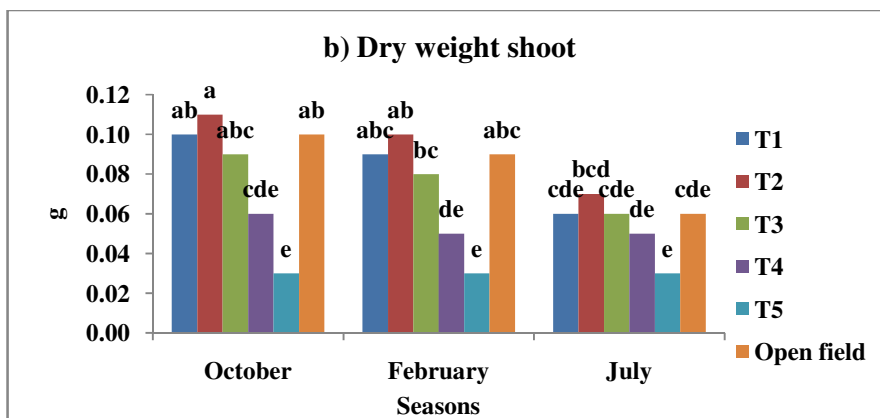
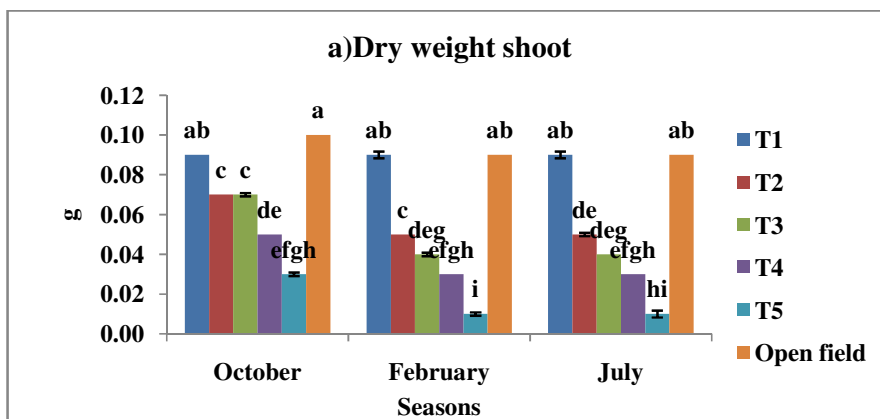


Figure 6. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on dry weight shoot in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

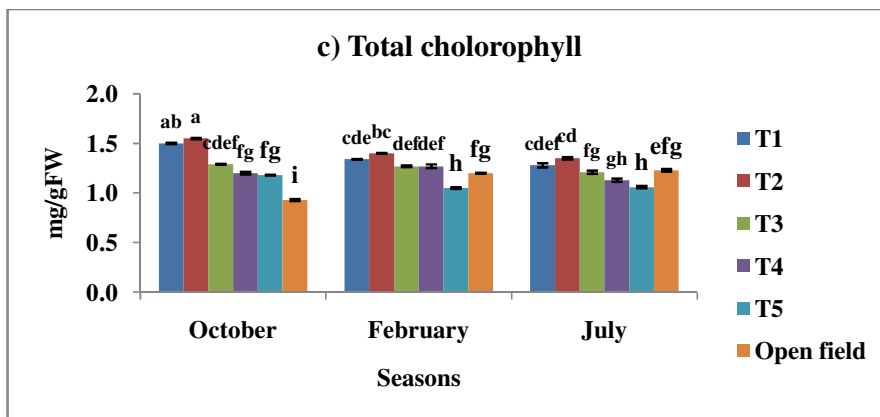
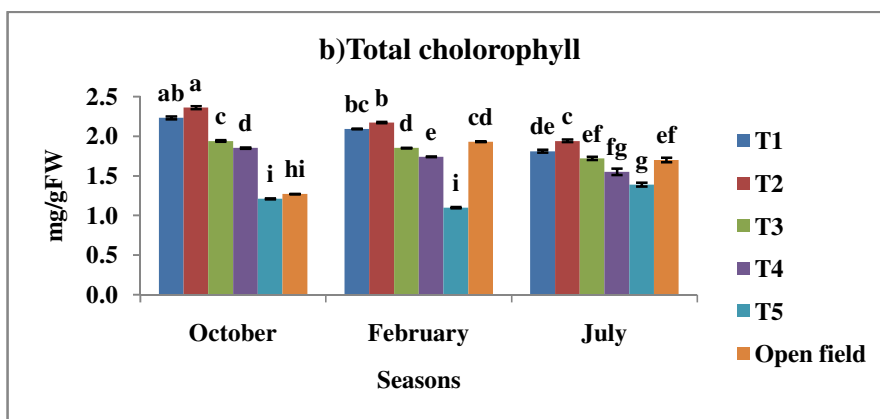
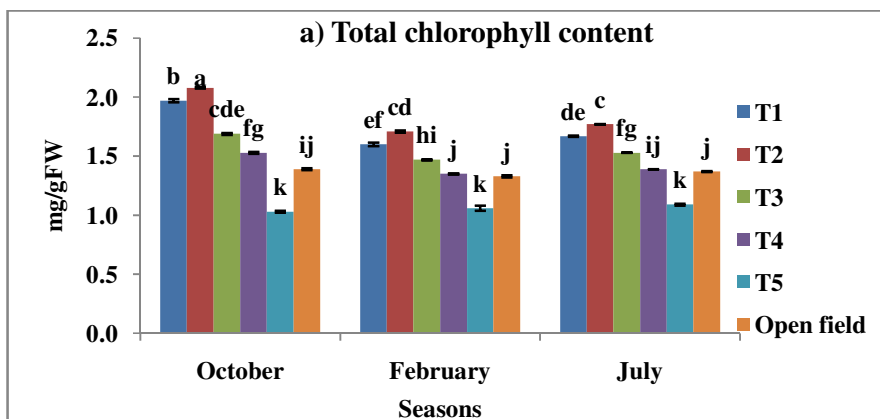


Figure 7. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on total chlorophyll content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

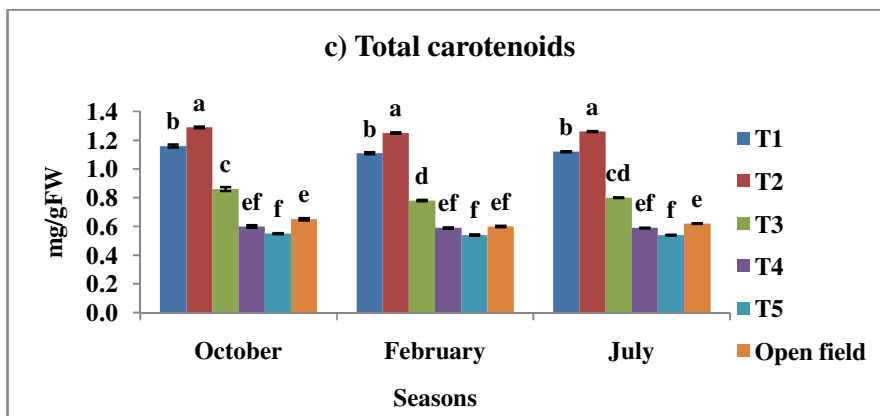
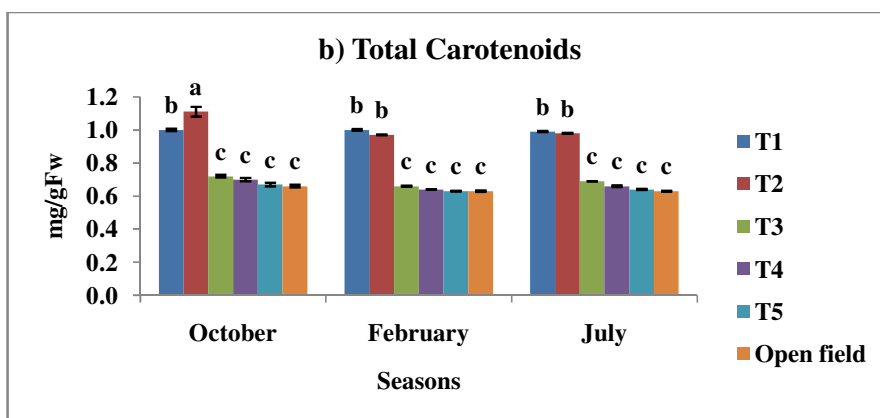
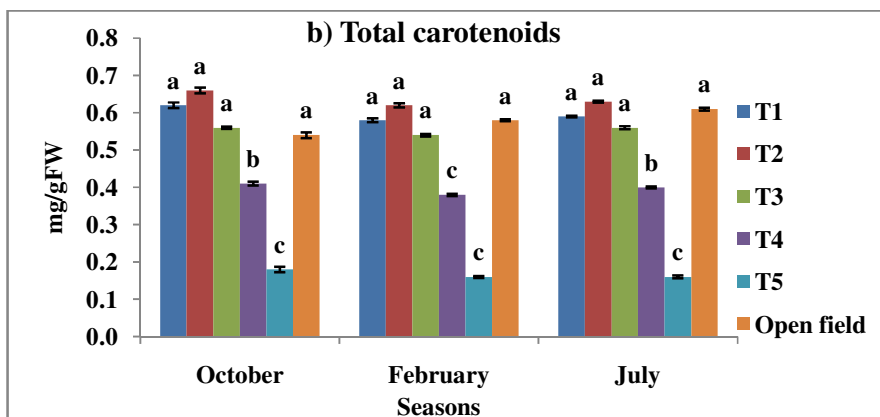


Figure 8. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on total carotenoids content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

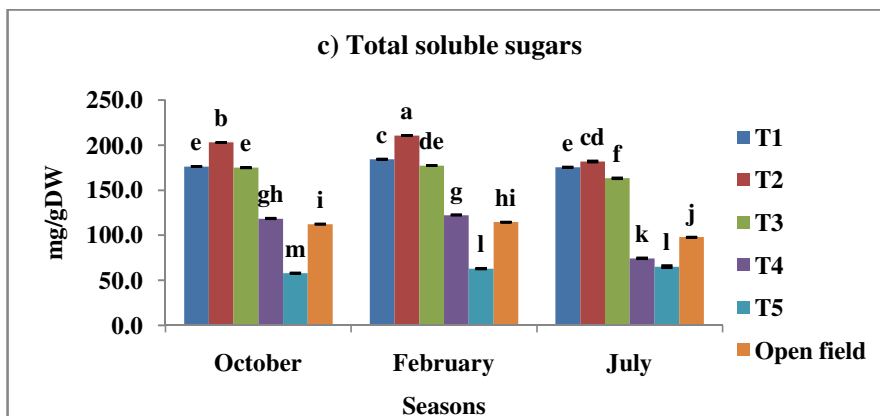
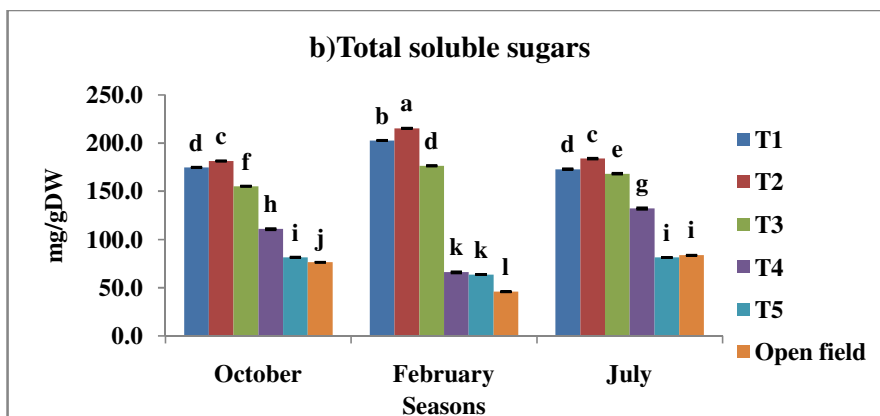
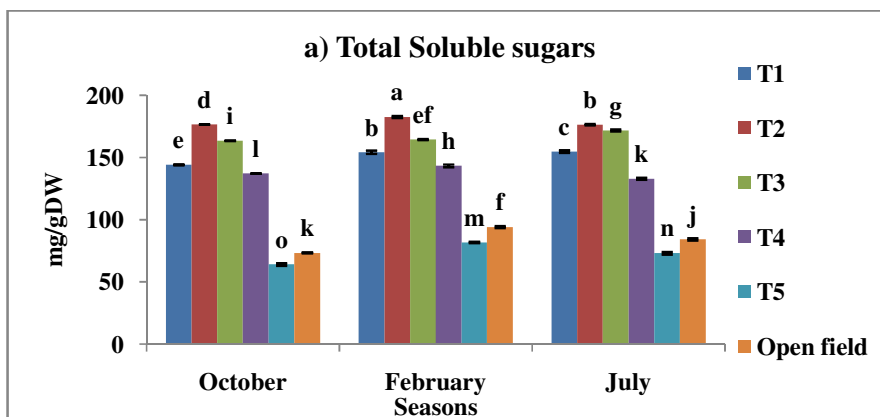


Figure 9. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on total soluble sugars content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

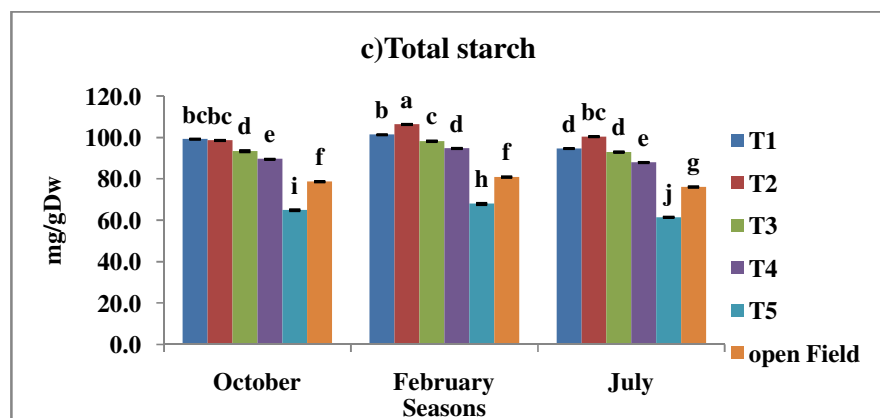
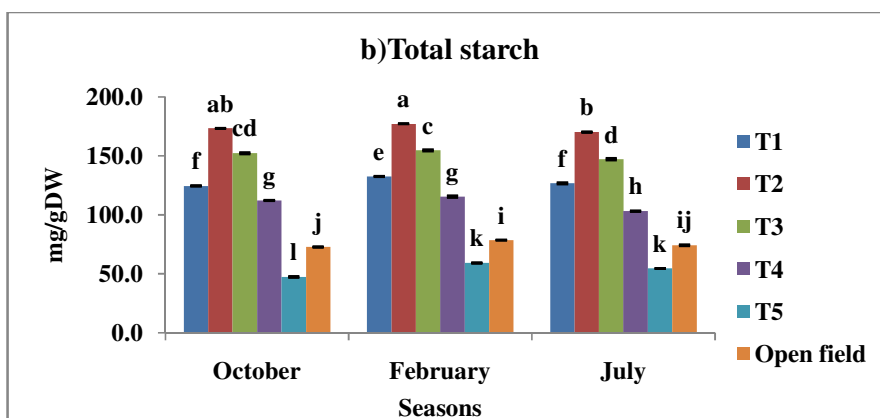
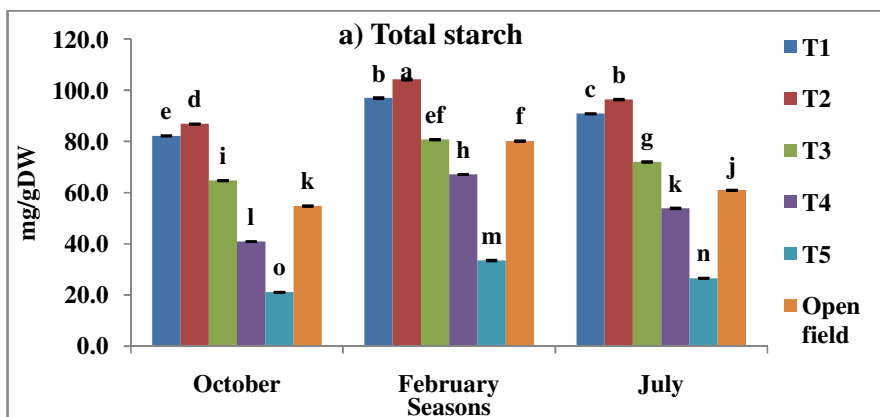


Figure 10. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on total soluble sugars content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

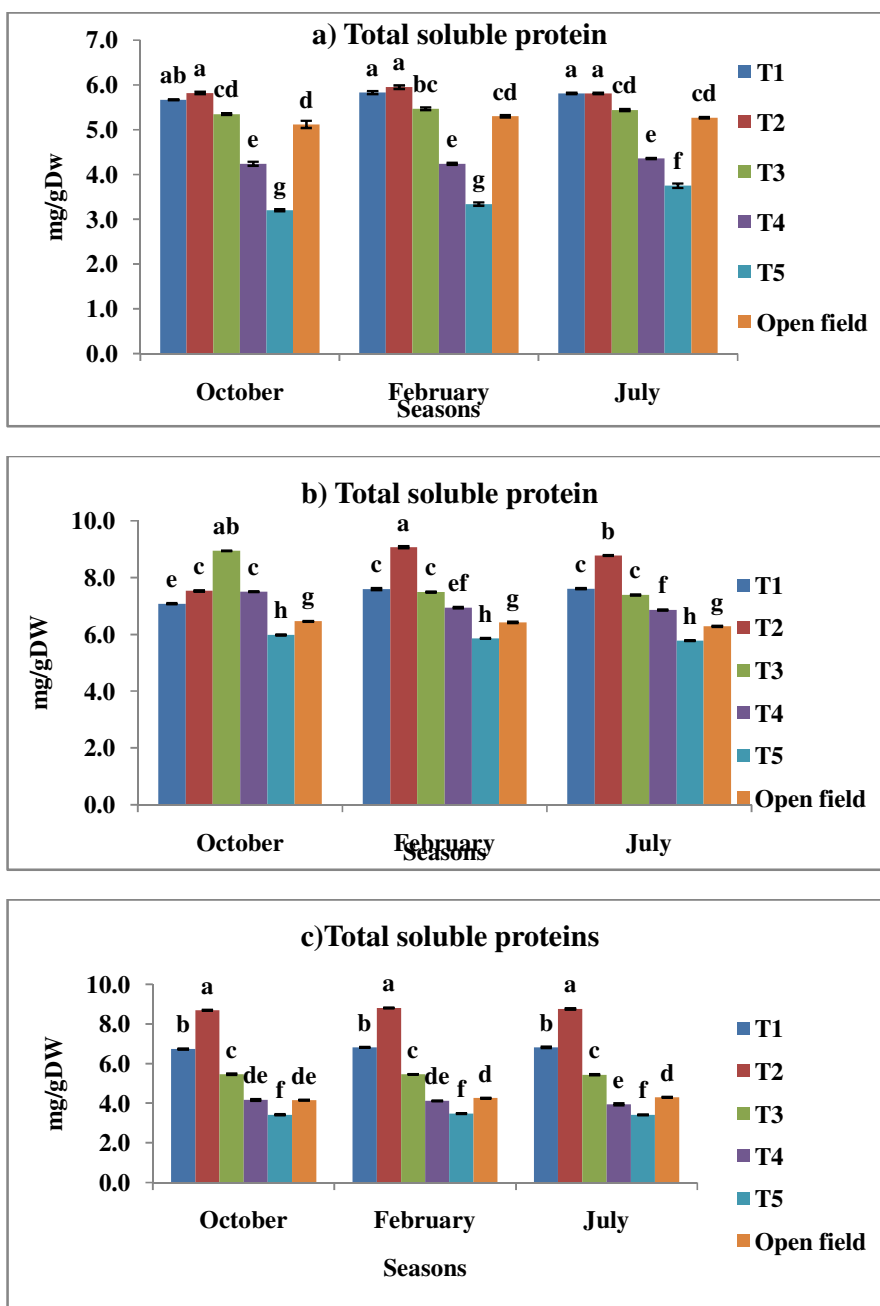


Figure 11. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on total soluble proteins content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

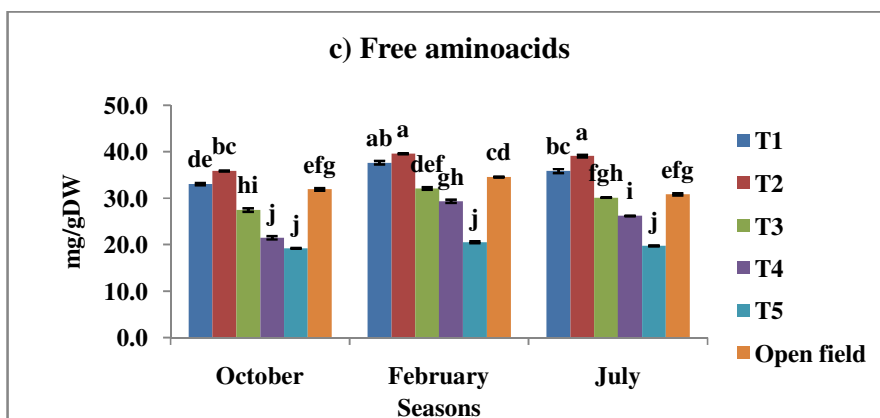
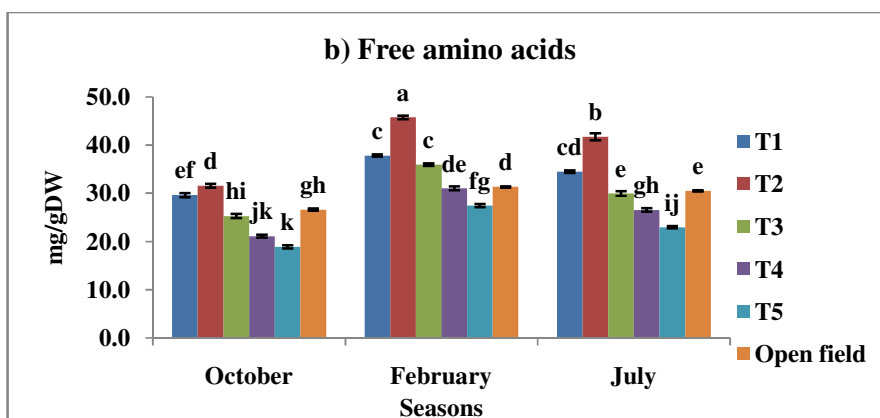
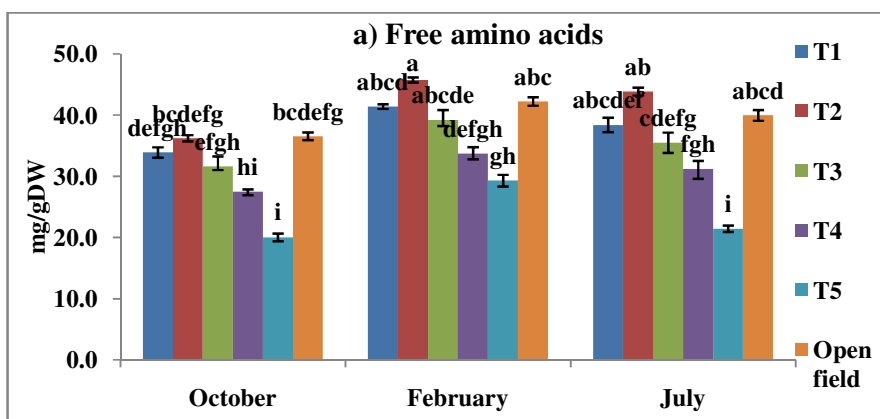


Figure 12. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on free amino acids content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

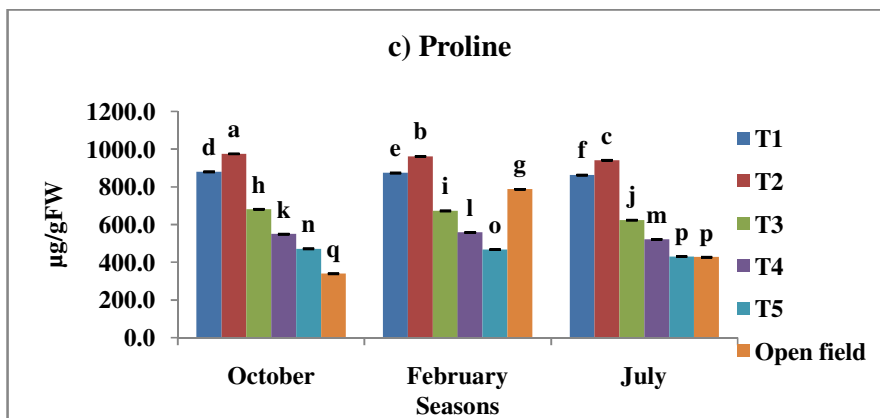
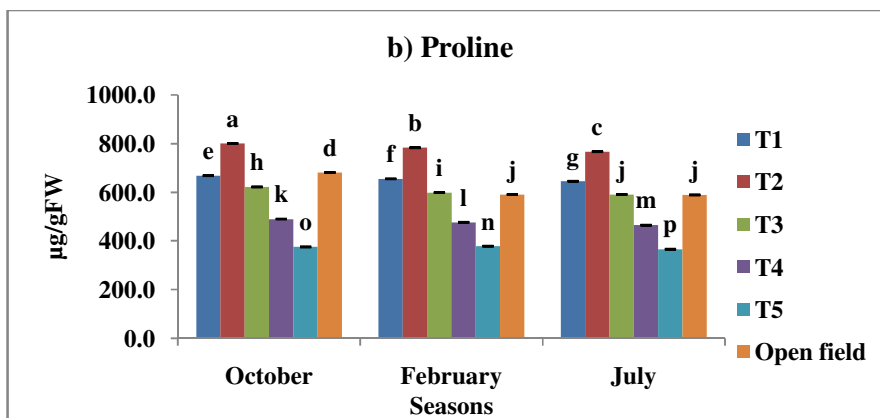
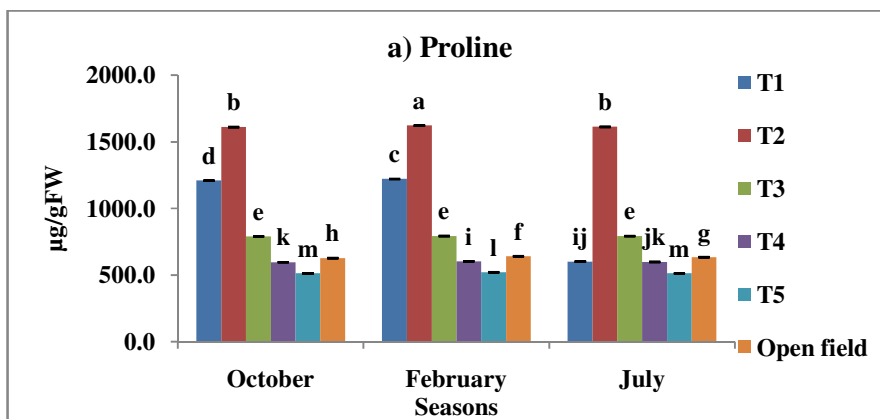


Figure 13. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on proline content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

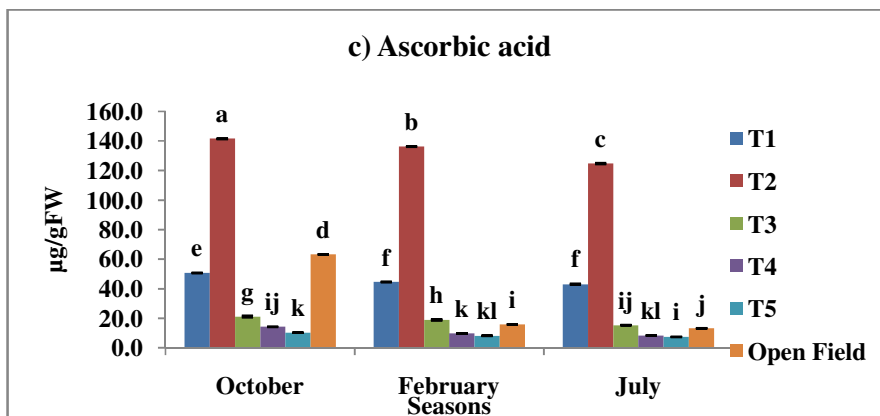
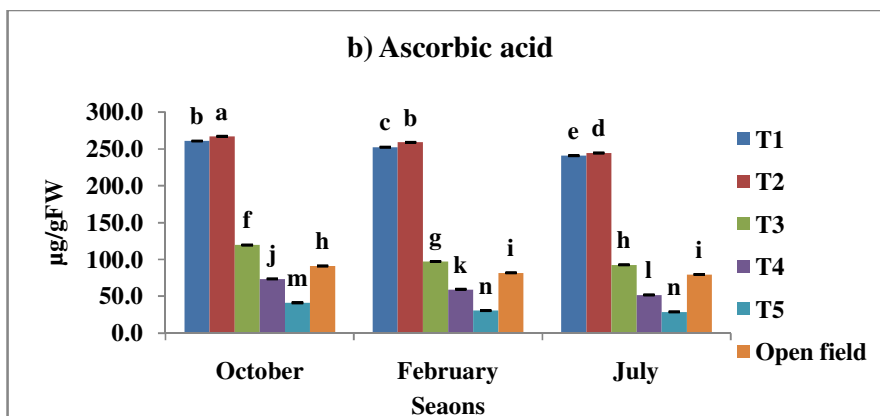
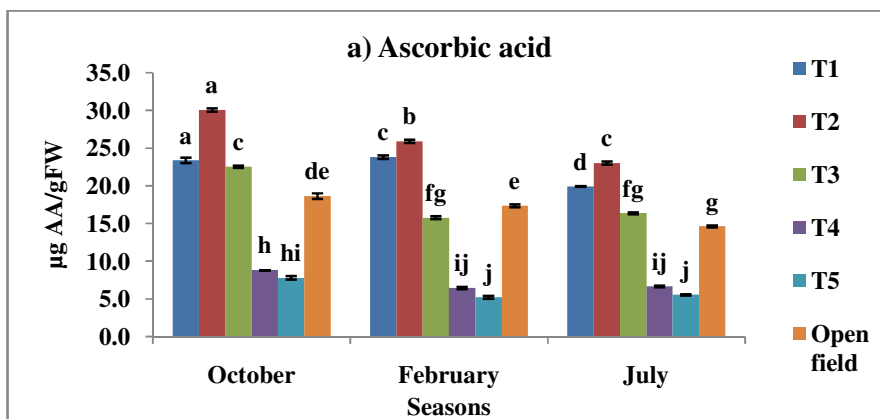


Figure 14. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on ascorbic acid content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

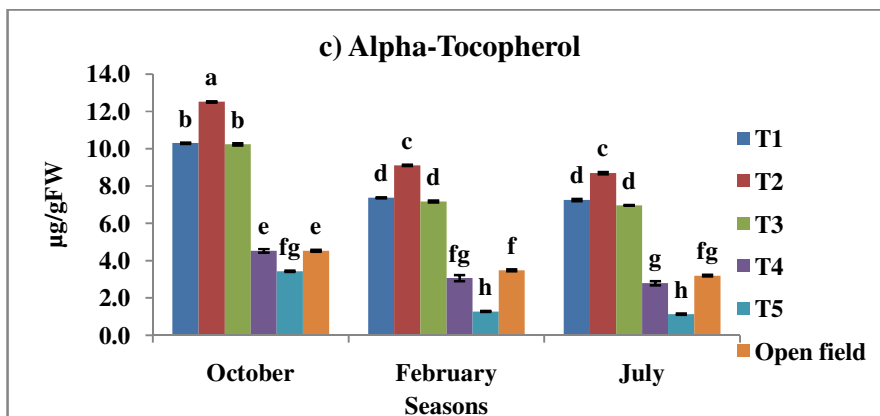
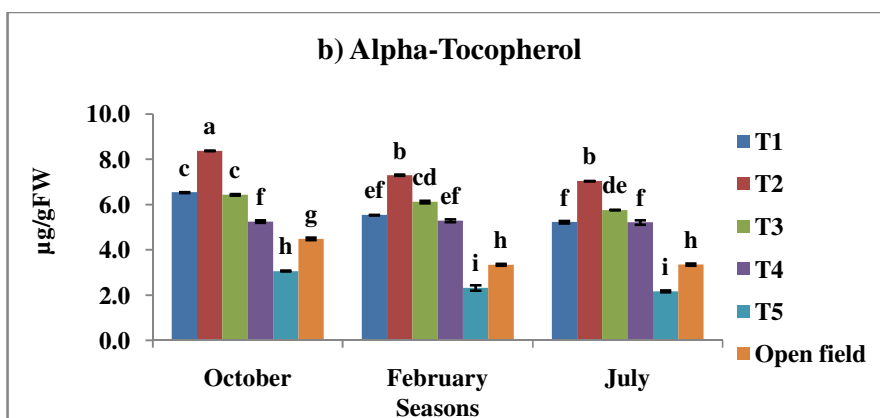
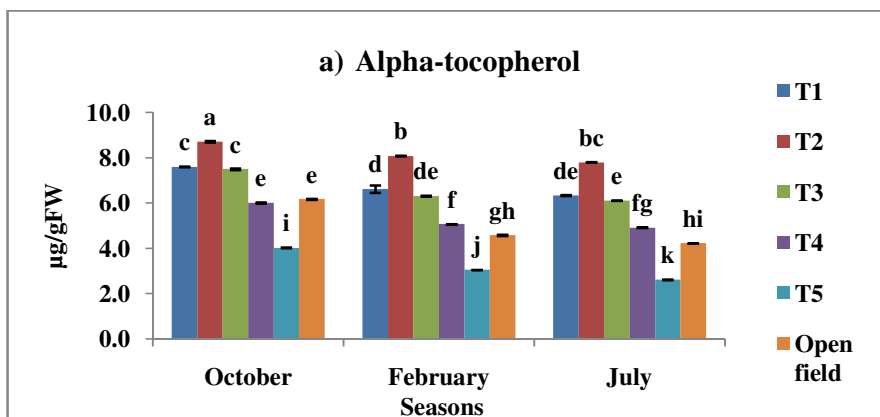


Figure 15. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on α -tocopherol content in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

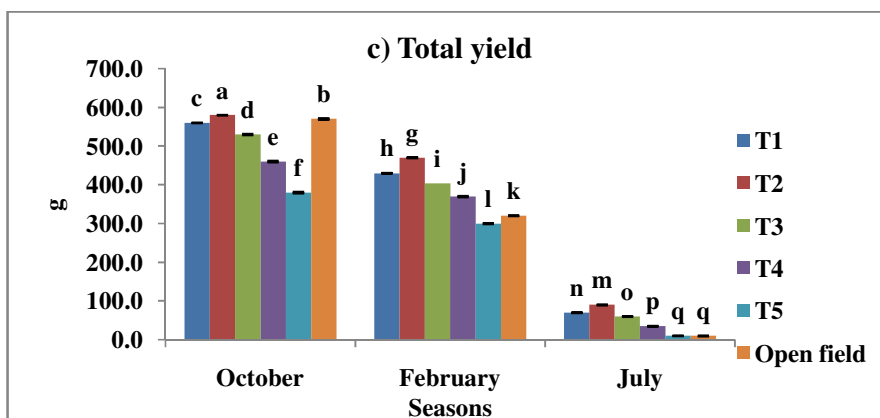
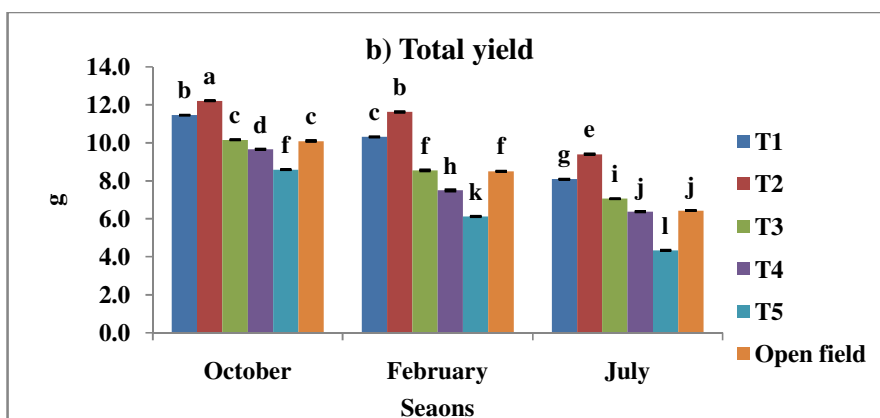
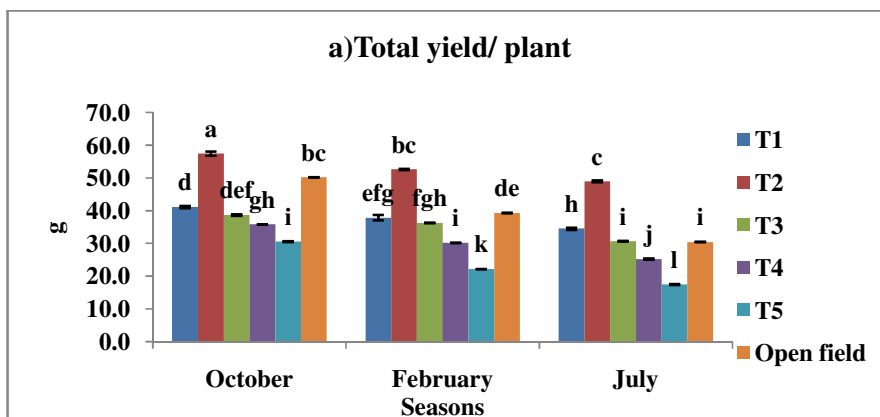


Figure 16. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on total yield per plant in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$, T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

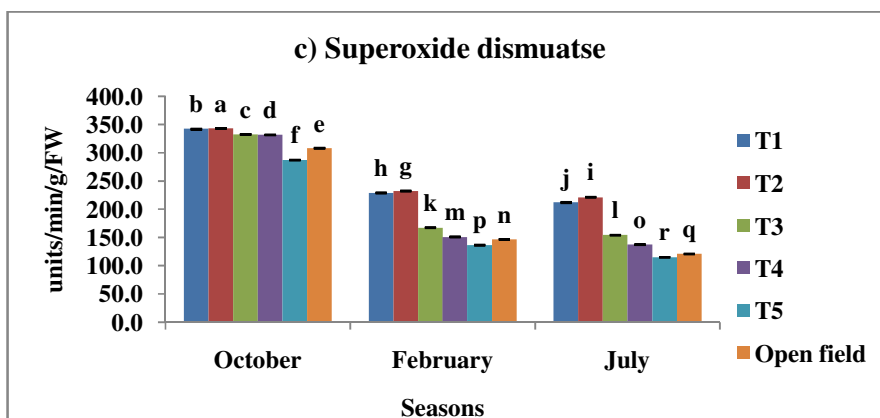
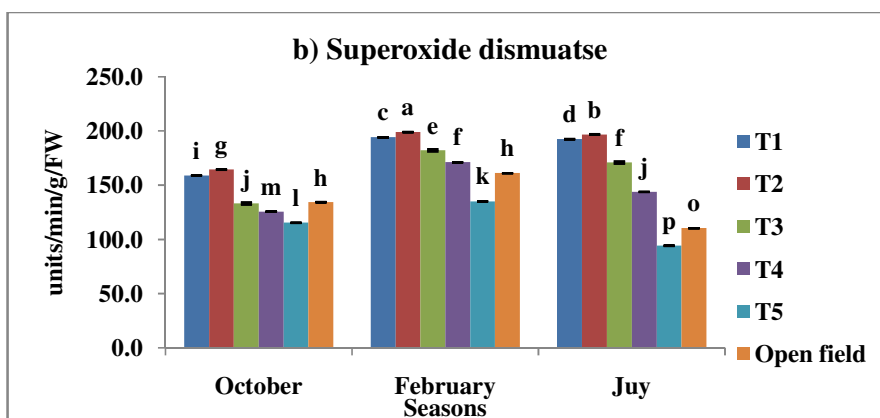
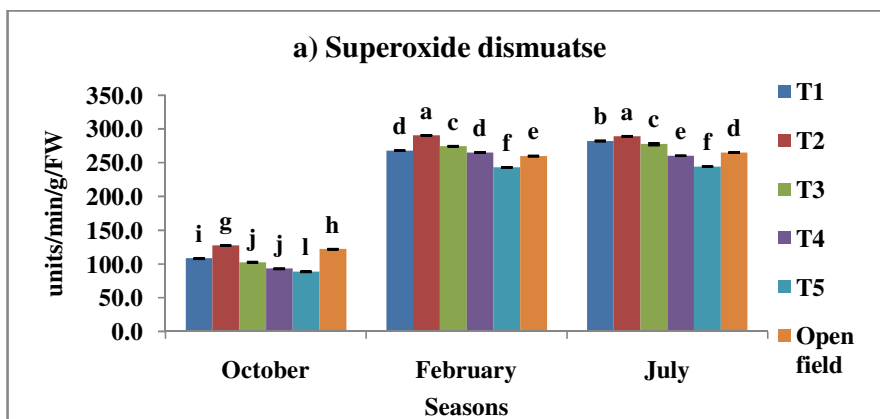


Figure 17. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on superoxide dismutase activity in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

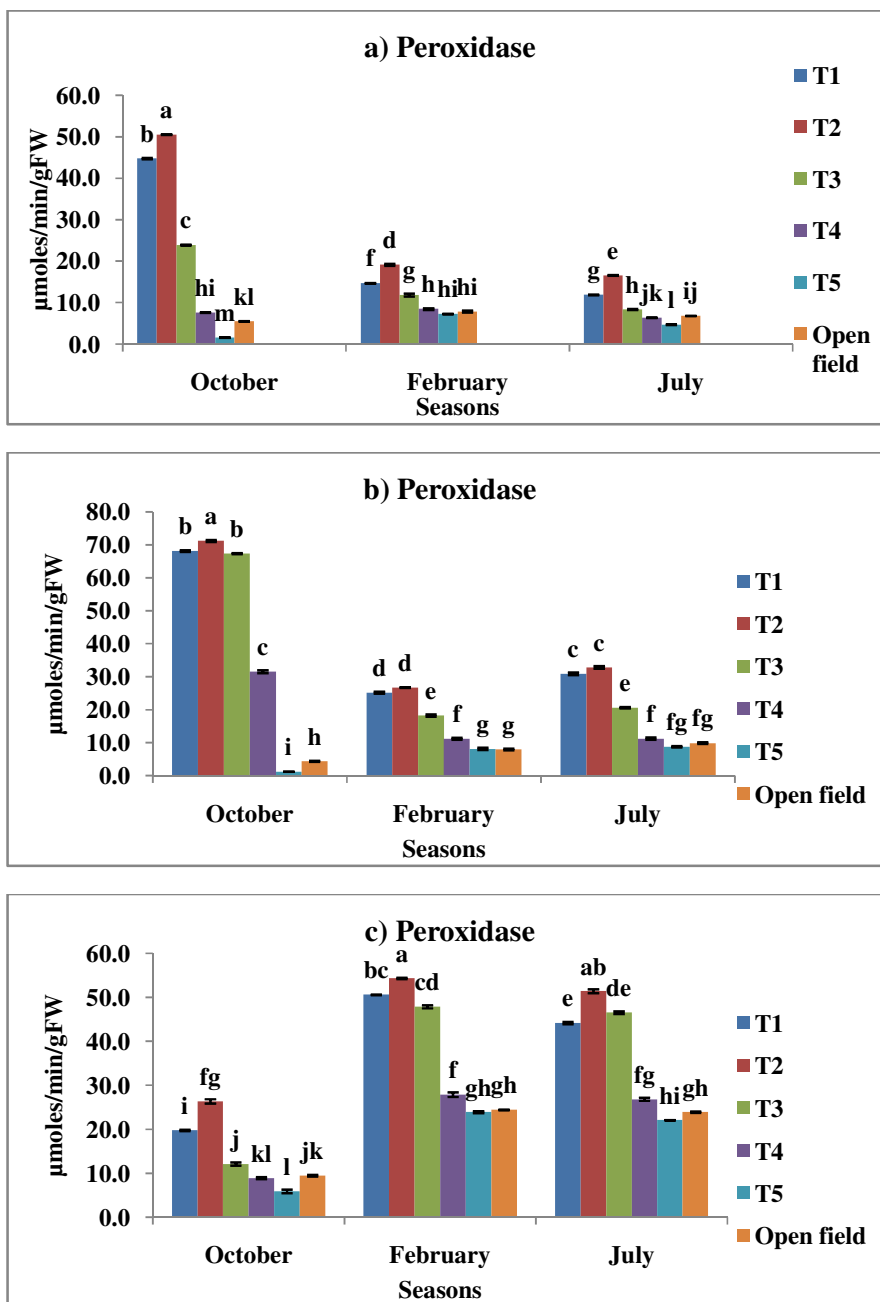


Figure 18. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on peroxidase activity in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

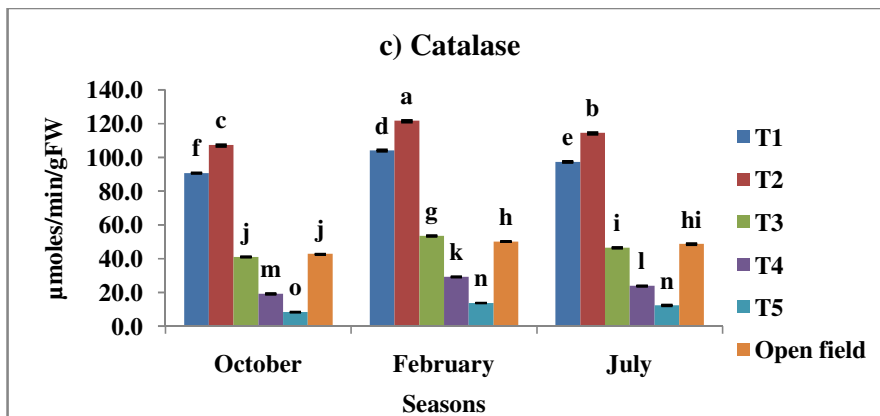
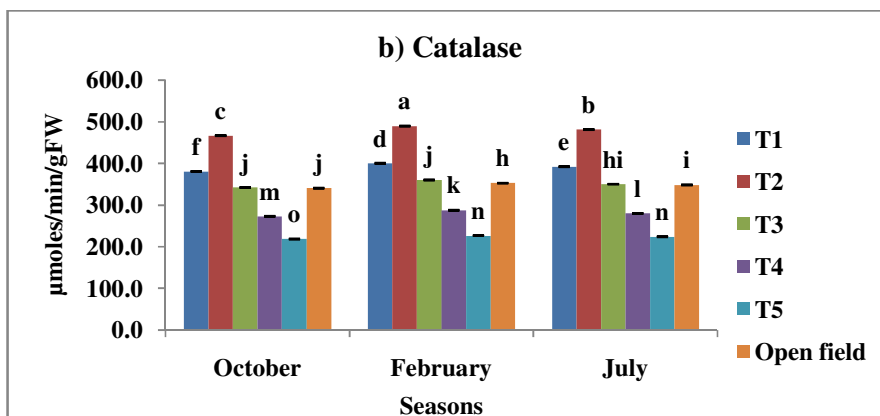
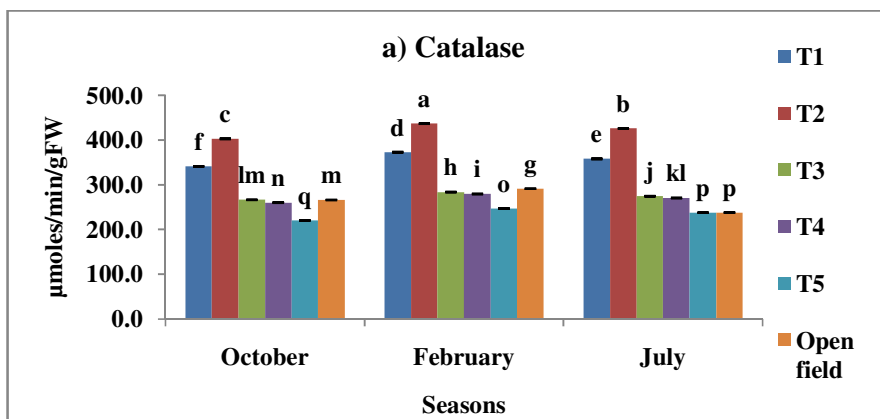


Figure 19. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on catalase activity in (a) spinach (b) coriander (c) lettuce.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

APPENDIX II

Two years data recorded on various physiological, biochemical parameters and enzymes activity in spinach, coriander and lettuce for the sessions 2016-17, 2017-18

Table1. Effect of nitrate and ammonium ratios on plant height

	Plant height (cm) Spinach						Plant height (cm) Coriander						Plant height (cm) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	October		February		July		October		February		July		October		February		July	
$\text{NO}_3^-/\text{NH}_4^+$ ratio	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
T1=100:0	26.96	26.60	23.84	23.78	20.17	20.11	23.05	21.46	20.25	19.24	18.77	18.61	20.12	20.04	17.37	17.20	11.64	11.28
T2=75:25	25.86	25.81	21.91	21.78	18.27	18.21	27.15	26.58	21.81	21.02	20.34	20.10	22.49	22.12	18.33	18.12	12.08	11.88
T3=50:50	20.08	20.04	18.14	18.12	16.77	16.72	21.98	20.73	18.09	17.79	16.14	15.95	18.45	18.18	16.35	16.18	11.18	11.15
T4=25:75	18.11	18.12	16.13	16.09	15.52	15.36	18.45	17.93	15.30	15.08	14.84	14.68	15.74	15.60	12.97	12.59	8.67	8.45
T5=0:100	13.85	13.76	11.90	11.84	11.24	11.18	13.94	13.38	9.03	8.19	8.25	8.15	12.54	11.95	8.43	8.38	6.67	6.46
Open field	19.72	19.70	17.26	17.20	12.26	12.21	18.19	18.23	12.11	11.59	11.33	11.29	15.53	15.38	8.77	8.52	6.37	6.31

Table2. Effect of nitrate and ammonium ratios on leaf water potential

	Leaf water potential (-MPa) Spinach						Leaf water potential (-MPa) Coriander						Leaf water potential (-MPa) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	October		February		July		October		February		July		October		February		July	
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
NO₃⁻/NH₄⁺ ratio																		
T1=100:0	3.01	2.99	3.44	3.40	3.40	3.36	3.33	3.34	2.93	2.98	2.47	2.83	2.50	2.62	2.94	2.60	2.87	2.46
T2=75:25	3.44	3.39	3.80	3.77	3.76	3.72	3.86	3.83	3.41	3.38	2.89	2.89	3.08	2.72	3.23	3.28	3.20	3.28
T3=50:50	2.42	2.39	3.15	3.11	3.10	3.07	2.63	2.61	2.81	2.86	2.30	2.29	2.10	2.27	2.86	2.90	2.71	2.85
T4=25:75	1.42	1.38	2.55	2.60	2.48	2.46	1.72	1.74	2.53	2.47	1.91	1.88	1.75	1.74	2.67	2.73	2.48	2.59
T5=0:100	1.39	1.39	2.63	2.50	2.50	2.44	1.68	1.64	2.47	2.56	1.87	1.89	1.65	1.90	2.65	2.86	2.46	2.79
Open field	1.47	1.46	2.94	2.91	2.51	2.48	1.77	1.81	2.41	2.56	1.84	1.94	1.62	1.66	2.54	2.65	2.42	2.48

Table3. Effect of nitrate and ammonium ratios on leaf area

	Leaf area (cm ²) Spinach						Leaf area (cm ²) Coriander						Leaf area (cm ²) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	October		February		July		October		February		July		October		February		July	
NO ₃ ⁻ /NH ₄ ⁺ ratio	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
T1=100:0	55.76	55.85	55.71	55.74	50.59	50.47	11.30	11.31	8.79	8.76	8.72	8.44	382.18	381.92	183.35	183.49	107.27	108.24
T2=75:25	56.08	56.26	55.75	55.76	51.48	51.39	16.33	16.42	15.18	14.90	14.84	14.75	396.77	396.40	199.58	199.48	118.28	117.61
T3=50:50	49.13	49.19	48.71	48.74	44.69	44.64	7.29	7.31	6.18	6.16	6.14	6.09	364.16	364.71	168.23	168.13	91.42	91.98
T4=25:75	44.32	44.35	44.28	44.29	41.31	41.20	4.81	4.78	2.96	2.93	2.86	2.76	301.91	301.78	153.41	152.76	79.34	78.32
T5=0:100	42.75	42.79	42.64	42.66	39.50	39.34	2.17	2.16	2.26	2.13	2.06	2.03	252.99	253.07	135.15	134.96	61.56	61.58
Open field	45.17	45.21	45.16	45.17	39.49	39.32	5.34	5.32	3.81	3.72	3.65	3.60	393.63	393.23	180.86	180.62	45.47	44.55

Table4. Effect of nitrate and ammonium ratios on fresh weight shoot

	Fresh weight shoot (g) Spinach						Fresh weight shoot (g) Coriander						Fresh weight head (g) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	October		February		July		October		February		July		October		February		July	
NO ₃ ⁻ /NH ₄ ⁺ ratio	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
T1=100:0	4.92	5.00	4.84	4.83	4.74	4.78	0.47	0.44	0.42	0.38	0.36	0.32	2.05	2.04	1.50	1.48	1.35	1.33
T2=75:25	5.81	5.82	5.70	5.71	5.43	5.47	0.55	0.53	0.47	0.45	0.39	0.36	2.99	3.00	2.15	2.14	1.43	1.40
T3=50:50	2.79	2.81	2.64	2.65	2.59	2.58	0.42	0.41	0.38	0.35	0.30	0.28	1.47	1.46	1.10	1.08	0.83	0.80
T4=25:75	2.13	2.15	2.12	2.15	1.87	1.93	0.34	0.31	0.31	0.28	0.24	0.22	1.37	1.35	0.85	0.83	0.71	0.71
T5=0:100	1.99	2.01	1.81	1.80	1.69	1.75	0.19	0.17	0.16	0.14	0.13	0.12	1.30	1.29	0.77	0.76	0.60	0.58
Open field	4.73	4.75	4.41	4.44	4.18	4.22	0.44	0.40	0.37	0.34	0.27	0.24	2.02	2.01	0.94	0.92	0.73	0.74

Table5. Effect of nitrate and ammonium ratios on fresh weight root

	Fresh weight root (g) Spinach						Fresh weight root (g) Coriander						Fresh weight root (g) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	0.35	0.35	0.34	0.34	0.34	0.35	0.05	0.05	0.05	0.04	0.05	0.04	0.13	0.13	0.12	0.13	0.12	0.13
T2=75:25	0.28	0.27	0.26	0.26	0.27	0.27	0.03	0.05	0.03	0.04	0.03	0.04	0.27	0.27	0.26	0.26	0.26	0.26
T3=50:50	0.27	0.26	0.25	0.26	0.26	0.26	0.03	0.04	0.03	0.02	0.03	0.03	0.12	0.12	0.11	0.12	0.12	0.12
T4=25:75	0.19	0.19	0.18	0.18	0.18	0.18	0.03	0.03	0.02	0.02	0.03	0.02	0.11	0.11	0.10	0.10	0.11	0.11
T5=0:100	0.10	0.10	0.09	0.09	0.09	0.10	0.02	0.02	0.02	0.01	0.02	0.02	0.08	0.08	0.07	0.07	0.08	0.08
Open field	0.38	0.39	0.37	0.37	0.35	0.35	0.07	0.09	0.05	0.05	0.02	0.02	0.28	0.28	0.26	0.26	0.16	0.16

Table 6. Effect of nitrate and ammonium ratios on dry weight shoot

	Dry weight Shoot (g) Spinach						Dry weight Shoot (g) Coriander						Dry weight Shoot (g) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
NO₃⁻/NH₄⁺ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	1.09	1.08	1.08	1.09	1.03	1.05	0.10	0.09	0.09	0.08	0.07	0.06	0.42	0.44	0.40	0.66	0.28	0.27
T2=75:25	1.29	1.28	1.23	1.25	1.18	1.19	0.12	0.11	0.10	0.10	0.08	0.07	0.67	0.68	0.64	0.41	0.32	0.31
T3=50:50	0.62	0.61	0.62	0.64	0.55	0.57	0.09	0.09	0.08	0.08	0.06	0.06	0.33	0.35	0.31	0.32	0.25	0.24
T4=25:75	0.47	0.46	0.45	0.46	0.41	0.41	0.07	0.05	0.06	0.05	0.05	0.05	0.31	0.32	0.29	0.30	0.24	0.22
T5=0:100	0.44	0.43	0.42	0.44	0.38	0.39	0.04	0.03	0.03	0.02	0.03	0.02	0.30	0.31	0.28	0.29	0.21	0.19
Open field	1.05	1.04	0.94	0.95	0.90	0.91	0.09	0.09	0.08	0.08	0.06	0.06	0.36	0.37	0.35	0.36	0.21	0.21

Table7. Effect of nitrate and ammonium ratios on dry weight root

	Dry weight root (g) Spinach						Dry weight root (g) Coriander						Dry weight root (g) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	0.09	0.09	0.09	0.09	0.09	0.08	0.05	0.04	0.01	0.01	0.01	0.02	0.03	0.03	0.02	0.03	0.02	0.02
T2=75:25	0.07	0.07	0.05	0.05	0.05	0.05	0.03	0.04	0.01	0.01	0.03	0.03	0.07	0.07	0.05	0.05	0.07	0.06
T3=50:50	0.07	0.07	0.04	0.04	0.04	0.04	0.03	0.02	0.01	0.01	0.01	0.02	0.03	0.03	0.02	0.02	0.02	0.02
T4=25:75	0.05	0.05	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.01	0.02	0.02
T5=0:100	0.03	0.03	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01
Open field	0.10	0.10	0.09	0.09	0.09	0.09	0.02	0.02	0.01	0.01	0.02	0.02	0.07	0.07	0.05	0.05	0.02	0.03

Table 8. Effect of nitrate and ammonium ratios on total chlorophyll content

	Total chlorophyll (mg/gFW) Spinach						Total chlorophyll (mg/gFW) Coriander						Total chlorophyll (mg/gFW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	1.98	1.96	1.68	1.65	1.61	1.58	2.25	2.21	2.11	2.07	1.79	1.82	1.50	1.50	1.34	1.35	1.27	1.27
T2=75:25	2.10	2.06	1.78	1.76	1.72	1.70	2.38	2.33	2.20	2.14	1.94	1.94	1.56	1.54	1.39	1.40	1.33	1.36
T3=50:50	1.71	1.66	1.53	1.54	1.48	1.46	1.92	1.95	1.84	1.86	1.72	1.72	1.27	1.31	1.26	1.27	1.21	1.22
T4=25:75	1.54	1.51	1.39	1.38	1.35	1.33	1.85	1.85	1.76	1.72	1.55	1.56	1.22	1.18	1.27	1.26	1.13	1.14
T5=0:100	1.04	1.01	1.10	1.08	1.07	1.04	1.21	1.22	1.12	1.09	1.39	1.39	1.13	1.23	1.06	1.04	1.05	1.06
Open field	1.43	1.35	1.38	1.35	1.33	1.30	1.26	1.28	1.95	1.91	1.68	1.72	0.93	0.93	1.20	1.21	1.22	1.23

Table 9. Effect of nitrate and ammonium ratios on total Carotenoids content

	Carotenoids (mg/gFW) Spinach						Carotenoids (mg/gFW) Coriander						Carotenoids (mg/gFW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	0.61	0.62	0.58	0.59	0.58	0.59	0.99	1.01	0.96	0.97	0.97	0.99	1.16	1.15	1.11	1.12	1.12	1.12
T2=75:25	0.64	0.67	0.61	0.63	0.61	0.64	1.11	1.12	0.98	1.02	0.98	1.00	1.28	1.29	1.24	1.26	1.25	1.26
T3=50:50	0.56	0.56	0.53	0.54	0.55	0.57	0.71	0.73	0.66	0.67	0.68	0.70	0.85	0.86	0.77	0.78	0.80	0.81
T4=25:75	0.40	0.42	0.37	0.38	0.39	0.40	0.69	0.71	0.64	0.65	0.66	0.66	0.60	0.60	0.58	0.59	0.58	0.60
T5=0:100	0.17	0.18	0.15	0.17	0.16	0.16	0.67	0.66	0.63	0.64	0.64	0.64	0.55	0.56	0.54	0.54	0.54	0.55
Open field	0.62	0.63	0.57	0.59	0.60	0.61	0.66	0.67	0.62	0.64	0.63	0.64	0.64	0.65	0.59	0.60	0.61	0.62

Table10. Effect of nitrate and ammonium ratios on total soluble sugars

	Total soluble sugars (mg/gDW) Spinach						Total soluble sugars (mg/gDW) Coriander						Total soluble sugars (mg/gDW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
	October		February		July		October		February		July		October		February		July	
NO₃⁻/NH₄⁺ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	163.54	163.47	164.52	164.45	171.80	171.74	174.77	174.64	202.80	202.51	172.78	172.93	176.80	175.77	184.72	183.79	175.26	175.37
T2=75:25	176.54	176.44	182.66	182.59	176.33	176.30	181.51	181.39	215.30	215.44	183.93	184.05	203.26	202.35	211.24	210.16	181.83	181.80
T3=50:50	144.21	144.01	154.31	154.19	154.85	154.79	155.29	155.13	176.12	176.43	168.06	168.34	175.04	174.70	177.63	177.11	162.81	163.35
T4=25:75	137.23	137.15	143.42	143.22	132.87	132.80	111.17	111.04	66.06	66.17	132.29	132.48	118.39	117.97	122.85	121.46	73.84	74.53
T5=0:100	64.07	63.92	81.83	81.75	73.11	72.98	81.48	81.45	63.70	63.75	81.29	81.54	58.78	56.90	63.35	62.37	64.71	65.52
Open field	73.40	73.26	94.19	94.03	84.29	84.20	76.47	76.43	45.65	45.91	83.56	83.75	112.55	111.91	114.77	113.91	97.61	97.76

Table11. Effect of nitrate and ammonium ratios on total starch

	Total starch (mg/gDW) Spinach						Total starch (mg/gDW) Coriander						Total starch (mg/gDW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
	October		February		July		October		February		July		October		February		July	
NO₃⁻/NH₄⁺ ratio																		
T1=100:0	82.17	82.17	96.96	96.95	90.90	90.70	126.64	122.51	133.62	131.44	127.62	125.83	176.80	175.77	184.72	183.79	175.26	175.37
T2=75:25	86.93	86.93	104.16	104.27	96.43	96.32	174.69	172.23	177.74	176.50	171.31	169.05	203.26	202.35	211.24	210.16	181.83	181.80
T3=50:50	64.71	64.71	80.78	80.59	72.25	71.79	152.15	152.26	155.40	154.07	148.25	145.98	175.04	174.70	177.63	177.11	162.81	163.35
T4=25:75	40.85	40.85	67.21	67.00	53.97	53.80	112.87	111.50	116.21	114.74	103.82	102.56	118.39	117.97	122.85	121.46	73.84	74.53
T5=0:100	21.13	21.13	33.60	33.39	26.69	26.51	48.55	46.01	59.77	58.60	55.25	53.97	58.78	56.90	63.35	62.37	64.71	65.52
Open field	54.81	54.81	80.29	80.11	61.03	60.89	73.84	71.58	79.41	77.79	74.83	73.78	112.55	111.91	114.77	113.91	97.61	97.76

Table12. Effect of nitrate and ammonium ratios on total soluble proteins

	Total soluble proteins (mg/gDW) Spinach						Total soluble proteins (mg/gDW) Coriander						Total soluble proteins (mg/gDW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	5.66	5.68	5.82	5.84	5.72	5.89	7.07	7.09	7.61	7.58	7.60	7.62	6.74	6.71	6.84	6.80	6.85	6.78
T2=75:25	5.80	5.83	5.94	5.96	5.87	5.74	7.52	7.54	9.08	9.06	8.77	8.79	8.70	8.69	8.83	8.76	8.80	8.71
T3=50:50	5.34	5.36	5.46	5.47	5.43	5.45	8.93	8.95	7.50	7.48	7.37	7.40	5.48	5.46	5.47	5.45	5.48	5.41
T4=25:75	4.22	4.25	4.23	4.25	4.35	4.37	7.49	7.50	6.95	6.93	6.85	6.87	4.18	4.16	4.13	4.10	4.00	3.89
T5=0:100	3.19	3.20	3.33	3.34	3.74	3.76	5.97	5.98	5.87	5.84	5.77	5.79	3.43	3.41	3.51	3.45	3.47	3.37
Open field	5.10	5.13	5.29	5.31	5.25	5.27	6.45	6.47	6.42	6.41	6.26	6.29	4.19	4.13	4.29	4.24	4.34	4.25

Table13. Effect of nitrate and ammonium ratios on free amino acids

	Free amino acids (mg/gDW) Spinach						Free amino acids (mg/gDW) Coriander						Free amino acids (mg/gDW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
NO₃⁻/NH₄⁺ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	34.02	33.77	41.49	41.31	38.61	38.19	30.01	29.23	38.69	36.99	35.01	33.96	33.55	32.53	37.82	37.48	36.33	35.41
T2=75:25	36.85	36.76	45.88	45.63	43.91	43.80	31.90	31.27	46.64	44.82	42.01	41.39	36.07	35.69	39.94	39.23	39.27	38.90
T3=50:50	31.66	31.54	39.23	39.15	35.55	35.42	25.55	25.01	36.60	35.29	30.48	29.42	27.84	27.20	32.16	32.07	30.20	30.05
T4=25:75	27.56	27.40	33.88	33.60	31.32	31.13	21.66	20.51	31.61	30.47	26.68	26.43	21.59	21.38	29.57	29.16	26.43	25.97
T5=0:100	20.20	19.86	29.39	29.19	21.47	21.41	19.05	18.73	28.00	26.89	23.17	22.70	19.33	19.09	20.99	20.12	20.05	19.45
Open field	36.72	36.36	42.27	42.16	40.03	39.86	26.95	26.30	31.95	30.69	30.61	30.39	32.39	31.41	34.86	34.30	31.16	30.53

Table14. Effect of nitrate and ammonium ratios on proline

	Proline (µg/gFW) Spinach						Proline (µg/gFW) Coriander						Proline (µg/gFW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
NO ₃ ⁻ /NH ₄ ⁺ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	627.81	627.26	641.72	641.60	633.73	633.64	668.00	667.76	654.81	654.96	645.67	645.23	880.55	879.06	874.66	873.89	862.66	862.79
T2=75:25	1611.25	1610.23	1622.28	1620.91	1613.68	1612.66	800.96	800.56	783.48	782.82	766.42	766.83	975.21	974.96	963.60	962.20	942.64	941.13
T3=50:50	790.10	789.45	793.40	792.84	792.47	792.00	622.17	621.79	598.92	598.01	590.99	590.76	681.53	681.31	673.24	672.18	624.92	623.00
T4=25:75	597.21	596.26	602.62	602.08	598.36	597.89	490.04	489.94	476.30	476.59	464.84	464.59	550.40	550.06	558.13	557.72	523.04	522.04
T5=0:100	512.14	511.23	520.79	519.91	512.14	511.76	375.40	375.31	378.61	379.00	366.51	366.15	471.93	471.15	468.02	467.56	430.56	429.75
Open field	1210.24	1208.26	1221.31	1220.08	601.04	599.88	681.63	681.09	591.11	590.23	588.86	588.78	339.30	339.22	788.55	787.70	427.70	426.90

Table15. Effect of nitrate and ammonium ratios on ascorbic acid content

	Ascorbic acid (µg/gFW) Spinach						Ascorbic acid (µg/gFW) Coriander						Ascorbic acid (µg/gFW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
	October		February		July		October		February		July		October		February		July	
NO₃⁻/NH₄⁺ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	30.07	30.02	23.84	23.77	19.78	20.04	260.75	260.72	252.45	252.36	240.69	240.59	50.24	51.10	43.66	45.39	43.12	42.95
T2=75:25	29.41	29.38	25.93	25.87	22.98	23.08	267.06	267.01	259.06	258.97	244.65	244.32	141.96	141.23	135.41	137.07	125.36	124.11
T3=50:50	22.57	22.51	15.82	15.74	16.22	16.47	119.60	119.58	97.29	97.12	92.73	92.61	21.82	20.52	16.98	20.76	15.13	15.40
T4=25:75	8.87	8.75	6.49	6.43	6.51	6.78	73.55	73.48	59.52	59.25	51.90	51.75	14.57	13.91	9.11	10.26	8.29	8.30
T5=0:100	7.82	7.77	5.25	5.19	5.33	5.73	41.06	41.01	30.52	30.42	28.57	28.74	10.46	10.08	7.70	8.67	7.13	7.58
Open field	18.65	18.61	17.41	17.30	14.40	14.85	91.03	90.95	81.80	81.67	79.70	79.65	64.41	62.15	16.31	15.37	13.22	13.28

Table16. Effect of nitrate and ammonium ratios on α -tocopherol

	α -Tocopherol ($\mu\text{g/gFW}$) Spinach						α -Tocopherol ($\mu\text{g/gFW}$) Coriander						α -Tocopherol ($\mu\text{g/gFW}$) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	7.52	7.68	6.59	6.65	6.30	6.39	6.84	6.23	5.75	5.33	5.32	5.12	10.40	10.20	7.61	7.11	7.34	7.15
T2=75:25	8.50	8.92	8.05	8.10	7.83	7.89	8.45	8.30	7.41	7.19	7.06	7.02	12.41	12.60	9.17	9.05	8.86	8.54
T3=50:50	7.29	7.70	6.28	6.34	6.07	6.14	6.67	6.19	6.22	6.00	5.92	5.61	10.25	10.23	7.19	7.14	7.04	6.90
T4=25:75	5.82	6.19	5.02	5.11	4.89	4.94	5.40	5.10	5.50	5.07	5.25	5.16	4.62	4.43	3.10	3.02	2.86	2.72
T5=0:100	3.96	4.09	3.01	3.08	2.59	2.62	3.10	3.03	2.39	2.23	2.22	2.11	3.55	3.31	1.30	1.26	1.16	1.09
Open field	6.12	6.21	4.58	4.58	4.23	4.21	4.56	4.41	3.58	3.10	3.44	3.25	4.44	4.63	3.68	3.28	3.31	3.09

Table17. Effect of nitrate and ammonium ratios on total plant yield

	Total yield (g/plant) Spinach Plant height (cm) Spinach						Total yield (g/plant) Coriander						Total yield (g/plant) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	41.11	40.21	37.89	35.43	34.50	32.26	11.46	10.32	10.31	8.56	8.09	5.87	560.00	545.0	430.00	427.50	70.00	67.5
T2=75:25	57.47	55.43	52.62	47.76	48.97	44.67	12.22	11.01	11.62	9.21	9.40	7.51	580.00	585.00	470.00	473.43	90.00	92.4
T3=50:50	38.71	35.43	36.3	34.45	30.68	27.87	10.16	8.54	8.55	7.11	7.06	5.67	530.00	532.00	404.00	402.23	60.00	57.8
T4=25:75	35.83	33.45	30.2	24.67	25.21	21.34	9.66	7.56	7.50	6.01	6.38	45.43	460.00	465.00	370.00	367.04	35.00	34.12
T5=0:100	30.58	32.05	22.18	18.76	17.46	14.54	8.58	6.78	6.12	5.09	4.34	5.01	380.00	382.00	300.00	297.76	10.00	8.06
Open field	50.22	46.54	39.35	35.56	30.44	27.87	10.09	11.01	8.50	9.01	6.43	5.02	570.00	567.00	320.00	321.45	10.00	7.5

Table18. Effect of nitrate and ammonium ratios on superoxide dismutase activity

	Superoxide dismutase (units/min/g/FW) Spinach						Superoxide dismutase (units/min/g/FW) Coriander						Superoxide dismutase (units/min/g/FW) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
	October		February		July		October		February		July		October		February		July	
NO₃⁻/NH₄⁺ ratio																		
T1=100:0	108.18	108.14	268.11	267.85	282.19	282.33	158.92	159.07	194.12	194.23	192.43	192.60	342.53	341.92	229.42	229.14	212.38	212.24
T2=75:25	127.62	127.55	290.81	290.66	289.20	289.31	164.32	164.59	198.90	198.93	196.76	196.89	343.55	343.24	232.43	232.14	221.47	221.13
T3=50:50	102.78	102.71	274.59	274.52	275.15	280.10	132.97	133.21	182.11	182.27	170.81	171.09	332.65	332.39	167.48	167.24	154.34	154.28
T4=25:75	93.06	92.91	265.35	265.27	260.25	260.46	125.41	125.81	171.10	171.33	143.78	144.01	331.66	331.51	151.04	150.79	137.54	137.17
T5=0:100	88.74	88.66	243.24	243.19	244.16	244.35	115.18	115.57	135.07	135.27	94.05	94.41	287.27	286.93	136.52	136.24	115.35	115.13
Open field	122.16	122.17	260.15	260.09	265.15	265.28	134.05	134.44	161.02	161.22	110.15	110.60	308.20	308.21	146.69	146.40	120.85	120.76

Table19. Effect of nitrate and ammonium ratios on peroxidase activity

	Peroxidase ($\mu\text{moles/min/gFW}$) Spinach						Peroxidase ($\mu\text{moles/min/gFW}$) Coriander						Peroxidase ($\mu\text{moles/min/gFW}$) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	44.77	44.73	11.92	11.86	14.73	14.63	68.42	67.86	25.56	24.79	31.00	30.82	19.42	20.16	50.59	50.67	44.59	43.85
T2=75:25	50.49	50.51	16.58	16.55	19.86	18.40	71.87	70.56	26.76	26.63	33.02	32.69	26.36	26.38	54.44	54.30	51.68	51.22
T3=50:50	23.94	23.82	8.36	8.32	11.83	11.85	67.65	67.16	18.33	18.15	20.84	20.39	12.04	12.22	48.16	47.63	46.65	46.46
T4=25:75	7.66	7.54	6.44	6.31	8.48	8.43	31.94	31.13	11.34	11.03	11.29	11.13	8.77	9.07	27.95	27.89	26.93	26.74
T5=0:100	1.62	1.49	4.72	4.61	7.32	7.22	1.23	1.16	8.21	8.03	8.79	8.72	5.82	5.98	23.98	23.89	22.14	22.05
Open field	5.55	5.46	6.87	6.76	7.90	7.82	4.22	4.47	7.96	7.89	9.93	9.82	9.48	9.41	24.56	24.33	24.01	23.86

Table20. Effect of nitrate and ammonium ratios on catalase activity

	Catalase ($\mu\text{moles/min/gFW}$) Spinach						Catalase ($\mu\text{moles/min/gFW}$) Coriander						Catalase ($\mu\text{moles/min/gFW}$) Lettuce					
	Main season		Off season				Main season		Off season				Main season		Off season			
	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018	2016	2017	2017	2018	2017	2018
$\text{NO}_3^-/\text{NH}_4^+$ ratio	October		February		July		October		February		July		October		February		July	
T1=100:0	341.39	341.23	359.59	356.51	372.82	372.62	381.18	380.52	400.65	399.69	392.67	391.57	91.00	90.66	104.54	103.79	97.50	97.27
T2=75:25	402.64	402.62	425.86	425.63	436.79	436.58	466.72	466.38	489.89	489.26	481.80	480.89	107.62	107.22	122.32	121.41	115.03	114.38
T3=50:50	266.62	266.56	274.56	274.49	283.87	283.78	342.37	341.89	360.25	360.08	350.73	349.71	41.58	40.63	53.88	53.16	46.98	46.00
T4=25:75	260.16	259.96	270.74	270.50	279.66	279.54	273.17	272.49	287.87	287.35	279.93	279.75	19.38	19.17	29.51	29.10	23.89	23.83
T5=0:100	220.28	220.25	238.11	237.89	246.95	246.74	218.65	218.26	226.48	226.12	223.94	223.60	8.56	8.38	14.01	13.59	12.51	12.24
Open field	266.37	266.21	284.34	284.18	291.68	290.59	340.70	340.43	352.80	352.73	348.44	348.04	42.99	42.52	50.75	49.64	48.70	48.73

APPENDIX III

**Monthly meteorological data pertaining to monthly temperature under hydroponics
and open field conditions at PAU,
Ludhiana, Punjab during the study period (2016,17,18)**

	Under polyhouse (Substrate hydroponics)	Under open field conditions
2016		
	Average temperature (°C)	Average temperature (°C)
September	28.3	29.7
October	24.4	25.9
November	16.5	19.9
December	12.5	15.4
2017		
January	15.8	12.9
February	19.6	16.2
March	20.3	19.9
April	24.3	28.5
May	27.3	32.0
June	26.5	31.5
July	25.7	31.1
August	27.5	30.3
September	23.4	28.8
October	26.5	25.9
November	20.7	18.1
December	18.6	14.2
2018		
January	18.6	12.5
February	20.5	16.0
March	22.9	21.6
April	23.6	27.8
May	25.7	31.6
June	26.7	32.4
July	25.3	32.4
August	24.7	30.7
September	25.3	28.0
October	24.9	24.2

LIST OF PUBLISHED/SUBMITTED RESEARCH PAPERS

Title	Journal Name	NAAS Rating	Status
Effects of variable $\text{NO}_3^-:\text{NH}_4^+$ ratios on growth, nutrition quality and antioxidants activity in spinach grown under polyhouse with intermittent supply of water and nutrients	Plant Physiology and Biochemistry	8.72	Submitted
effect of proportion of NO_3^- and NH_4^+ in nutrient solution and growing seasons on yield and quality in coriander	Israel Journal of Plant Sciences	6.67	Submitted
effect of nitrogen forms on physiological and biochemical constituents of lettuce grown under polyhouse during different seasons	Indian Journal of Horticulture	6.10	Submitted

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Effects of variable $\text{NO}_3^-:\text{NH}_4^+$ ratios on growth, nutrition quality and antioxidants activity in spinach grown under polyhouse with intermittent supply of water and nutrients

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Abstract

The effect of variable NO_3^- to NH_4^+ ratios in the nutrient solution on growth, nutritional quality and yield attributes of spinach (cv. Punjab green) grown under polyhouse conditions was evaluated. Simultaneously crop was raised in open field by conventional practices. Crop was sown at three dates viz; October (main season) and February and July (off-season) respectively. N 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 from each replication of respective treatments at 30 days interval starting from date of sowing. At each harvesting, growth (fresh and dry biomass of shoot, plant height, leaf area and total yield per plant) nutritional and quality parameters (total chlorophyll, carotenoids, soluble sugars, starch, soluble proteins, free amino acids and proline) and antioxidant enzyme activities (SOD, CAT, POD) were determined. In main as well as in off season, the yield was significantly more in polyhouse than in open field sown crop. Under polyhouse, maximum yield was obtained in the plants provided with nutrient solution T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) Further, the crop provided with nutrient solutions having higher proportion of NO_3^- (i.e., T1, T2, T3) had higher values of quality parameters and highest antioxidants activities than the open field crop. It is suggested that NO_3^- to NH_4^+ ratio of 75:25 improve the growth, antioxidants activity, nutritional and quality attributes and is thus most suitable for spinach growth under polyhouse system.

Key words: Ammonium, nitrate, proline, proteins, quality, spinach, yield.

Abbreviations: NO_3^- , Nitrate; NH_4^+ , Ammonium; SOD, Superoxide Dismutase; POD, Peroxidase; CAT, Catalase; N, Nitrogen.

Introduction

Leafy vegetables are rich sources of antioxidants, minerals, vitamins and other nutritional components. Those nutrient attributes are enhanced through manipulation of cultural practices such as controlled temperature, light and mineral nutrient levels (Barickman and Kopsell 2016). Spinach (*Spinach oleracea* L.) is one of the major leafy vegetables and a good source of vitamin A, B1, B2, and C, as well as minerals such as calcium (Ca), iron (Fe), and magnesium (Mg). As consumers are progressively becoming conscious of beneficial and nutritional aspects of vegetables, there is a huge demand for off season vegetables in local as well as international market. This demand can be met by

growing vegetables under protected conditions like polyhouse. This results in maximum production using less agricultural area, improves quality and also fetches huge profits to farmers.

N is an essential mineral required in large quantities. It plays an important role in determining quality of crop as it is a constituent of proteins, related with synthesis of many metabolites and nucleic acids. According to Marschner (2012) N metabolism can be regulated by supply, demand for growth, and amino acid content in plant. Plants can use N in two forms, i.e. cation (NH_4^+), or anion (NO_3^-) (Wang et al., 2009). Most of plant prefers NO_3^- because high concentration of NH_4^+ is toxic for its metabolism. NO_3^- has the advantage of being a storage form in plants with no necessity to be assimilated in the roots. However, uptake of NO_3^- ions by the plants as sole source of N results in an efflux of OH^- ions in the nutrient solution lead to increase in alkalinity of nutrient solution. This can also lead to imbalance in nutrient absorption by the plant and hence has negative effects on the plant growth and metabolism. NO_3^- affects the growth of tea plant roots when it is the only source of N (Ruan et al., 2007). A very high concentration of nitrate has been found to be associated with respiratory problems and to be formation of the carcinogenic compound in human digestive tract.

When both NH_4^+ and NO_3^- are supplied, pH stat may be achieved by similar rates of H^+ production (NH_4^+ assimilation) and H^+ consumption (NO_3^- assimilation) and thus has a very low energy requirement (Wang et al., 2009). Controlling the N supply by varying the ratio of NO_3^- and NH_4^+ could be an efficient way for changing the relative uptake of anions and cations (Sonneveld 2002). For most plant species, NO_3^- supply combined with low quantities of NH_4^+ favors growth, but the response depends on the species and the age of the plant (Guo et al., 2012, Saavatian et al. 2014). Maximum growth rates and higher plant yields can be achieved with combined supply of both NH_4^+ and NO_3^- . In spinach, treatment comprising 100:0 (NO_3^- : NH_4^+) gave the highest biomass yield (Wang et al., 2005) while Zhang et al., (1990) observed highest yield in plants supplied with NO_3^- and NH_4^+ in the ratio of 50:50 and 75:25 (NO_3^- -N: NH_4^+ -N).

Application of N with different NO_3^- -N/ NH_4^+ -N ratios will influence different nutritional and quality parameters of vegetables. Therefore, practically controllable nitrification and understanding the relationship between NO_3^- -N/ NH_4^+ -N ratio could be the mainstay to boost the characteristics of leafy vegetables. The present study was conducted with the objective to standardize a suitable NO_3^- : NH_4^+ ratio in nutrient formulation for growth, nutritional, quality and yield attributes of spinach under polyhouse conditions with intermittent water and nutrient supply.

Materials and methods

Experimental site

The study was conducted during the years 2016-17 and 2017-18 in the poly houses at the College of Agricultural Engineering and Technology, the Vegetable Research Farm, the 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 meters above the sea level.

Experimental material and procedures

The seeds of spinach (cultivar. Punjab Green) 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, in open field conditions (control) and under polyhouse conditions with intermittent supply of water and nutrients. Under the open field conditions the seeds were planted in 2m² plot as per recommended practices (Anonymous 2016).

In polyhouse, seeds were directly sown in plastic pots 30 cm (diameter)×30 cm (height) on a coco peat: perlite: vermiculite (3:1:1) medium (Table 1). The potting mixture for the experiments was obtained from local suppliers. After emergence, the seedlings were thinned to retain four per pot. For each season, crop was harvested one month after sowing. At harvesting, foliage was cut close to the base of the plant with a pair of scissors. Thereafter, two more harvestings were done at an interval of one month each. Data represented for various parameters is a mean value of 2 years.

	Season		Off season
	October	February	July
Month of sowing	3 rd week of October (2016 and 2017)	2 nd week of February (2017 and 2018)	2 nd week of July (2017 and 2018)
Month of harvesting	3 rd week of January (2017 and 2018)	1 st week of May (2017 and 2018)	1 st week of October (2017 and 2018)

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

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 average day maximum temperatures were 25.2°C during season crop (October- January), 28.1°C during the off season crops (February-May) and 28.3°C (July-October). The average day minimum temperatures were 11.4°C during October- January, 16.7 °C during February-May) and 16.9°C (July-October). The range of temperature for season crop was between 18.2°C and 25.2°C and for off- season crop, it was between 16.2°C and 28.3°C.

Treatments	NO ₃ ⁻ /NH ₄ ⁺ ratio	Source of nutrients (mmol L ⁻¹) (mg L ⁻¹)						
		KNO ₃	Ca(NO ₃) ₂ .4H ₂ O	CaCl ₂ .2H ₂ O	(NH ₄) ₂ SO ₄	KH ₂ PO ₄	MgSO ₄ .7H ₂ O	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

Table 2. Compositions (mmol L⁻¹) of nutrient solutions with different NO₃⁻/NH₄⁺ ratios but the same N concentration (8 mmol L⁻¹)

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

micronutrients formulation was used. There were five treatments with varying ratios of NO_3^- -N and NH_4^+ -N while keeping the total N content constant (Table.2) NO_3^- was supplied as $\text{Ca}(\text{NO}_3)_2$ and NH_4^+ as $(\text{NH}_4)_2\text{SO}_4$, respectively. The pH of the solution was adjusted to 6.0 ± 0.2 with dilute sodium hydroxide (NaOH) or hydrochloric acid (HCl). Approximately 2L nutrient solution was added manually to each pot during the season crop and approximately 3 L during off season. Nutrient solution was made fresh every 10 days.

Leaf analysis

Growth parameters

Fresh weight of shoot: After 30-35 days of growth, leaves were harvested their fresh weight was measured in g.

Dry weight of shoot (g): Plant shoots were dried in air oven at 60°C for 48 hours and their weight was measured in g.

Plant height: The length of the main stem was measured in cm from the ground level to the tip of the top most leaf using a cm scale.

Leaf area: For measuring leaf area, all leaves were collected and passed through LI-COR(LI-3100) leaf area measuring machine.

Total yield per plant: For yield, fresh leaves from each plant were collected separately at an interval of one month and weighed. Yield was expressed in g per plant.

Leaf water potential

After one month of sowing, fresh leaves of spinach were taken for the estimation of leaf water potential (measured with instrument (PSYPRO, ELI Tech Group WESCOR®). Leaf discs were cut with a cork borer of 6mm diameter from leaves and placed in the disc chamber of the instrument for 30 seconds to obtain the stable readings).

Nutritional , quality attributes and antioxidants activity

Total chlorophyll and carotenoids (Anderson and Boardman, 1964) were estimated from freshly harvested leaves. Estimation of total soluble sugars and total starch, total soluble proteins and free amino acids was done from dried leaves as per Dubois et al., (1956), Lowry et al., (1951) and Lee and Takahashi (1966) respectively. Proline was determined as per Bates et al., (1973).

Ascorbic acid, alpha- tocopherol, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) were estimated from the fresh leaves as per Ranganna (1986), Jayaraman (1981), Marklund & Marklund (1974), Shannon et al., (1966) and Chance & Maehly (1955).

Statistical analysis

Data obtained from this experiment were analyzed by factorial design using SPSS (Version 16.0 SPSS) software. Significant differences ($p \leq 0.05$) were determined by Tukey's test, and were marked with lower-case letters in figures.

Results and discussions

Growth parameters

Fresh, dry weight of shoot

During all the three growing seasons, maximum mean fresh weight recorded in the plants which were supplied with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and plants provided with treatment

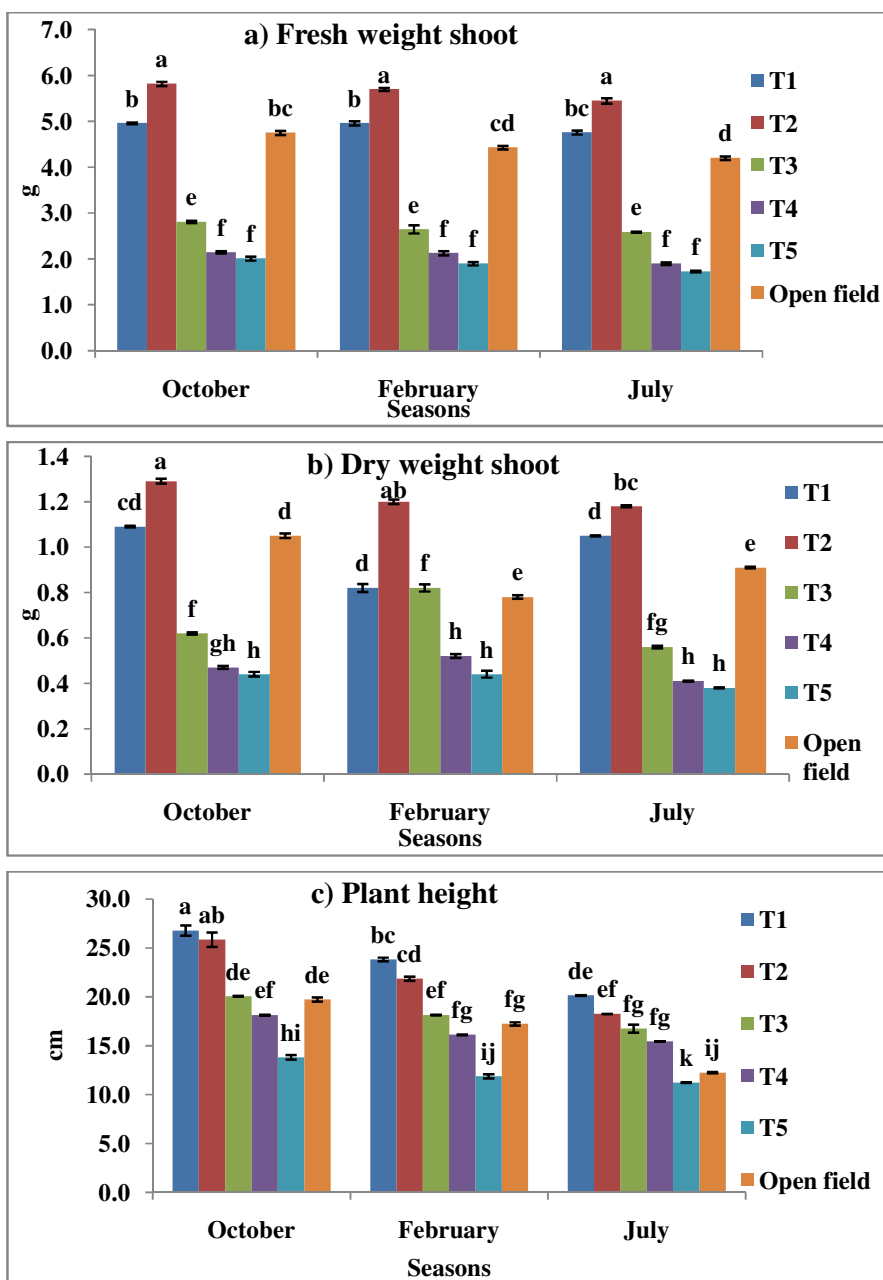


Figure 1. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Fresh weight shoot (b) Dry weight shoot (c) Plant height.

T1 ($\text{NO}_3^-:\text{NH}_4^+::100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+::50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+::25:75$), T5 ($\text{NO}_3^-:\text{NH}_4^+::0:100$).

T5 ($0:100 = \text{NO}_3^-/\text{NH}_4^+$) recorded with least fresh weight (Figure 1a). Likewise dry weight of shoot was maximum in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Figure 1b). Least

dry weights was observed in the plants provided with treatments T4 (25:75 =NO₃⁻/NH₄⁺) and T5 (0:100 =NO₃⁻/NH₄⁺). Least fresh and dry weight at and above 50% NH₄⁺ concentrations may be attributed to the greater supply of carbohydrates to roots for assimilation and detoxification of ammonium (Marschner 2011). The strategy of the plant, namely to lower free ammonium in its tissues by assimilation, requires extra C (carbohydrates), which is then not available for growth (Britto and Kronzucker 2013). Fresh biomass increased by 60.5% as the ratio of NH₄:NO₃ -N decreased from 100% to 0% (Barickman and Kopsell 2016; Kopsell et al., 2007). Zhang et al., (2005) in spinach, Chen et al., (2011) in soyabean and Kim et al., (2006) in rocket salad has also observed the same results where the maximum fresh and dry biomass was recorded for the treatment T2 (75:25 =NO₃⁻/NH₄⁺). In gerbera plant supply of NO₃⁻ and NH₄⁺ in the ratios of 80:20 and 60:40 in the nutrient solutions resulted in highest and lowest production of fresh and dry biomass, respectively (Khalaj et al., 2017).

Total yield varied significantly with supply of different N treatments.

Plant height

Plant height is a selection criterion for screening vigorous plants, and is an index of growth and development. During all the three seasons, (October, February and July) maximum mean plant height was observed in the plants which were provided with treatment T1 (NO₃⁻/NH₄⁺::100:0), whereas least plant height was recorded for the treatment T5 (NO₃⁻/NH₄⁺ = 100:0) (Figure 1c). Shoot height of melon plants was decreased when supplied with NO₃⁻ and NH₄⁺ in the ratio of 0:100 (Na et al., (2014). Supply of NO₃⁻-N and NH₄⁺-N in the ratio of 75:25, resulted in increased plant height in strawberry (Tabatabaei et al., 2006), rocket salad (Kim et al., 2006) and soyabean (Chen et al., 2011). Gonzalez-Garcia et al., (2009) in chives Zou et al., (2017) in *Polygonum Odoratum* obtained maximum plant height when plants were supplied with NO₃⁻ and NH₄⁺ in the ratio of 80:20.

Leaf area

Application of treatments T1 (NO₃⁻/NH₄⁺ =100:0) and T2 (NO₃⁻/NH₄⁺::75:25) resulted in maximum mean leaf area during October and February growing crop and for July grown crop maximum leaf area was found in plants provided with treatment T2(NO₃⁻/NH₄⁺::75:25) while the plants provided with treatment T4 (NO₃⁻/NH₄⁺ =25:75) and T5 (NO₃⁻/NH₄⁺ =0:100) recorded with minimum mean leaf area during all the three growing seasons.(Figure 2a). Our results are in agreement with Cao et al., (2010) in ryegrass, Osorio et al.,(2003) in taro and Young and Ryong (2001) in petunia who observed that leaf area was higher under 75/25 (NO₃⁻/NH₄⁺) ratio. For chives and *Polygonum Odoratum* maximum leaf area was obtained when plants were supplied with a ratio of 80/20 (NO₃⁻/ NH₄⁺) in nutrient solution (Gonzalez-Garcia et al.,2009, Zho et al., 2017).

Total yield per plant

During all the three growing seasons (October, February and July) maximum yield per plant was recorded in the plants which were provided with treatments T2 (75:25 =NO₃⁻/NH₄⁺) (Figure 2b). Polyhouse grown spinach, provided with treatment T2 (75:25 =NO₃⁻/NH₄⁺) reported 14.40%, 33.72%

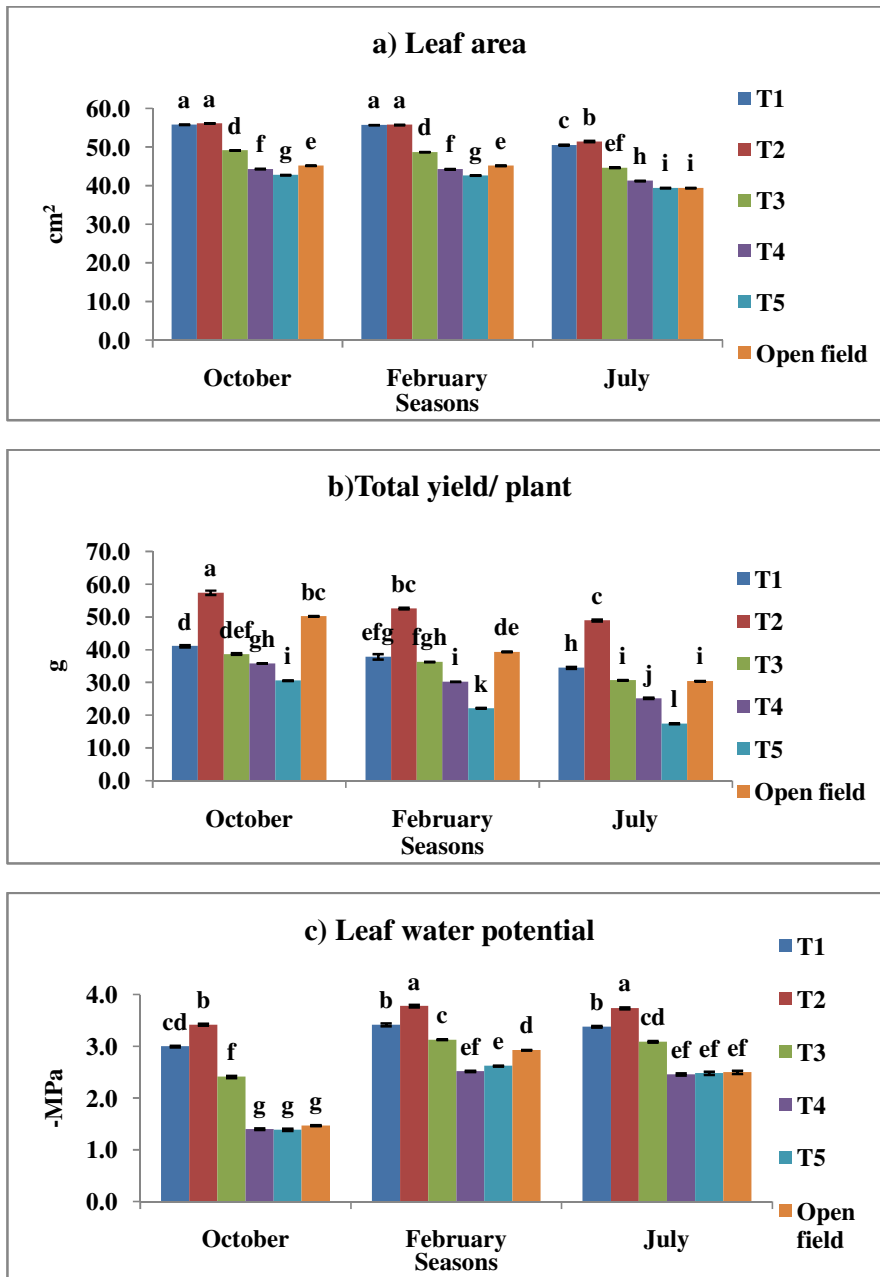


Figure 2. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Leaf area (b) Total yield per plant (c) Leaf water potential.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

and 60.87% more yield than soil grown spinach in October, February and July, respectively. Likewise in taro plants greater biomass was obtained when supplied with nutrient solutions NO_3^- and NH_4^+ in the ratio of 75:25 (Osorio et al., 2003), in lettuce (Wang and Shen 2011), in pepper (Bar-Tal et al., 2001) and in French beans (Guo et al., 2002).

Leaf water potential

In October sown crop (main season) lower values (more negative) of water potential were recorded in leaves supplied with treatments T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) as compared to open field conditions and plants supplied with treatments T4 ($\text{NO}_3^-:\text{NH}_4^+::25:175$) and T5($\text{NO}_3^-:\text{NH}_4^+::0:100$) maintained the higher leaf water potentials (less negative values) (Figure 2c). In off season crops (February and July) lower values (more negative) of water potential were recorded in leaves supplied with treatments T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) followed by T1($\text{NO}_3^-:\text{NH}_4^+::100:0$) as compared to open field conditions and plants supplied with treatments T4 ($\text{NO}_3^-:\text{NH}_4^+::25:175$) and T5($\text{NO}_3^-:\text{NH}_4^+::0:100$) maintained the higher leaf water potentials (less negative values). The reduction in water potential is a consequence of low water uptake and hydraulic flow rates within plants, or of high rates of water loss in white clover (Lee et al., 2009). Lower amount of leaf water potential in the leaves of plants provided with treatment T1, T2 and T3 ($\text{NO}_3^-/\text{NH}_4^+ = 100:0, 75:25, 50:50$) was due to accumulation of cytoplasmic osmolytes like proline and is thought to help in reducing the cellular water potential to a level below the external water potential. It enables the water to move into the cell while at the same time minimizing potentially deleterious increases in ionic strength (Hossain et al., 2014).

Nutritional and quality attributes

Total chlorophyll and Carotenoid content

The crop supplied with different nutrient solutions showed significant variation in total chlorophyll content (Figure 3a) during all the three growing seasons (October, February and July). Maximum mean total chlorophyll content was recorded for the plants supplied with nutrient solution T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) and plants provided with nutrient solution T5 ($\text{NO}_3^-:\text{NH}_4^+::0:100$) had minimum mean total chlorophyll content during all the three seasons.

Carotenoids content (Figure 3b) during the main season (October) and off seasons (February and July) was maximum in the plants provided with T1 ($\text{NO}_3^-:\text{NH}_4^+::100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) and T3 ($\text{NO}_3^-:\text{NH}_4^+::50:50$). No significant differences were observed between the carotenoids content of the plants supplied with nutrient solutions T1 to T3 and open field sown crop during all the three seasons. Chlorophylls are an important quality parameter because they contribute to leaf colour and have a direct effect on the consumer preference. Similarly carotenoids have an important role in human nutrition, because they act as antioxidants (Maiani et al., 2009). Similar observations of increased chlorophyll and carotenoids content in crop supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratio of 75:25 have been reported in swiss chard (Barickman and Kopsell 2016) and in kale (Kopsell et al., 2007). It has been observed that total chlorophyll contents of *Polygonum odoratum* seedlings supplied with $\text{NO}_3^-:\text{NH}_4^+$ in the ratios of 90:10 or 80:20 was significantly higher than the seedlings which were provided with NH_4^+ as sole source of N (i.e. $\text{NO}_3^-/\text{NH}_4^+=0:100$) (Zou et al., 2017).

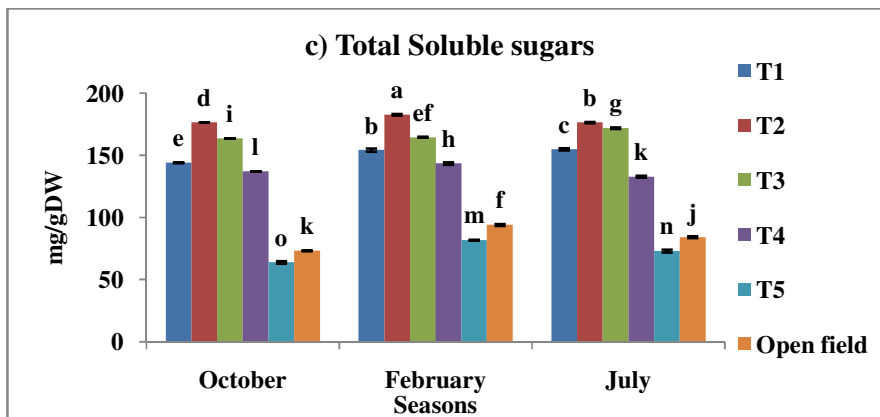
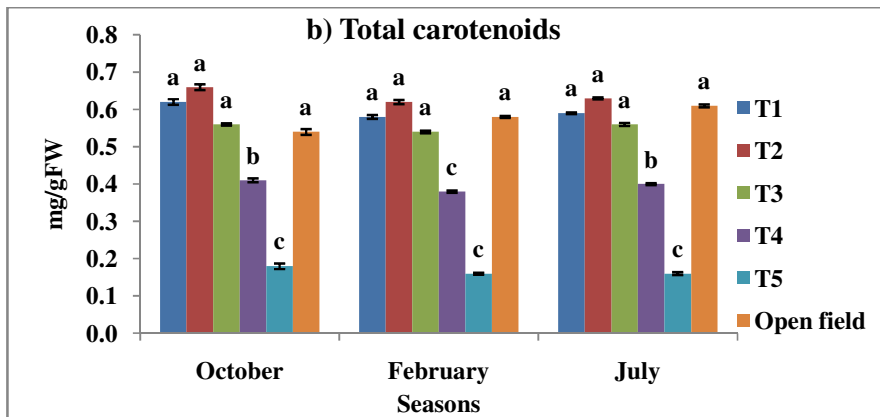
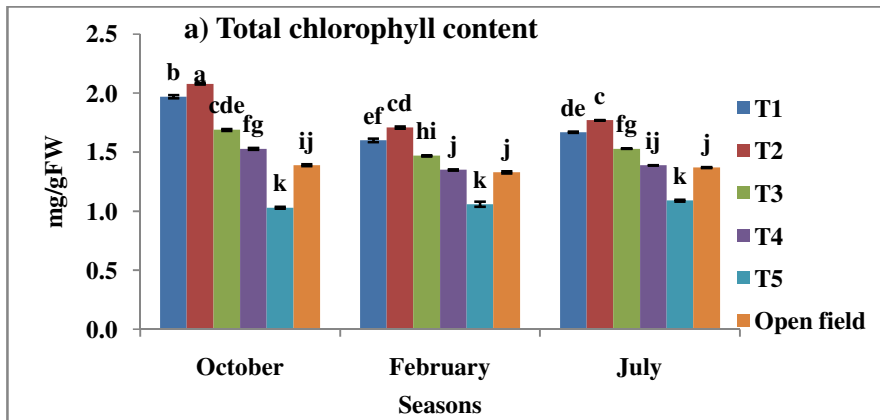


Figure 3. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Total chlorophyll content (b) Total carotenoids (c) Total soluble sugars.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

Total soluble sugars and starch content

Total soluble sugars (Figure 3c) content was maximum in the plants supplied with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+ :: 75:25$) while, those plants which were supplied with T5 ($0:100=\text{NO}_3^-/\text{NH}_4^+$) treatment had minimum total soluble sugars accumulation during all the three seasons (Table 5). It showed that total soluble sugars (TSS) content were significantly ($P \leq 0.05$) decreased when concentration of NH_4^+

in the nutrient solution was above 25%. Moreover plants grown under green house were able to accumulate more sugars and starch as compared to open field conditions. Total soluble sugars accumulation in the plants which were supplied with treatment T2 (75:25::NO₃⁻/NH₄⁺) was increased by 140.64%, 94.05% and 109.28% in October, February and July sown crops respectively as compared to crop sown in open field conditions in the same seasons.

In the main season (October) the plants supplied with ratios of 100:0 (T1)(NO₃⁻/NH₄⁺) in the nutrient solutions had maximum accumulation of total starch(Figure 4a) than the plants which were supplied with T5 (NO₃⁻/NH₄⁺=0:100) treatments. In off seasons crop (February and July), starch content was maximum till the proportion of ammonium (NH₄⁺) was 0 or 25% in the nutrient solution (i.e.T1& T2) and plants had the least total starch content which were supplied with treatment T5 (NO₃⁻/NH₄⁺=0:100). Likewise in Chinese chive seedlings total soluble sugars were increased when the NO₃⁻ to NH₄⁺ ratio in the nutrient solution was reduced and the ratio of 75:25(NO₃⁻/NH₄⁺) (Sun et al., 2014). Contents of total soluble sugars and starch were increased when the proportion of NH₄⁺ in the supplied nutrient solution was 25% in tomato (Yang et al., 2003). Zhang et al.,(1990) has also reported that contents of soluble sugar were increased when spinach plants were supplied with a ratio of 75:25 (NO₃⁻-N to NH₄⁺-N). A NO₃⁻/NH₄⁺ ratio of 80:20 increased the total soluble sugars content in Chinese kale(Zhu et al., 2018)and a ratio of 6: 1.9 (NO₃⁻/NH₄⁺) in *Corchorus olitorius* (Giro and Ferrante 2016).

Total soluble proteins and Free amino acids

Among the treatments the highest total soluble protein content in all the three seasons occurred in leaves of plants which were supplied with nutrient solutions containing NO₃⁻ and NH₄⁺ in the ratios of 100:0, 75:25 respectively. The plants supplied with treatment T5=0:100 (NO₃⁻/NH₄⁺) nitrogen solution and the plants raised in open field conditions had the lowest levels of soluble protein content (Figure 4b).

In February and July sown crop, free amino acids content accumulated maximum in plants provided with treatments T2 and T1(NO₃⁻/NH₄⁺:: 75:25,100:0) in the nutrient solution and the plants supplied with treatment T5=0:100 (NO₃⁻/NH₄⁺) nitrogen solution maintained the lowest levels of free amino acids content (Figure 4c). In October sown crop, free amino acids content was found maximum in the plants provided with nutrient solution T2(NO₃⁻/NH₄⁺::75:25). Zhu et al., (2018) observed that a nutrient solution with NO₃⁻/NH₄⁺ in the ratio of 80:20 increased the total soluble proteins and amino acids in Chinese kale. It has been observed in spinach plants, that total soluble protein and amino acids content were decreased when they were supplied with higher proportion of ammonium (NO₃⁻-N: NH₄⁺-N= 1.5:3) than the nitrate in the nutrient solution (Dominguez- Valdivia et al., 2008). In the previous study, it has been observed that the content of total soluble protein and free amino acids were highest in the plants which were supplied with NO₃⁻-N: NH₄⁺-N in the ratio of 75:25 in the chive (Sun et al. 2014) and in *Camellia oleifera*(Wang et al., 2018).

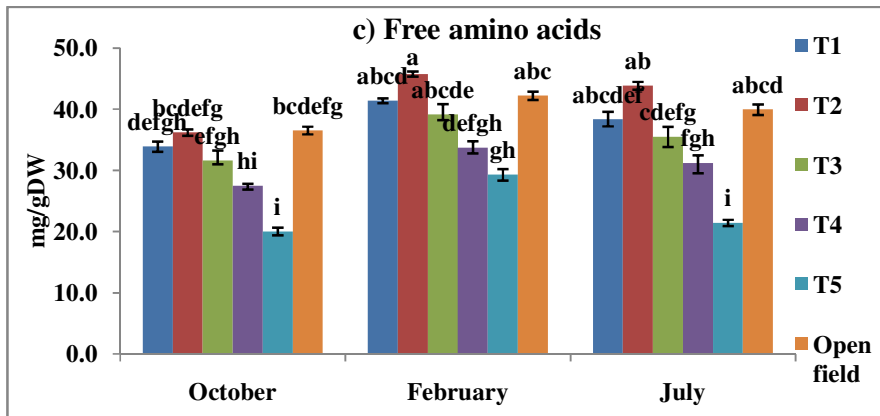
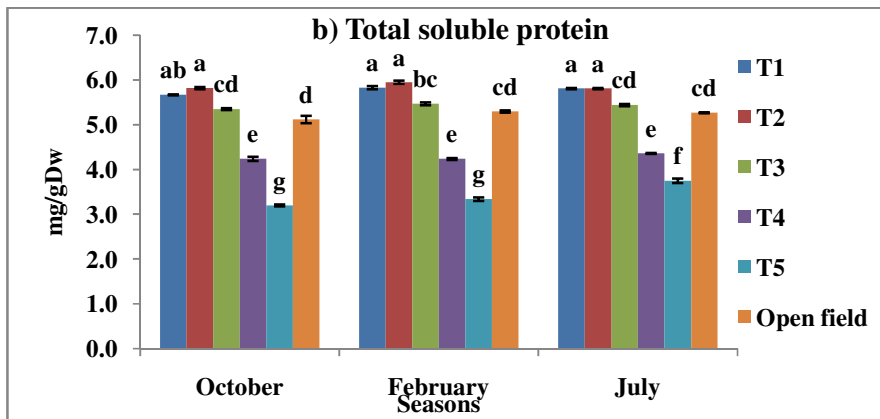
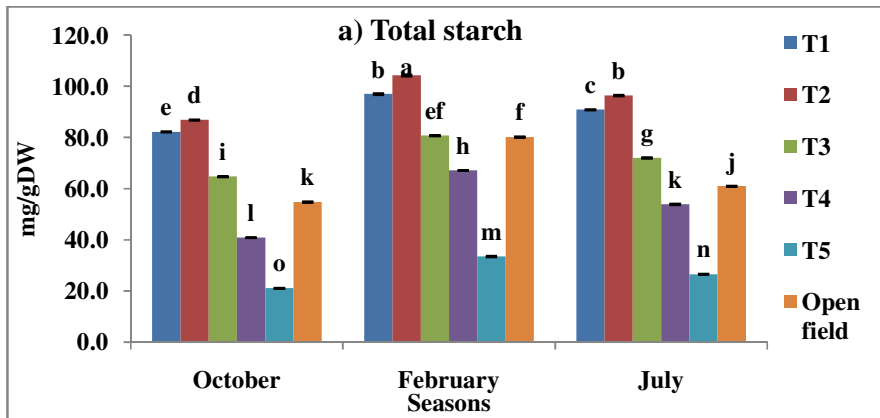


Figure 4. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Total starch (b) Total soluble proteins (c) Free amino acids.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

Proline content

Proline content (Figure 5a) was found to be significantly increased when concentration of NH_4^+ in the nutrient solution was upto 25% (T1 and T2) and further there was a decline in proline accumulation at a very higher proportion of ammonium (50 to 100%) in the nutrient solution. Among the treatments maximum mean proline content in October and February growing crop was recorded for the plant

supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratios of 75:25 followed by 100:0 whereas in July grown crop maximum mean proline content was recorded for the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$). Plants supplied with nutrient solution in the ratio of 0:100 ($\text{NO}_3^-/\text{NH}_4^+$)

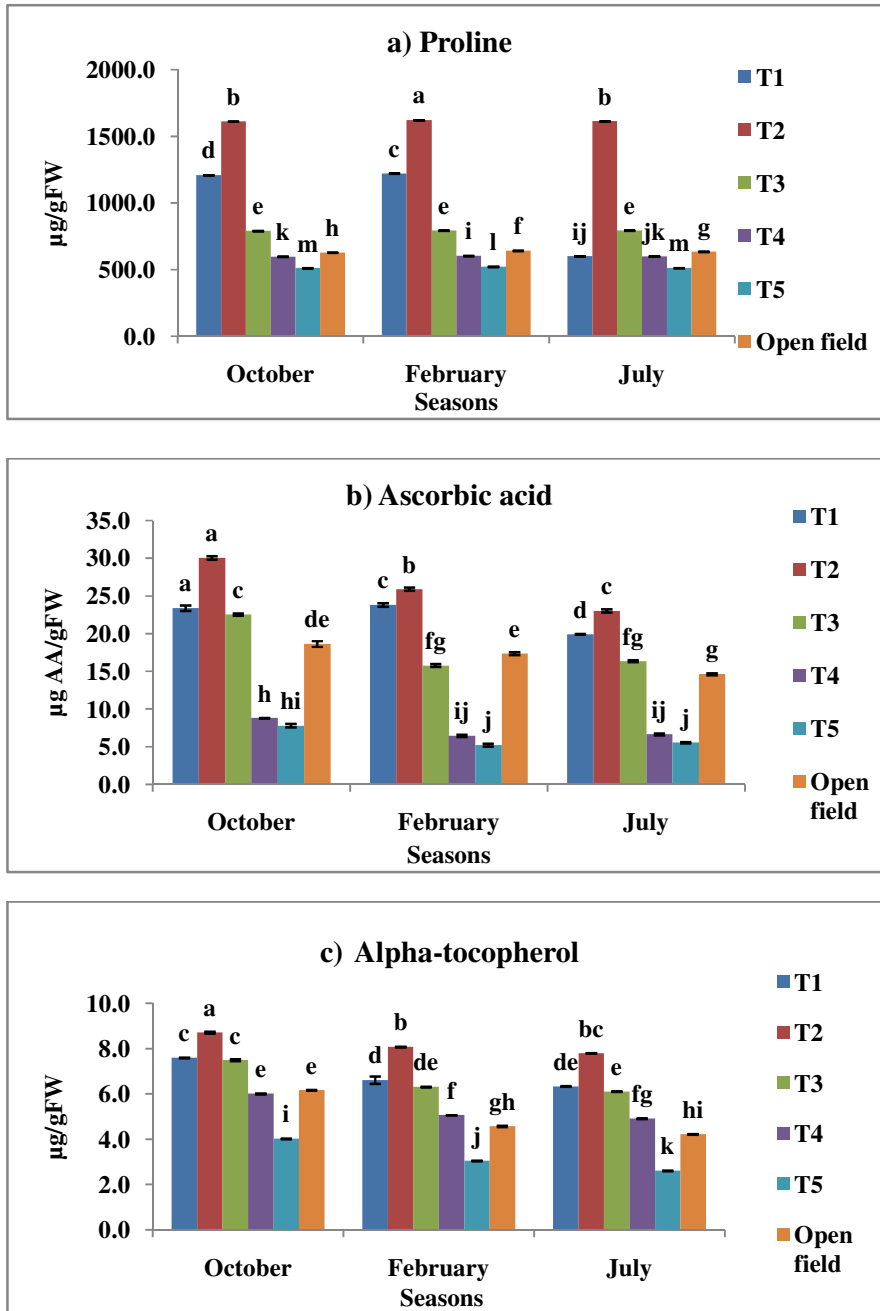


Figure5. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Proline content (b) Ascorbic acid content (c) Alpha-tocopherol content.

T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

maintained the lowest level of proline during all the three growing seasons. Plants which were provided with treatment T2 i.e. a ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) in the nutrient solution accumulated

156.72%, 152.78% and 154.46% more proline than soil grown spinach in October, February and July seasons, respectively. Sun et al., (2014) has reported the same results that ratio of 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) led to maximum accumulation of proline in Chinese chive seedlings.

Ascorbic acid

During main season (October) maximum mean ascorbic acid content was found in the plants provided with treatment T1 and T2 ($\text{NO}_3^-/\text{NH}_4^+::100:0$, 75:25). During off season (February and July) sown crop, maximum mean ascorbic acid content was recorded in the plants supplied with treatments T2($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Figure 5b). Buchanan and Omaye (2013) have observed the same results in lettuce. Increased ascorbic acid content was obtained with the supply of NO_3^- and NH_4^+ in the ratio of 80:20 in Chinese kale (Zhu et al., 2018). Highest ascorbic acid content was obtained in basil with nutrient solution 3 ($\text{NO}_3^-:4/ \text{NH}_4^+:0.5$) and 1 ($\text{NO}_3^-:3.5/ \text{NH}_4^+:1.5$), respectively (Saadatian et al., 2014).

Alpha-tocopherol content

Alpha- tocopherol content in October, February and July sown crop was significantly higher in plants supplied with treatments T2 (75:25= $\text{NO}_3^-/\text{NH}_4^+$) and the plants which were provided with treatment T5 (0:100= $\text{NO}_3^-/\text{NH}_4^+$) had the least mean alpha- tocopherol content (Figure 5c). Buchanan and Omaye (2013) has obtained the same results where they found that hydroponically grown lettuce plants accumulated more α -tocopherol than soil grown lettuce.

Antioxidants activities

SOD (superoxide dismutase) activity was significantly more in off seasons (February and July) crop than the main season crop (October) (Figure 6a). During February and July sown crop, maximum SOD activity was seen in the plants which were provided with treatments T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and T3 ($\text{NO}_3^-/\text{NH}_4^+::50:50$) and plants had least SOD activity provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). In main season (October) sown crop maximum mean SOD activity was reported in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) and plants had the least SOD activity provided with T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) nutrient solution.

POD (Peroxidase) activity during main season (October) was significantly maximum in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$). During off seasons (February and July) grown crop maximum POD activity was found in plants provided with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) (Figure 6b) plants provided with treatment T5 ($\text{NO}_3^-/\text{NH}_4^+ =0:100$) and grown in open field conditions recorded with minimum level of POD activity.

CAT activity (Figure 6c) was recorded maximum in the plants which were supplied with T2 ($\text{NO}_3^-/\text{NH}_4^+ =75:25$) followed by T1 ($\text{NO}_3^-/\text{NH}_4^+ =100:0$) treatments and plants provided T5 ($\text{NO}_3^-/\text{NH}_4^+ =0:100$) maintained the lowest level of CAT activity, during all the three seasons. CAT activity during off seasons was significantly maximum than main season sown crop. Reactive oxygen species (ROS) which cause the lipid peroxidation of cell membranes are detoxified by the plant

antioxidant enzymes e.g. SOD, POD, and CAT (Gulen et al., 2008). Activities of anti-oxidant

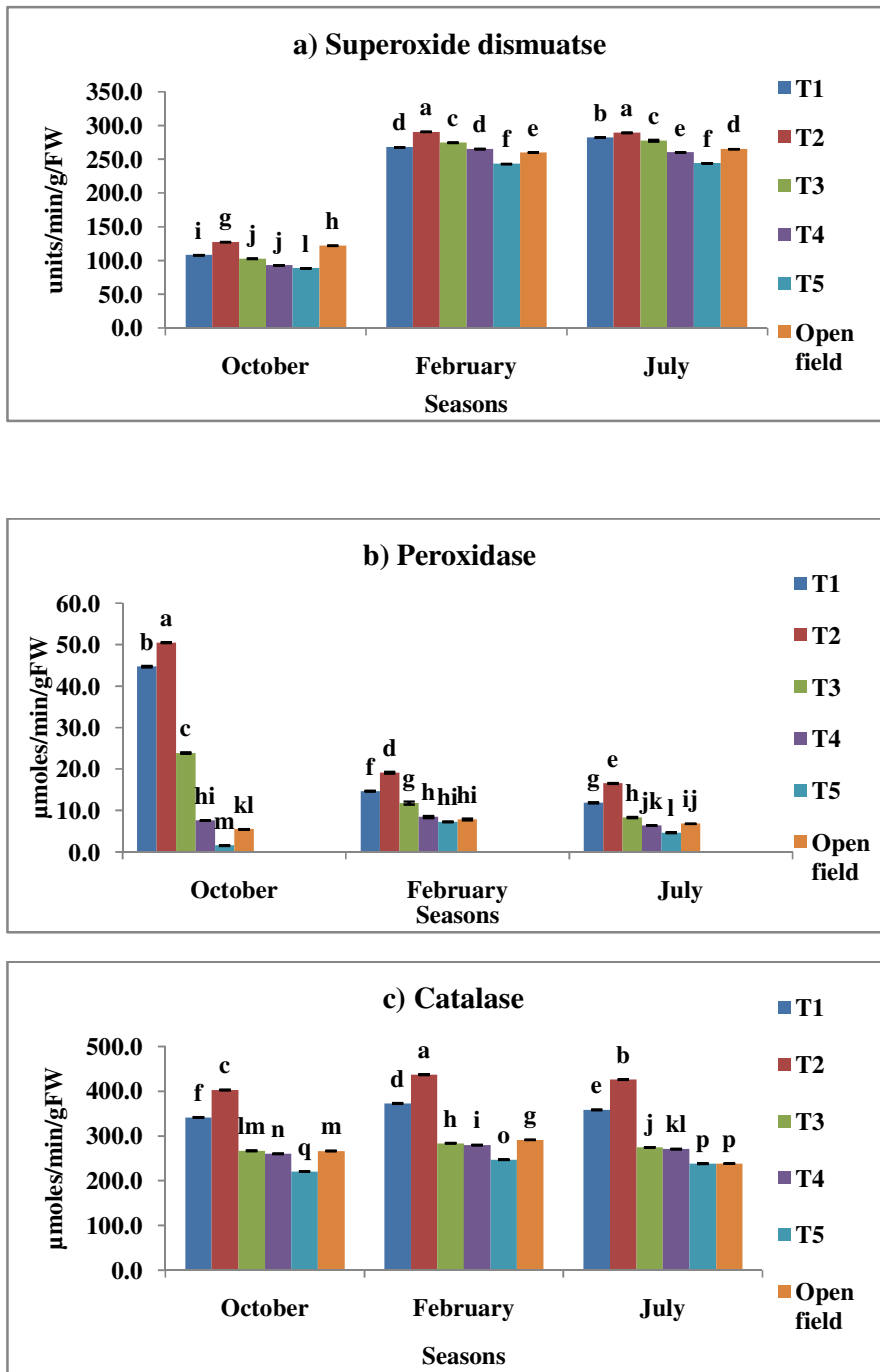


Figure 6. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Superoxide dismutase activity (b) Peroxidase activity (c) Catalase activity. T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

enzymes were influenced significantly with N forms, and their activities of plants treated with NH_4^+ mixed nutrient solution were significantly higher than those with the sole NO_3^- source of N treatment

(Polesskaya et al., 2004). An increase in activities of anti-oxidant enzymes was observed in *Polygonatum odoratum* which were grown aeroponically with low and moderate proportion of NH_4^+ mixed solutions (90:10= NO_3^- : NH_4^+) treatment compared with all nitrate N. NH_4^+ is an important material to synthesize amino acids, nevertheless, its superfluous usage would be toxic to plant (Zou et al., 2017). Consequently, ($\text{NO}_3^-/\text{NH}_4^+ = 75:25$) treatment, which had a low or moderate NH_4^+ proportion, helped in promoting the activities of anti-oxidant enzymes in the present investigation. Our results are also in agreement with the findings of Sun et al., (2014) in Chinese chive where the optimum ratios of NO_3^- -N to NH_4^+ -N for protecting Chinese chive against oxidative stress are 50:50 and 75:25. Boschiero et al., (2019) has reported the same results in sugarcane.

Conclusion

In conclusion, overall, for each date of sowing, maximum yield was obtained in plants provided with nutrient solution T2 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$). In main season as well as off season sown spinach crop, the yield was significantly more in polyhouse than open field sown crop. Further, the crop provided with nutrient solutions having higher proportion of NO_3^- had higher values of quality parameters and maximum antioxidant activity than the open field crop. Hence providing nutrient solutions with appropriate ratios of $\text{NO}_3^-:\text{NH}_4^+$ improves the yield and quality traits in polyhouse grown spinach. It is therefore suggested that $\text{NO}_3^-/\text{NH}_4^+$ ratios of 75:25 could promote growth, increase yield and improve the nutritional quality of spinach under polyhouse system.

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EFFECT OF PROPORTION OF NO₃⁻ AND NH₄⁺ 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 on yield and quality of Coriander (cv. Punjab Sugandh) grown under polyhouse conditions was evaluated. Crop was sown on three dates viz., October (main season), February and July (off-season) respectively. N (Nitrogen) was supplied in variable ratios of NO₃⁻ to NH₄⁺ 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 harvesting, growth (fresh and dry weight of shoot, plant height, leaf area and yield), quality parameters (total chlorophyll and carotenoids, total soluble sugars and starch, total soluble proteins and free amino acids, proline, ascorbic acid, α -tocopherol contents), and activity of antioxidant enzymes superoxide dismutase, catalase, peroxidase were determined. Irrespective of the seasons, the yield was significantly more in polyhouse and among the nutrient solutions, the solution T2 recorded higher yield as well as growth and quality parameters during all the season. It is suggested that NO₃⁻ to NH₄⁺ ratio of 75:25 in the nutrient solution improves the both quality, and yield and is thus most suitable for Coriander crop.

Key words: Ascorbic acid, α -tocopherol, Coriander, Growth, Proline, yield.

Introduction

Leafy vegetables are the sources of antioxidants, minerals, vitamins and other nutritional contents. 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). In a polyhouse, the crop is generally grown in soilless media. Many plants such as Chinese cabbage (Song et al, 2011) and cucumber (Roosta and Schjoerring 2008) prefer higher proportion of nitrate to ammonium nutrient solution. N metabolism can be regulated by supply, demand for growth, and amino acid content in plant. Plant composition can be changed by N more than any other mineral element (Marschner 2012). 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 quality in coriander raised as 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 $30^\circ-56'$ North latitude, $75^\circ-52'$ 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 (control) and another under polyhouse. Under the open field conditions the crop was raised in 2m^2 plot as per recommended practices (Anonymous 2016). In polyhouse, seeds were directly sown in plastic pots 30 cm (diameter) \times 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°C and 11.4°C during main season (October- January), 28.1°C and 16.7°C during the off season crops (February-May) and 28.3°C 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 8mmolL^{-1} (Table.2). NO_3^- was supplied as $\text{Ca}(\text{NO}_3)_2$ and NH_4^+ as $(\text{NH}_4)_2\text{SO}_4$, 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. Approximately 2L nutrient solution was added manually to each pot during the main season crop and approximately 3 L during off season.

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 till the tip of the topmost leaf with a centimetre scale. 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.

Quality attributes

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

Statistical analysis

Data obtained from this experiment were analyzed by factorial design using SPSS (Version 16.0 SPSS) software. Significant differences ($p \leq 0.05$) were determined by Tukey's test, and were marked with lower-case letters in figures.

Results and discussions

Growth parameters

Maximum leaf area (Fig. 1.a), plant height (Fig. 1.b), fresh and dry weight of shoot (Fig. 1.c; 1.d) were recorded in the crops sown in main season and within each season, polyhouse grown crop showed higher values than the soil grown crop. Among the nutrient solutions, the plants provided with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) had the maximum leaf area, plant height and fresh and dry weight of shoot while least values for these parameters were observed in the plants supplied with nutrient solution T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$). This decrease in growth at higher supply of NH_4^+ (50% or above) (viz; treatments T4 and T5) in nutrient solution may be due to the fact that 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 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). Increased plant growth under polyhouse can be related to controlled environmental conditions along with optimum supply of nutrients and water to the plants which led to more vegetative growth.

Proline and Leaf water potential

Among the treatments, maximum proline content in all the seasons was recorded in the plants supplied with nutrient solution containing $\text{NO}_3^-/\text{NH}_4^+$ in the ratios of 75:25 (Fig. 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) (Fig. 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). 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 which 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 (NO_3^- : NH_4^+ ::75:25) (Fig.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 (Fig.2.b) Carotenoids content in the plants supplied with treatments T3, T4 and T5 did not differ significantly during all the three seasons. It means 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 (NO_3^- : NH_4^+ ::75:25) resulted in maximum chlorophyll and carotenoid content in both main and off season crop. Previous studies have also reported maximum chlorophyll and carotenoids content in crop supplied with $\text{NO}_3^-/\text{NH}_4^+$ 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 (Fig.2.c). The total starch content was maximum in the plants supplied with nutrient solution T2 at all dates of sowing (Fig.2.d). It means 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 $\text{NO}_3^-/\text{NH}_4^+$ ratio of 80:20 increased the total soluble sugars content in Chinese kale (Zhu et al. 2018).

Total soluble proteins and free amino acids content

In October sown crop, maximum mean total soluble proteins content recorded for the plants which were supplied with treatment T3 ($\text{NO}_3^-/\text{NH}_4^+:: 50:50$), whereas in off season crop, plants provided with nutrient solutions T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) resulted in maximum mean total soluble proteins content (Fig.2.e). During all the three seasons, plants provided with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+:: 75:25$) had the maximum total free amino acids content (Fig.2.f). Likewise, ratios of 80:20 and 75:25 ($\text{NO}_3^-/\text{NH}_4^+$) 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 acids content has been reported more in wheat under heat stress (Kaur et al. 2018).

Ascorbic acid and α -tocopherol content

In main as well as in off season, among the nutrient solutions maximum ascorbic content was recorded in the plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Fig.3.a). It means 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 containing NO_3^- and NH_4^+ in the ratio of 80:20 in Chinese kale (Zhu et al. 2018) and a ratio of 3.5:1.5 ($\text{NO}_3^-:\text{NH}_4^+$) in basil (Saadatian et al. 2014). α -tocopherol content followed the same trend as ascorbic acid content i.e., it was maximum in the plants which were supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Fig.3.b).

Ascorbic acid and α -tocopherol content in the leaves were higher in main season crop raised under polyhouse. Phillips 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 (Buchmann and Omaye 2013).

Antioxidants activity

During all the three seasons maximum SOD, POD and CAT activities were recorded in plants provided with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) (Fig.3.c.;3d.;3e.). 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 ($\text{NO}_3^-: \text{NH}_4^+::90:10$) 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 activity 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 main season sown crop. Whereas, 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 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) 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 (Fig.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).

Conclusion

In conclusion, in main season as well as off season sown coriander crop, the yield was significantly more in polyhouse than open field sown crop. Further, the crop provided with nutrient solutions

having higher proportion of NO_3^- had higher values of quality parameters and maximum antioxidant activity than the open field crop. Hence providing nutrient solutions with appropriate ratios of $\text{NO}_3^-:\text{NH}_4^+$ improves the yield and quality traits in polyhouse grown coriander. From the present study it is therefore suggested that nutrient solution having $\text{NO}_3^-/\text{NH}_4^+$ ratios of 75:25 could promote growth, increase yield and improve the nutritional quality of coriander raised under polyhouse conditions.

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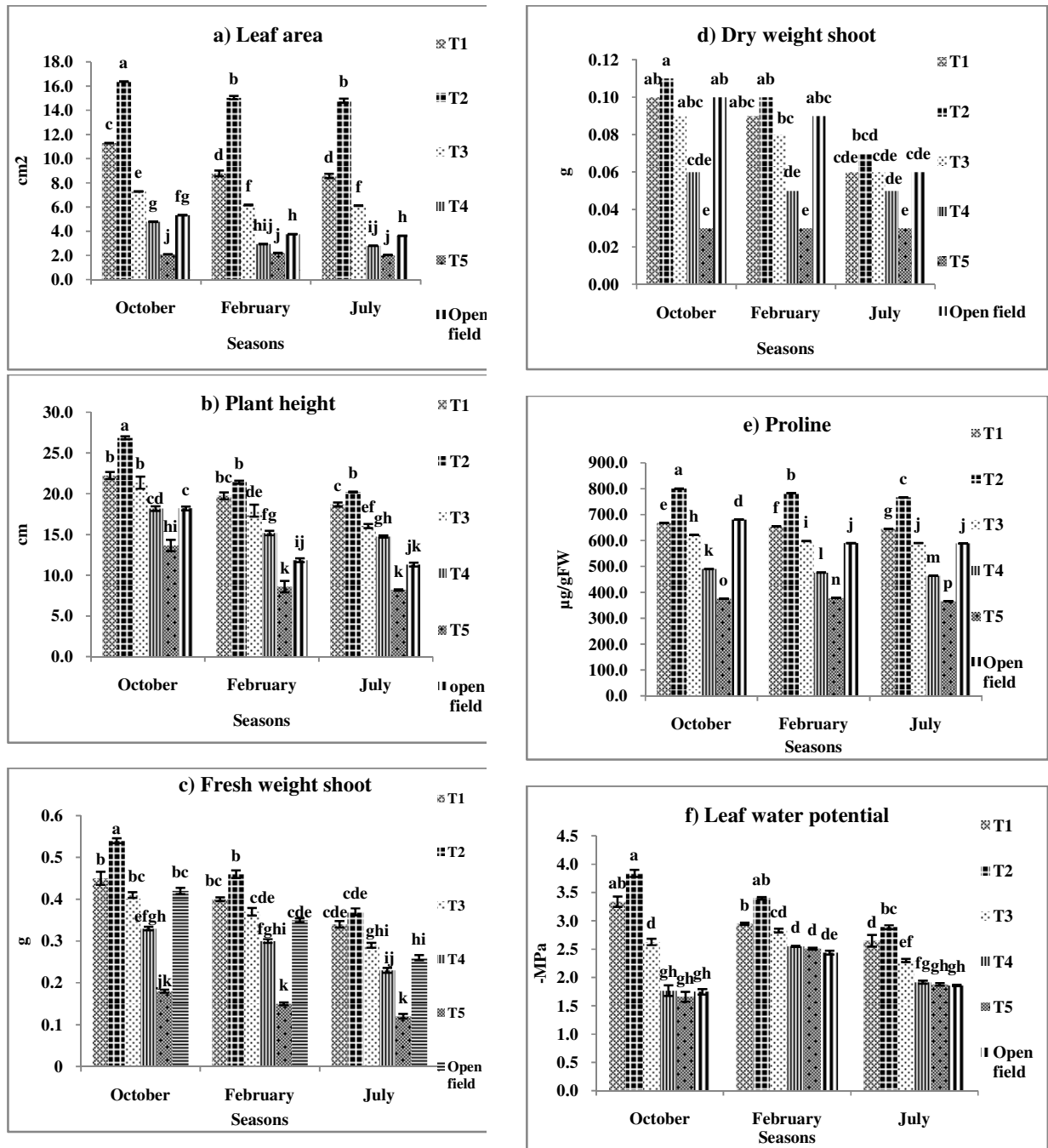


Figure 1. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Leaf area (b) Plant height (c) Fresh weight shoot (d) Dry weight shoot (e) Proline content (f) Leaf water potential
T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

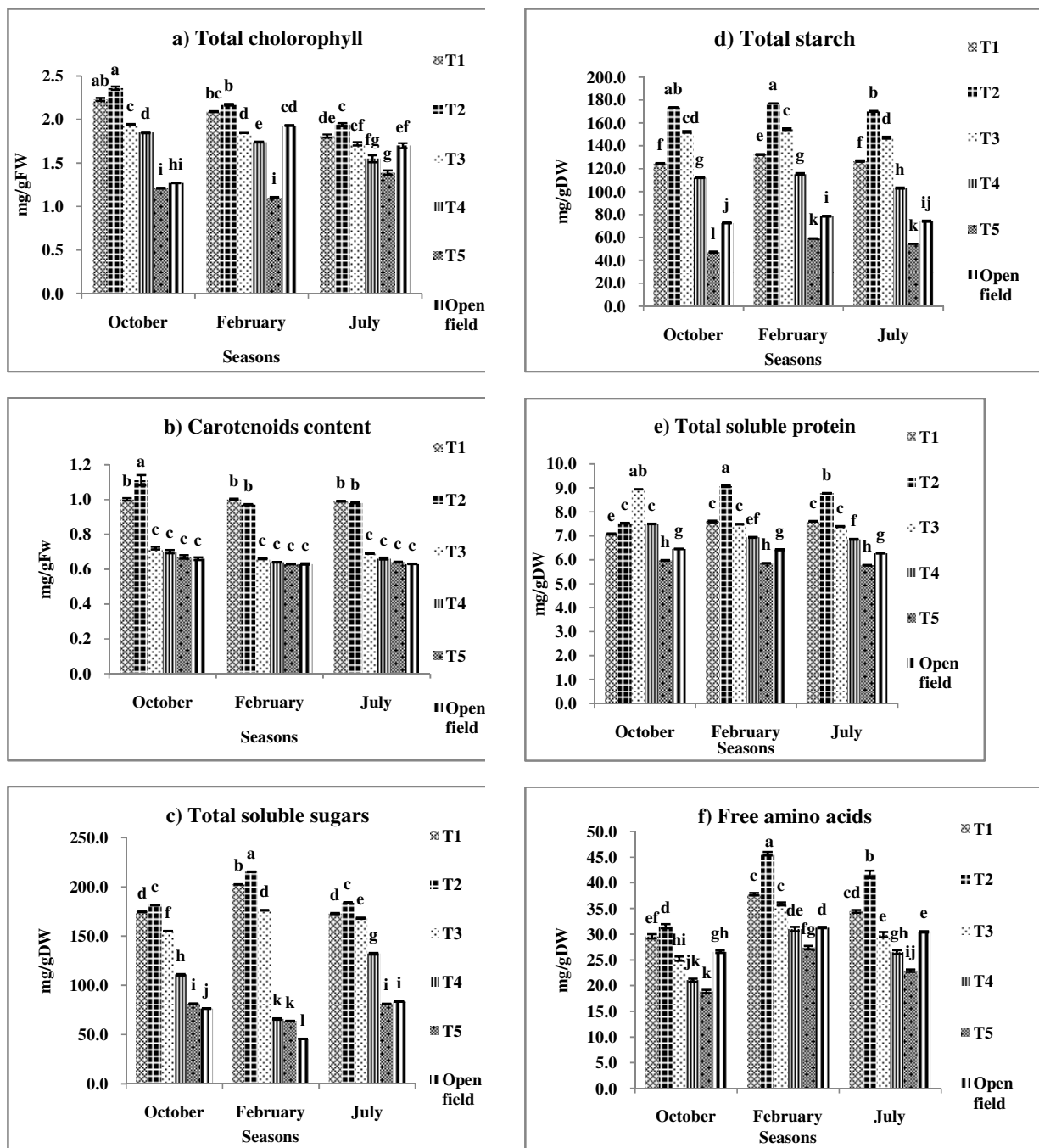


Figure 2. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Total chlorophyll content (b) Total Carotenoids content (c) Total soluble sugars (d) Total starch (e) Total soluble proteins (f) Free amino acids content T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

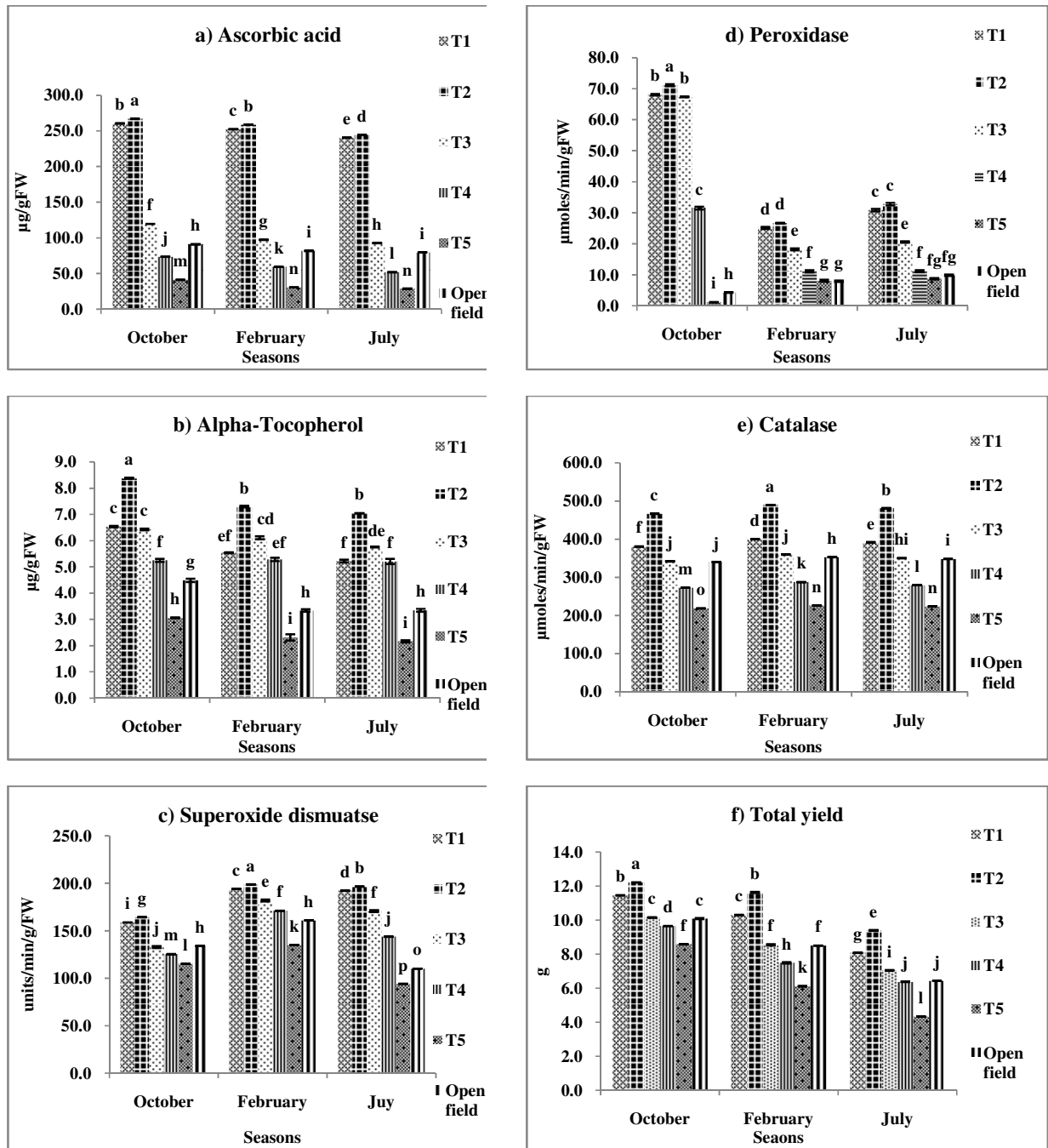


Figure 3. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Ascorbic acid content (b) Alpha-tocopherol content (c) SOD activity (d) POD activity (e) CAT activity (f) Total yield/plant T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

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

	Main season	Off season	
	October	February	July
Month of sowing	3 rd week of October (2016 and 2017)	2 nd week of February (2017 and 2018)	2 nd week of July (2017 and 2018)
Month of harvesting	3 rd week of January (2017 and 2018)	1 st week of May (2017 and 2018)	1 st week of October (2017 and 2018)

Table 2. Compositions (mmol L⁻¹) of nutrient solutions with different NO₃⁻/NH₄⁺ ratios

Treatments	NO ₃ ⁻ /NH ₄ ⁺ ratio	Source of nutrients (mmolL ⁻¹)						
		KNO ₃	Ca(NO ₃) ₂ .4H ₂ O	CaCl ₂ .2H ₂ O	(NH ₄) ₂ SO ₄	KH ₂ PO ₄	MgSO ₄ .7H ₂ O	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.

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EFFECT OF NITROGEN FORMS ON PHYSIOLOGICAL AND BIOCHEMICAL CONSTITUENTS OF LETTUCE GROWN UNDER POLYHOUSE DURING DIFFERENT SEASONS

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Abstract

The effect of different nitrate to ammonium ratios ($\text{NO}_3^- : \text{NH}_4^+:: 100:0, 75:25, 50:50, 25:75$ 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 was harvested 3 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 (SOD, CAT, 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: Ascorbic acid, biochemical parameters, growth, lettuce, and yield.

Introduction

Soilless cultivation of vegetables provides better control of plant growth and development as compared to traditional greenhouse production in soil. Lettuce is in demand throughout the year as it is popular as a vegetable salad (Maboko & Du Plooy, 2008) being rich in calcium, iron and vitamin A and is a diet food because of its low kilo joule content (Maboko, 2007).

The total nutrient concentration of the nutrient solution used in soilless culture is one of the most important aspects for successful vegetable production. Too high levels of nutrients induce osmotic stress, ion toxicity and nutrient imbalance, while too low levels generally lead to nutrient deficiencies (Falovo et al., 2009). Mineral nutrient supply directly affects the crop yields and quality during critical growth stages. Optimum N application is essential for optimum plant growth, development, yield and quality (Mehlangu et al 2016). In previous studies, 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. 2009), lettuce (Wang and Shen 2011), Chinese kale (Song et al. 2012) and Chinese cabbage (Hu et al. 2015). There is very little information on effect of the nutrient solution compositions on lettuce raised in different seasons in the polyhouse 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 polyhouse grown lettuce in relation to the cultivation season.

Material 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' and 243 meters above the sea level). The seeds of lettuce (cultivar Iceberg) were obtained from 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 field area of department of Vegetable Science as per schedule given in Table1 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 2m² plot as per recommended practices (Anonymous 2016). 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).

2.1 Polyhouse conditions and nutrient solution

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.*, 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 NO₃⁻ and NH₄⁺ while keeping the total N content constant (Table.2). NO₃⁻ was supplied as Ca (NO₃)₂ and NH₄⁺ as (NH₄)₂ SO₄, 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 was added manually to each pot during the main season crop and during off season, respectively.

2.2 Growth parameters: 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 water potential 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 oven at 60°C for constant weight and the dry weight was recorded.

2.3 Biochemical parameters: From the freshly harvested leaves, total chlorophyll and Carotenoids content was determined by the method of *Anderson and Boardman* (1964). Proline, Ascorbic acid and α -tocopherol were estimated from the fresh leaves by the method of *Bates et al* (1973), *Ranganna* (1986) and *Jayaraman* (1981), respectively. Dried leaves were analyzed for the estimation of total soluble sugars and total starch (*Dubois et al.*, 1956), total soluble proteins (TSP) (*Lowry et al.*, 1951) and free amino acids (FAA) (*Lee and Takahashi*, 1966). Enzyme activities of SOD (*Marklund & Marklund*, 1974), POD (*Shannon et al.*, 1966), CAT (*Chance & Maehly*, 1955) were determined, from fresh leaves.

2.4 Statistical analysis: Data obtained from this experiment were analyzed by factorial design using SPSS (Version 16.0 SPSS) software. Significant differences ($p \leq 0.05$) were determined by Tukey's test, and were marked with lower-case letters in figures.

Results and discussions:

Plant growth parameters:

Plant growth was determined in the form of fresh and dry biomass of head (Fig. 1.a; 1.b), plant height (Fig. 1.c) and leaf area (Fig. 1.d). Maximum plant growth was recorded in the main season (October) crop and within each season, crop sown in polyhouse had more growth than the soil grown crop. 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 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$). Higher ratios of ammonium (50% to 100%) (T4 and T5) decreased these plant growth parameters. A similar decrease in plant growth at higher concentration of NH_4^+ in nutrient solution was attributed to salinity of nutrient solution that caused restrictions in plant water flux. Reduction in fresh weight of plants due to high NH_4^+ in the nutrient solution has also been reported in purslane plants (Szalai et al, 2010) and lettuce (Andriolo et al 2006).

Proline and Leaf water potential

During all the three growing seasons, less leaf water potential (more negative values) were observed in the plants which were supplied with nutrient solutions T2 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$) and less negative leaf water potential values (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 ($\text{NO}_3^-:\text{NH}_4^+:: 25:75$) and T5 ($\text{NO}_3^-:\text{NH}_4^+:: 0:100$) (Fig. 1.e).

Proline content (Fig. 1.f) in lettuce leaves was highest in plants supplied with nutrient solution T2 ($\text{NO}_3^-:\text{NH}_4^+:: 75:25$) during all the three seasons. 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 2012). In October (main season), sown crop, the proline content of the plants grown in polyhouse conditions 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. 1.f) 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.

Total chlorophyll and Carotenoids:

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 carotenoids content as compared to other treatments while 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 plant growth and decreased chlorophyll content, whereas low proportions of NH_4^+ increased chlorophyll content e.g., in duckweed (Huang *et al* 2013). An increase in chlorophyll and carotenoids content in crop supplied with $\text{NO}_3^-/\text{NH}_4^+$ in the ratio of 75:25 have been reported in kale (Kopsell *et al.*, 2007) and swiss chard (Barickman and Kopsell, 2016). Main season (October) crop supplied with nutrient solutions T1 and T2 had more total chlorophyll content than off season (February and July) sown crop supplied with same nutrient solutions.

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

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

Table 2. Compositions (mmol L⁻¹) of nutrient solutions with different NO₃⁻/NH₄⁺ ratios

Treatments	NO ₃ ⁻ /NH ₄ ⁺ ratios	Source of nutrients (mmolL ⁻¹)						
		KNO ₃	Ca(NO ₃) ₂ .4H ₂ O	CaCl ₂ .2H ₂ O	(NH ₄) ₂ SO ₄	KH ₂ PO ₄	MgSO ₄ .7H ₂ O	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.

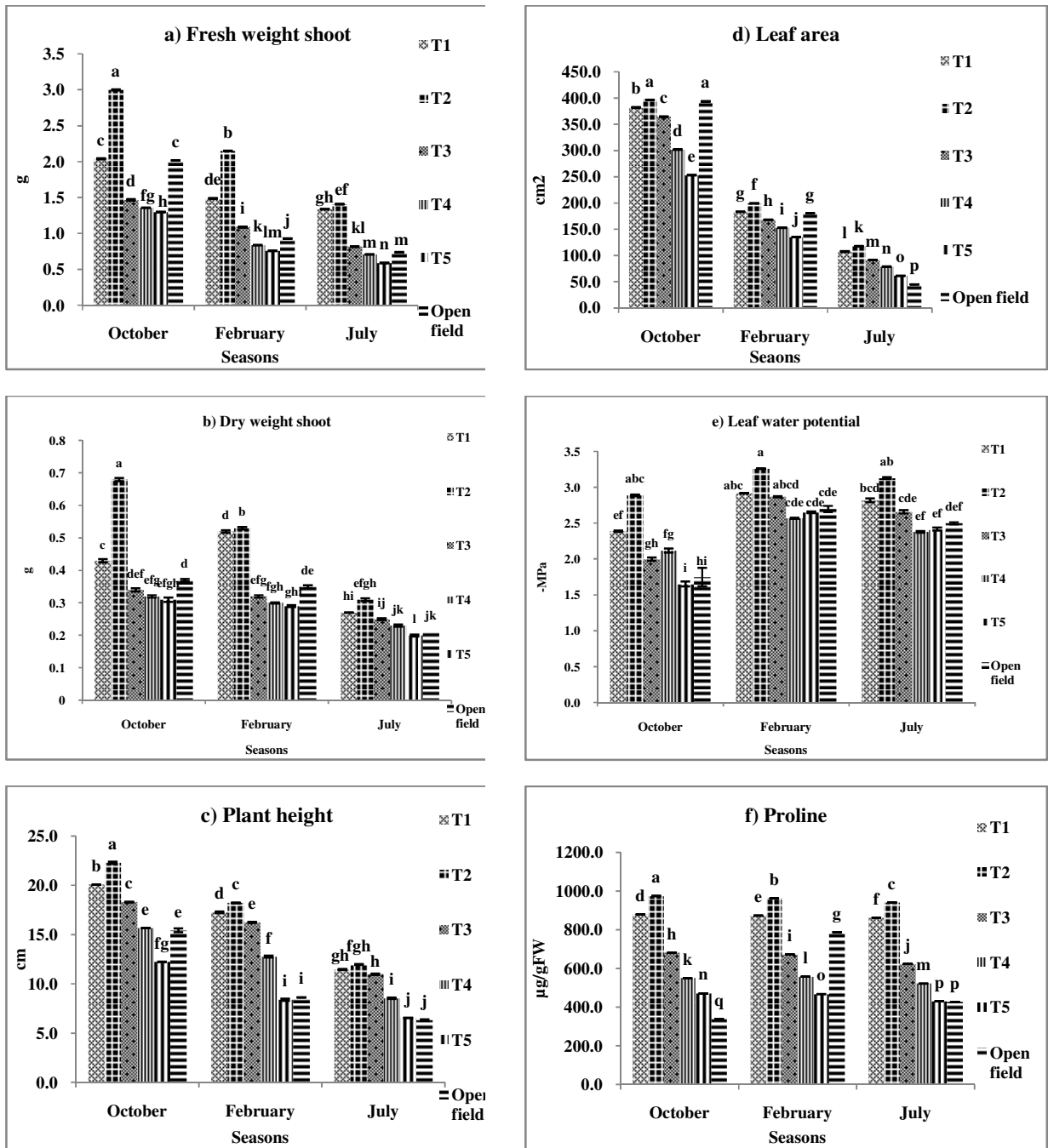


Figure 1. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Fresh weight shoot (b) Dry weight shoot (c) Plant height (d) Leaf area (e) Leaf water potential (f) Proline content T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$). Open field

Total soluble sugars and starch:

The T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) nutrient solution promoted the accumulation of total soluble sugars and starch content in all three seasons followed by treatment T1 ($\text{NO}_3^-:\text{NH}_4^+::100:0$). In contrast, the plants supplied with nutrient solution T5 ($\text{NO}_3^-:\text{NH}_4^+::0:100$) had the lowest soluble sugar and starch content contents (Fig. 2.c; 2.d). Total soluble sugars content in plants supplied with nutrient solution T2 in polyhouse grown plants was 80.70%, 84.28% and 86.13% more than 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 leafy lettuce grown with high nutrient concentration could be related to an increase in tissue vegetable respiration (Fallavo et al 2009).

Total soluble proteins and free amino acids content:

Contents of both total soluble proteins (TSP) and free amino acids (FAA) varied with the form of nitrogen used in nutrient medium (Fig. 2.e; 2.f). Both TSP and FAA contents generally increased with the lower proportion of NH_4^+ and then decreased when NH_4^+ in the nutrient solution was above 25%. Plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) and T5 ($\text{NO}_3^-/\text{NH}_4^+::0:100$) 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 soil grown crop. Similarly, increased TSP and FAA have been observed in Chinese kale (Zhu et al., 2018) supplied with nutrient solution having 20% NH_4^+ and in chives (Sun et al., 2014) supplied with 25% NH_4^+ in nutrient solution. Off season crop had more accumulation of both TSP and FAA and that may be due to high temperature (as a stress signal) prevailing during off season sown crop than main season crop. An, increased TSP and FAA content have also been reported in wheat under heat stress (Kaur et al., 2018).

Ascorbic acid and α -tocopherol content:

In each season, among the treatments, content of ascorbic acid and α -tocopherol were significantly higher in the plants supplied with treatment T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) in the nutrient solution than the other 4 treatments (Fig.3.a; 3.b). Previous studies have reported higher amounts of both alpha and gamma-tocopherols in leaves of purslane (Szalai et al. 2010) and rapeseeds oil (Hussain et al. 2014), 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 increase of tocopherol is dependent on 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 and α -tocopherol content than soil grown lettuce (Buchmann and Omaye, 2013). 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 (2018) 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 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 2016). High temperatures and low amounts of light resulted in low vitamin C content (Lee and Kader, 2000) since vitamin C content in many vegetables

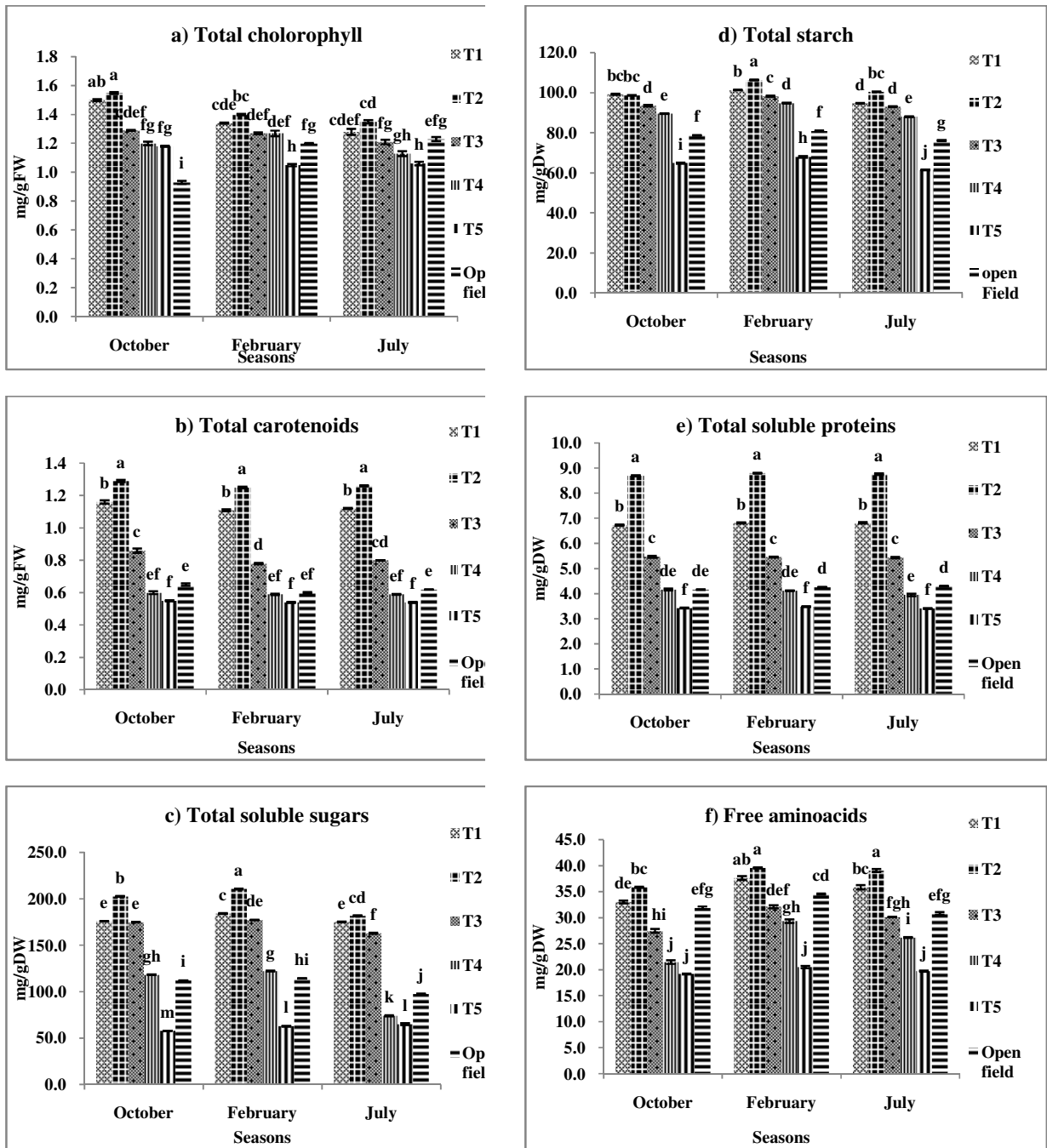


Figure 2. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Total chlorophyll content (b) Total Carotenoids content (c) Total soluble sugars (d) Total starch (e) Total soluble proteins (f) Free amino acids content T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

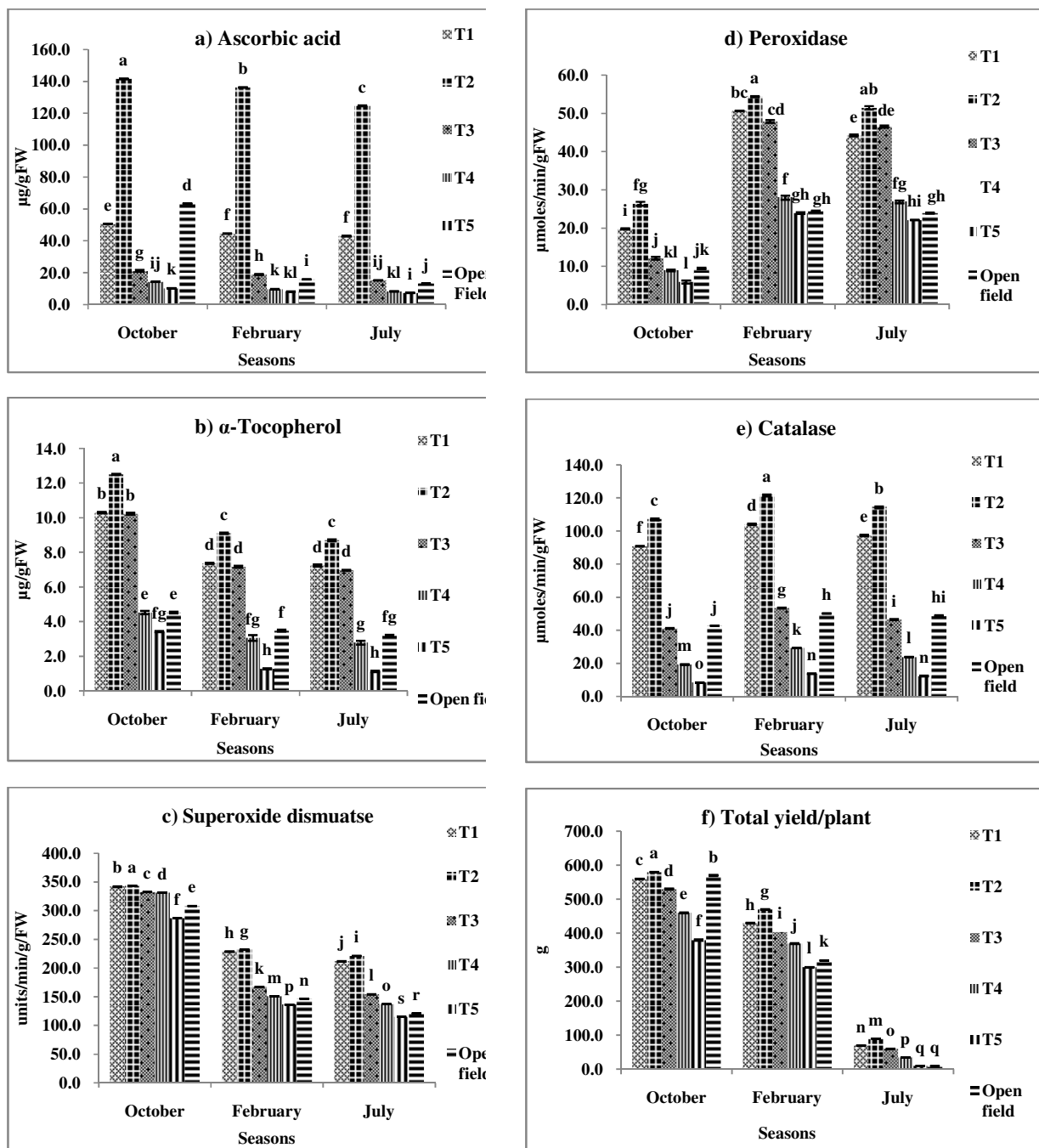


Figure 3. Effects of different $\text{NO}_3^-:\text{NH}_4^+$ on (a) Ascorbic acid content (b) Alpha-tocopherol content (c) SOD activity (d) POD activity (e) CAT activity (f) Total yield/plant T1 ($\text{NO}_3^-:\text{NH}_4^+ :: 100:0$), T2 ($\text{NO}_3^-:\text{NH}_4^+ :: 75:25$), T3 ($\text{NO}_3^-:\text{NH}_4^+ :: 50:50$), T4 ($\text{NO}_3^-:\text{NH}_4^+ :: 25:75$) T5 ($\text{NO}_3^-:\text{NH}_4^+ :: 0:100$).

is significantly affected by the temperature and total available heat and also by the amount and intensity of light.

Antioxidants activity:

The main season sown crop showed more SOD activity than off season sown crop (Fig.3.c). Irrespective of the season, SOD activity of plants supplied with nutrient solution T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) was significantly higher than the other treatments. Similarly POD activity was maximum in the plants supplied with treatment T2 ($\text{NO}_3^-:\text{NH}_4^+::75:25$) in the nutrient solution (Fig.3.d). But unlike activity of SOD, the activity of POD in off season sown crop was more than that of main season sown crop. CAT activity (Fig.3.e) in lettuce leaves followed the same trend as POD activity. Minimum SOD, POD and CAT activity among all the treatments recorded in the plants supplied with nutrient solution T5 ($\text{NO}_3^-:\text{NH}_4^+::0:100$). Antioxidants activity in polyhouse grown crop was higher than the soil grown crop Luna et al. (2013) and Sofo et al. (2016) have suggested that antioxidant properties of lettuce may be affected by the fertilizers applied and growing systems, while Fallovo et al. (2011) have suggested that apart from nutrient solution composition, growing season may also affect chemical composition of lettuce.

Total yield per plant: Among the seasons, main season (October) sown crop had the higher yield than off season (February and July) sown crop. Irrespective of the season, yield of polyhouse grown plants supplied with nutrient solution T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) was more than soil grown crop (Fig. 3.f). The yield enhancement in polyhouse grown crop supplied with treatment T2 ($\text{NO}_3^-/\text{NH}_4^+::75:25$) over open field crop was 1.75%, 46.88% and 800.00% respectively in October, February and July sown crops. Similar biomass enhancement in crops supplied with nutrient solution having NO_3^- to NH_4^+ in the ratio of 75:25 have been reported in lettuce (Wang and Shen, 2011), pepper (Bar-Tal et al., 2001) and French beans (Guo et al., 2002).

Conclusion: In conclusion, in main season as well as in off season sown lettuce crop, the yield was significantly more in polyhouse 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 $\text{NO}_3^-:\text{NH}_4^+$ improves the yield and biochemical traits in polyhouse grown lettuce. It is therefore suggested that $\text{NO}_3^-/\text{NH}_4^+$ ratios of 75:25 could promote growth, increase yield and improve the biochemical traits in lettuce.

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Title of Ph. D's Dissertation : Effect of nitrate and ammonium ratios on
physiological and quality attributes of
hydroponically grown leafy vegetables.
Awards/Distinctions/Fellowships/Scholarships : Recipient of University Merit fellowship
Ph.D (Botany) (2017-2018)
: Award of honour for highest OCPA in
Master's programme for the session
2014-2015.
: Recipient of University Merit fellowship
MSc. (Botany) (2013-2015)
: Awarded with Gold medal (Shri Puran
Anand Adlakha Medal) in M.Sc.
: Award of honour for securing highest
OCPA in B.Sc. for the year 2012 and
2013.
: ICAR- ARS-NET (2017) qualified