

## Full Length Research Paper

# Yield and quality of garden cress affected by different nitrogen sources and growing period

Özlem Tuncay<sup>1\*</sup>, Dursun E iyok<sup>1</sup>, Bülent Ya mur<sup>2</sup> and Bülent Okur<sup>2</sup><sup>1</sup>Ege University, Faculty of Agriculture, Department of Horticulture, 35100, Izmir-Turkey.<sup>2</sup>Ege University, Faculty of Agriculture, Department of Soil Sciences, 35100, Izmir-Turkey.

Accepted 10 April, 2019

The effects of different months of the year and nitrogen sources on garden cress (*Lepidium sativum* L.) yield, quality and nitrate accumulation were investigated during the years 2002 and 2003. In both years, seeds were sown on the first days of September, October, November, January, February and March. Three different nitrogen sources were used: Farmyard (cattle) manure (100 ton·ha<sup>-1</sup>), Ca(NO<sub>3</sub>)<sub>2</sub>-15.5% N (150 kg N·ha<sup>-1</sup>) and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-21% N (150 kg N·ha<sup>-1</sup>). Yield, leaf color, dry matter, vitamin C, total glucosinolate content and nitrate accumulation were assessed. No interaction between sowing date and nitrogen form was observed for any of the assessed parameters. Growing period affected all parameters significantly. Plants obtained from January and February sowings resulted in better yield, leaf color, dry matter and vitamin C content. But the nitrate contents also increased. Highest total glucosinolate content was observed during the warmer months. Nitrate nitrogen application increased yield, leaf greenness, vitamin C and nitrate content while farmyard manure application raised dry matter and total glucosinolate contents. Nitrate content of the garden cress plants did not exceed 391 mg kg<sup>-1</sup> fresh weight, which is below the accepted daily intake of 3.7 mg nitrate per kg<sup>-1</sup> bodyweight set by European Commission's Scientific Committee on Food.

**Key words:** *Lepidium sativum* L., farmyard manure, nitrate, glucosinolate, vitamin C.

## INTRODUCTION

Fresh fruits and vegetables are an important element of human nutrition. World Health Organisation Panel on Diet, Nutrition and Prevention of Chronic Diseases recommended an individual intake of at least 400 g or five servings of fruits and vegetables every day (WHO, 2003). Several epidemiological studies showed that there is an inverse relationship between the consumption of fruits and vegetables and cancer and cardiovascular diseases (World Cancer Research Fund, 1997). Recent research suggests that vegetables are more effective compared to fruits in preventing chronic diseases such as cancer, stroke (Su and Arab, 2006), cardiovascular diseases (Cox et al., 2000) and non-insulin dependent diabetes (Williams et al., 1999). Raw vegetables are theoretically more beneficial because of the better preserved vitamins and phytochemicals. With the consumption of salads and raw vegetables, it is possible

to meet daily vitamin C, E, B-6 and folate recommendations (Su and Arab, 2006).

Since people are encouraged to increase their salad consumption, there is also a need to diversify the vegetables to be used in salads. Adams et al. (2005) showed that variety in salads tend to increase the salad intake in elementary school students. Recently, garden cress (*Lepidium sativum* L.), a member of Brassicaceae, has gained more interest from consumers and producers (Zhan et al., 2009), and can be a good choice for salads with its peppery taste, and health promoting substances such as glucotropaeolin, a glucosinolate compound and the precursor of benzyl isothiocyanate (Kassie et al., 2002) and sterols (Conforti et al., 2009).

Several pre-harvest factors including climatic conditions and available nutrients affect the yield and quality of vegetables (Lee and Kader, 2000). The yield declining effect of sub and supraoptimal temperatures in leafy vegetables was reported by different authors (Pavlou et al., 2007; Stagnari et al., 2007; Dufault et al., 2009). Temperature also affects the vitamin and phytochemical content of plants. Vitamin C content of broccoli (*Brassica*

\*Corresponding author. E-mail: [Ozlem.tuncay@ege.edu.tr](mailto:Ozlem.tuncay@ege.edu.tr).  
Tel/Fax: +90 232 388 1865

*oleracea* L. var. *Italica*) tends to increase under low temperature stress (Schonhof et al., 2007; Lee and Kader, 2000). High temperatures are reported to increase total glucosinolate (T-GLS) content of different Brassica crops (Cartea and Velasco, 2008).

Nitrogen is an important component of many structural, genetic and metabolic compounds such as chlorophyll and amino acids in plant cells. Nitrogen deficiency is a limiting factor for plant growth. But the excessive use of nitrogen fertilizers in order to guarantee yield, increases the risk of nitrate accumulation, since accumulation of nitrate in plants occurs when the nitrate uptake exceeds its reduction and assimilation. Although there are conflicting views on the long-term health risks of nitrate intake (L'hirondel and L'hirondel, 2002; Addiscott, 2005; Powlson et al., 2008), reducing dietary nitrate intake as a preventive measure is advisable (Santamaria, 2006).

Several factors such as plant species, the dose and form of nitrogen, nitrification, availability of other nutrients, climatic conditions and water availability affect nitrate accumulation (Umar and Iqbal, 2007; Santamaria, 2006). The most studied factor is the nitrogen fertilization, especially the rate. Nitrogen form was also studied by several authors; some of the studies were conducted in soilless culture systems (Zhang et al., 2007; Kim et al., 2006; Santamaria et al., 1999; 1998b; Elia et al., 1998) and others in soil (Stagnari et al., 2007; Wang et al., 2008; Wang and Li, 2003). The use of nitrogen in ammonium form decreased nitrate content, due to the higher availability of nitrogen in nitrate based fertilizers (Umar and Iqbal, 2007). Organic fertilizers also reduce nitrate accumulation (Zhou et al., 2000).

Temperature and light intensity also affect nitrate content of plants. Nitrate reductase activity increases in high light intensity conditions, and therefore decreases nitrate accumulation (Umar and Iqbal, 2007). Santamaria et al. (2001) reported an interaction between light intensity, temperature and nitrogen availability in rocket salad (*Eruca sativa* Mill.). Even low nitrogen availability increases the nitrate content of rocket salad in low light and high temperature conditions, while in high light intensity and temperature conditions, only high doses of nitrogen cause an increase in nitrate content.

The aim of the present research was to study the effects of farmyard manure and two mineral fertilizers on garden cress yield and quality grown through autumn to spring.

## MATERIALS AND METHODS

### Plant material and growing conditions

The study was carried out in the experimental fields of Ege University, Faculty of Agriculture, Department of Horticulture, Izmir (38°27' N; 27°13' E; 26 m above sea level, sandy clay loam) during the years 2002 and 2003. Climatic conditions during the experiment are given in Figures 1a and b. Garden cress (*L. sativum* L.) seeds (1 g m<sup>-2</sup>) were sown to 2 m<sup>2</sup> beds with a 10 cm space between rows

to give a final number of ca. 450 plants m<sup>2</sup> on the first days of each month from January to March and September to November in both years. Prior to sowing, different nitrogen (N) sources, namely, farmyard (cattle) manure (100 ton·ha<sup>-1</sup>), Ca(NO<sub>3</sub>)<sub>2</sub> (150 kg N·ha<sup>-1</sup>) and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (150 kg N·ha<sup>-1</sup>), were uniformly mixed with the topsoil. Phosphorus (120 kg P<sub>2</sub>O<sub>5</sub>·ha<sup>-1</sup>, as triple superphosphate) and potassium (180 kg K<sub>2</sub>O·ha<sup>-1</sup>, as K<sub>2</sub>SO<sub>4</sub>) were also applied in the case of chemical nitrogen source. The properties of the soil and farmyard manure are given in Table 1. Irrigation, weeding and chemical spraying were applied when needed.

### Harvest and sample preparation

All plants in beds were hand harvested by cutting the plants as close to the soil as possible, when 90% of the plants reached to 7 to 10 leaf stage (Table 2). The plants were put in plastic bags and weighed to determine the yield, and brought to laboratory in cool boxes, where they were washed first with tap water, then twice with deionized water. Excess water was removed with a domestic salad spinner.

### Quality determination

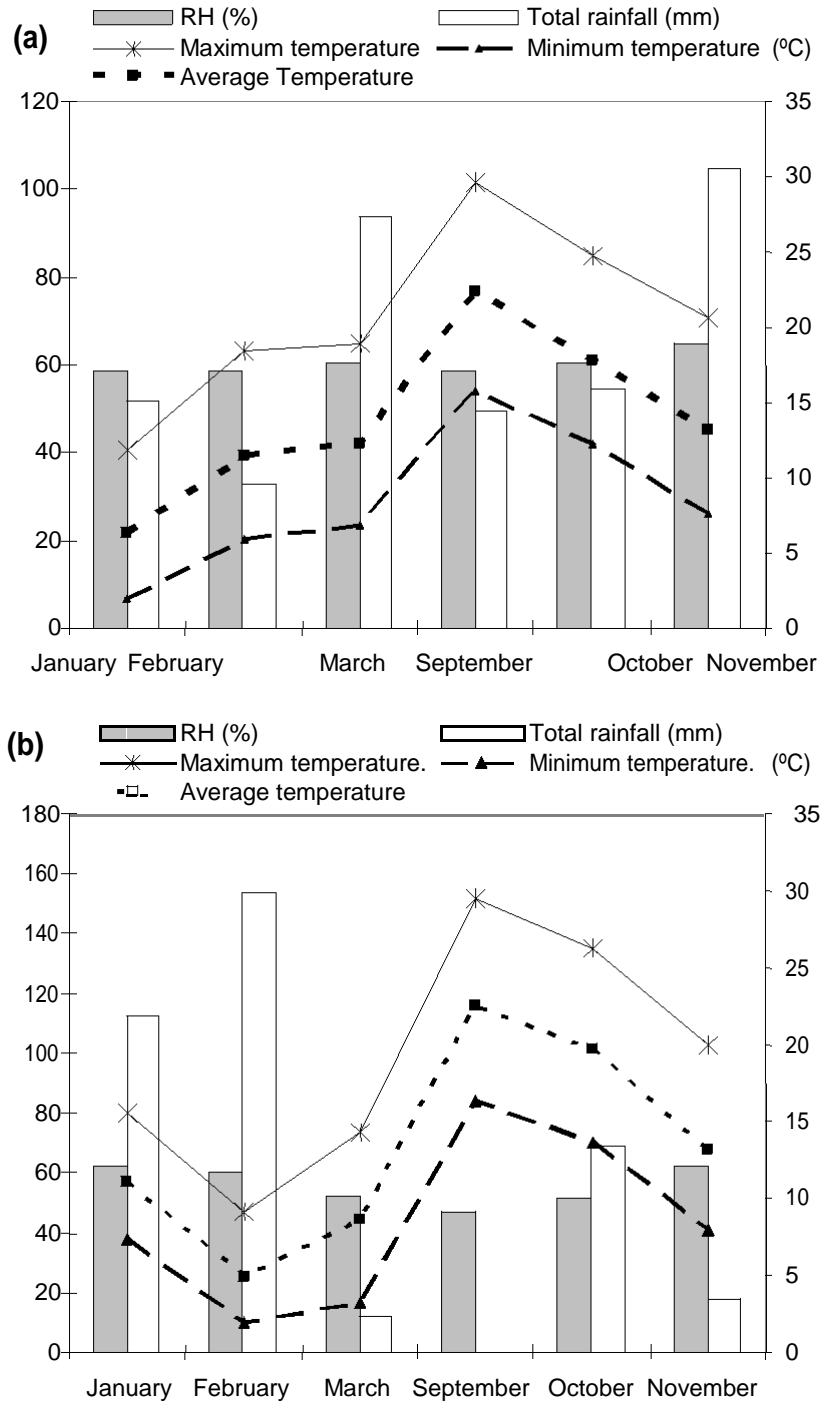
Leaf color was measured with a Minolta CR 300 Colorimeter (Minolta, Japan) as CIE (Commission Internationale De L'eclairage) L\*a\*b\*. Colorimeter was calibrated with the standard white tile prior to measurements. A minimum of 20 plants were measured for each treatment, and the color was characterized by lightness (L\*), Hue angle (H°=tan<sup>-1</sup>(b\*/a\*)) and chroma.

Dry matter was determined by drying the samples in an oven at 65°C until constant weight was obtained.

Vitamin C was determined according to Pearson (1970) colorimetrically with some modifications. 25 g of garden cress leaves were homogenized in a Waring Blender (Waring Products Inc., Connecticut, USA) with 100 ml of 0.4% aqueous oxalic acid solution, filtrated through Whatman No 1 paper and centrifuged for 10 min at 7000 rpm. The centrifuged solution was diluted 10 folds with 0.4% oxalic acid. 1 ml of sample was mixed with 9 ml 2,6 dichlorophenol-indophenol (Merck KGaA, Germany), and immediately the absorbance was measured with a spectrophotometer (Varian Cary Bio 100, Australia) at 518 nm against the solution of sample mixed with distilled water (1:10). Standard curve was constructed by using L(+) ascorbic acid (Merck KGaA, Germany) solutions with known concentrations, and vitamin C content of the samples calculated against the standard curve.

Total glucosinolate content was determined according to the method described by Szmigielska et al. (2000). Total glucosinolates were extracted by adding ca. 50 ml of boiling water to 10 g of fresh sample. Samples were boiled in a waterbath at 95°C for another 20 min, cooled, made up to volume and centrifuged at 7000 rpm for 10 min, and the supernatant was filtered through 0.45 µm Millipore filters. 200 µl of the filtrate was mixed with 3.80 ml of freshly prepared sodium tetrachloropalladate (Sigma-Aldrich Chemie, Germany) solution (0.588 mg L<sup>-1</sup> in water). The mixture was left for 30 min at room temperature for the reaction, and the absorbance was measured at 450 nm with Cary 100 UV/Vis spectrophotometer. A standard curve was constructed with known concentrations of Sinigrin Monohydrate (Sigma-Aldrich Chemie, Germany), and total glucosinolate content of samples were calculated against the standard curve.

Nitrate content of fresh leaves was determined colorimetrically according to the method of Balke and Reekers (1955). 5 g of fresh leaves were homogenized in a Waring Blender with 50 ml of distilled water, transferred to 100 ml volumetric flasks, made up to volume and filtered through white ribbon filter paper. 2.5 ml of the filtrate was transferred to a 100 ml volumetric flask and 0.1 g 2,4



**Figure 1.** Climatic conditions during (a) 2002 and (b) 2003.

xylene and 22.5 ml 90% sulphuric acid (both from Sigma-Aldrich Chemie, Germany) added. 15 min later, 60 ml of deionized water was added. After the solution was cooled, it was transferred to a separation funnel and 30 ml of diethylether (Sigma-Aldrich Chemie, Germany) added, mixed well, and diethylether phase was discarded. 10 ml of 5% NaOH (Sigma-Aldrich Chemie, Germany) was added to the remaining phase, shaken by hand for 1 min and the absorbance value of the colored phase was measured at 433 nm. Nitrate content was calculated against the standard curve constructed by known concentrations of potassium nitrate (Sigma-

Aldrich Chemie, Germany).

#### Statistical analysis

Data were subjected to analysis of variance according to a split-plot design with three replicates (main plot: sowing time; subplot: nitrogen sources) by SPSS v11 for Windows (SPSS Inc., USA), and significantly different means were separated by Duncan's multiple range test ( $P=0.05$ ).

**Table 1.** Some properties of the soil and farmyard (cattle) manure used in the experiments.

Soil		Farmyard (Cattle) manure	
Soil texture	Sandy clay loam	pH	9.10
pH	7.36	Total soluble salts (%)	1.92
Total soluble salts (%)	0.059	Organic matter (%)	34.13
CaCO <sub>3</sub> (%)	3.60	Total C (%)	19.80
Organic matter (%)	2.06	Total N (%)	1.11
Total N (%)	0.100	C/N	17.84
Available P (mg•kg <sup>-1</sup> )	4.2	Total P (%)	0.76
Available K (mg•kg <sup>-1</sup> )	460	Total K (%)	1.50
Available Ca(mg•kg <sup>-1</sup> )	3750	Total Ca (%)	7.47
Available Mg (mg•kg <sup>-1</sup> )	56	Total Mg (%)	0.76
Available Fe (mg•kg <sup>-1</sup> )	52	Total Na (%)	0.18
Available Cu (mg•kg <sup>-1</sup> )	4.60	Total Cu (mg kg <sup>-1</sup> )	1.62
Available Zn (mg•kg <sup>-1</sup> )	0.90	Total Zn (mg kg <sup>-1</sup> )	294
Available Mn (mg•kg <sup>-1</sup> )	26.00	Total Mn (mg kg <sup>-1</sup> )	93.10
Total S (mg•kg <sup>-1</sup> )	1630.91	Total S (mg kg <sup>-1</sup> )	742.02

**Table 2.** Days needed after sowing garden cress seeds to reach the 7 to 10 leaf stage during the experiment.

Growing periods	Days after sowing	
	2002	2003
January	56	51
February	48	62
March	47	59
September	30	29
October	38	36
November	45	48

## RESULTS

Growing period by nitrogen source interaction did not have a statistically significant effect on any of the assessed parameters in both years of the experiment. Growing period affected yield significantly ( $P < 0.001$ ), and although yield was slightly higher in 2003, a similar pattern was observed in both years (Tables 3 and 4). The highest yields were obtained in February and January, followed by March and November. The lowest yielding months were September and October. The yield average of January and February was 30% higher than the average of March and November, and 45% higher than the average of September and October. The effect of nitrogen source was also statistically significant ( $P < 0.05$ ).

Highest yield was obtained when  $\text{NO}_3^-$ -N was applied, although not differing from yield obtained when  $\text{NH}_4^+$ -N was applied; farmyard manure gave the lowest yield, although not significantly different from yield obtained when  $\text{NH}_4^+$ -N was applied.  $\text{NO}_3^-$ -N application increased

yield by 18% compared to farmyard manure.

In both years, similar leaf color values were observed and all the color parameters were affected by growing period ( $P < 0.001$ ) (Tables 3 and 4). Garden cress grown in March and September had lighter colored leaves compared to the other months. They had higher lightness and chroma values, which indicates a lighter and more vivid color, and although not statistically significant different in March of 2002, lower hue<sup>o</sup> values, the indication of a more yellowish-green color. When all color parameters considered together, plants grown in January and November had darker green leaves. Nitrogen source did not have a statistically significant effect on leaf lightness values, but the effect on hue<sup>o</sup> and chroma was significant ( $P < 0.05$  and  $P < 0.01$ , respectively). The plants grown with farmyard manure had yellowish-green leaves compared to those grown with other fertilizers. Farmyard manure application resulted

brighter colored leaves compared to  $\text{NO}_3^-$ -N application.

Both growing period and nitrogen source affected the dry matter content of garden cress significantly ( $P < 0.001$  and  $P < 0.05$ , respectively) (Tables 5 and 6). The dry matter content changed between 8.24 and 11.12% in 2002, and between 8.09 and 10.91% in 2003. Plants grown in colder months had higher dry matter content compared to others. The highest dry matter content was observed in January and decreased with the increasing temperature in both years. In comparison to  $\text{NO}_3^-$ -N application, the use of farmyard manure increased dry matter content by 14.5%. There are researches reporting that nitrogen source neither affects dry matter content and February nor from plants grown in September, October and November. Farmyard manure application

**Table 3.** Changes in yield, color and dry matter of garden cress during 2002.

Growing period	Yield (g m <sup>-2</sup> )	Lightness	Hue <sup>o</sup>	Chroma
January	3180.5 <sup>a1</sup>	46.46 <sup>c</sup>	139.0 <sup>a</sup>	31.5 <sup>c</sup>
February	3223.0 <sup>a</sup>	48.16 <sup>bc</sup>	131.9 <sup>bc</sup>	34.6 <sup>b</sup>
March	2484.8 <sup>b</sup>	51.61 <sup>a</sup>	130.7 <sup>ab</sup>	36.4 <sup>ab</sup>
September	1692.3 <sup>c</sup>	52.24 <sup>a</sup>	132.3 <sup>b</sup>	36.8 <sup>a</sup>
October	1711.6 <sup>c</sup>	48.79 <sup>b</sup>	133.6 <sup>b</sup>	35.0 <sup>ab</sup>
November	2453.3 <sup>b</sup>	47.05 <sup>bc</sup>	140.7 <sup>a</sup>	31.9 <sup>c</sup>
Significance <sup>2</sup>	***	***	***	***
Nitrogen source				
Farmyard manure	2260.3 <sup>b</sup>	49.50	132.2 <sup>b</sup>	35.4 <sup>a</sup>
NH <sup>+</sup> <sub>4</sub> -N	2438.8 <sup>ab</sup>	49.36	136.0 <sup>a</sup>	34.5 <sup>ab</sup>
NO <sup>-</sup> <sub>3</sub> -N	2673.6 <sup>a</sup>	48.30	136.0 <sup>a</sup>	33.2 <sup>b</sup>
Significance	*	n.s.	*	**

<sup>1</sup>Means separated with different letters in columns differ significantly according to Duncan's multiple range test.

<sup>2</sup>\*, \*\*, \*\*\* statistically significant at P<0.05, P<0.01 and P<0.001 levels, respectively.

**Table 4.** Changes in yield, color and dry matter of garden cress during 2003.

Growing period	Yield (g m <sup>-2</sup> )	Lightness	Hue <sup>o</sup>	Chroma
January	3265.1 <sup>a1</sup>	46.4 <sup>c</sup>	139.3 <sup>a</sup>	30.9 <sup>c</sup>
February	3299.6 <sup>a</sup>	48.1 <sup>bc</sup>	132.2 <sup>b</sup>	33.8 <sup>b</sup>
March	2514.9 <sup>b</sup>	51.5 <sup>a</sup>	130.9 <sup>b</sup>	35.5 <sup>ab</sup>
September	1731.4 <sup>c</sup>	52.1 <sup>a</sup>	132.6 <sup>b</sup>	36.0 <sup>a</sup>
October	1755.6 <sup>c</sup>	48.7 <sup>b</sup>	133.9 <sup>b</sup>	34.2 <sup>ab</sup>
November	2541.4 <sup>b</sup>	47.0 <sup>bc</sup>	140.3 <sup>a</sup>	31.3 <sup>c</sup>
Significance <sup>2</sup>	***	***	***	***
Nitrogen source				
Farmyard manure	2315.2 <sup>b</sup>	49.26	132.5 <sup>b</sup>	34.7 <sup>a</sup>
NH <sup>+</sup> <sub>4</sub> -N	2499.6 <sup>ab</sup>	49.42	135.9 <sup>a</sup>	33.8 <sup>ab</sup>
NO <sup>-</sup> <sub>3</sub> -N	2739.2 <sup>a</sup>	48.19	136.2 <sup>a</sup>	32.4 <sup>b</sup>
Significance	*	Ns	*	**

<sup>1</sup>Means separated with different letters in columns differ significantly according to Duncan's multiple range test.

<sup>2</sup>\*, \*\*, \*\*\* statistically significant at P<0.05, P<0.01 and P<0.001 levels, respectively.

(Santamaria et al., 1998a; Kopsell et al., 2007; Kim et al. 2006; Elia et al., 1998), and the increase in dry matter in organic products (Magkos et al., 2003), which is in accordance with our results.

The effect of growing period on vitamin C content of garden cress leaves was statistically significant in both years, ranging between 0.669 and 0.892 mg g<sup>-1</sup> FW (fresh weight), in 2002, and 0.664 and 0.879 mg g<sup>-1</sup> FW, in 2003 (Tables 5 and 6). Nitrogen source also affected vitamin C content significantly (P<0.05). NO<sup>-</sup><sub>3</sub>-N resulted in a slight increase in vitamin C content compared to farmyard

manure application, both years.

In 2002, total glucosinolate content of garden cress leaves changed between 9.97 and 12.79 mmol kg<sup>-1</sup> DW (dry weight), and both the effects of growing period and nitrogen source were statistically significant (P<0.01 and P<0.05, respectively) (Table 5). The highest total glucosinolate content was obtained in September, October and November. Plants grown in January and February had the lowest total glucosinolate content. Plants grown in March had total glucosinolate content not statistically different neither from plants grown in January. In the second year of the experiment, total glucosinolate

**Table 5.** Changes in vitamin C, total glucosinolate and nitrate content of garden cress during 2002.

Growing period	Dry matter (%)	Vitamin C (mg g <sup>-1</sup> FW)	Total glucosinolates (mmol kg <sup>-1</sup> DW)	Nitrate (mg kg <sup>-1</sup> FW)
January	11.12 <sup>a1</sup>	0.882 <sup>a</sup>	9.97 <sup>b</sup>	391.36 <sup>a</sup>
February	10.98 <sup>ab</sup>	0.892 <sup>a</sup>	10.03 <sup>b</sup>	324.21 <sup>c</sup>
March	9.52 <sup>bc</sup>	0.778 <sup>ab</sup>	11.00 <sup>ab</sup>	349.72 <sup>b</sup>
September	8.35 <sup>c</sup>	0.669 <sup>c</sup>	12.79 <sup>a</sup>	253.90 <sup>f</sup>
October	8.24 <sup>c</sup>	0.676 <sup>c</sup>	12.76 <sup>a</sup>	269.64 <sup>e</sup>
November	9.64 <sup>abc</sup>	0.770 <sup>ab</sup>	12.53 <sup>a</sup>	306.77 <sup>d</sup>
Significance <sup>2</sup>	***	**	**	***
Nitrogen source				
Farmyard manure	10.28 <sup>a</sup>	0.706 <sup>b</sup>	12.37 <sup>a</sup>	309.61 <sup>b</sup>
NH <sup>+</sup> <sub>4</sub> -N	9.66 <sup>ab</sup>	0.801 <sup>ab</sup>	11.80 <sup>ab</sup>	313.16 <sup>b</sup>
NO <sup>-</sup> <sub>3</sub> -N	8.98 <sup>b</sup>	0.827 <sup>a</sup>	10.62 <sup>b</sup>	325.04 <sup>a</sup>
Significance	*	*	*	**

<sup>1</sup>Means separated with different letters in columns differ significantly according to Duncan's Multiple Range test.

<sup>2</sup>\*, \*\*, \*\*\* statistically significant at P<0.05, P<0.01 and P<0.001 levels, respectively.

**Table 6.** Changes in vitamin C, total glucosinolate and nitrate content of garden cress during 2003.

Growing period	Dry matter (%)	Vitamin C (mg g <sup>-1</sup> FW)	Total glucosinolates (mmol kg <sup>-1</sup> DW)	Nitrate (mg kg <sup>-1</sup> FW)
January	10.91 <sup>a1</sup>	0.879 <sup>a</sup>	10.53 <sup>b</sup>	382.42 <sup>a</sup>
February	10.79 <sup>ab</sup>	0.814 <sup>ab</sup>	9.76 <sup>b</sup>	342.39 <sup>b</sup>
March	9.31 <sup>bc</sup>	0.763 <sup>ab</sup>	9.72 <sup>b</sup>	317.31 <sup>c</sup>
September	8.09 <sup>c</sup>	0.664 <sup>c</sup>	13.39 <sup>a</sup>	249.65 <sup>e</sup>
October	8.18 <sup>c</sup>	0.691 <sup>c</sup>	11.21 <sup>ab</sup>	263.94 <sup>e</sup>
November	9.45 <sup>abc</sup>	0.709 <sup>c</sup>	11.02 <sup>ab</sup>	301.14 <sup>d</sup>
Significance <sup>2</sup>	***	*	*	***
Nitrogen source				
Farmyard manure	10.10 <sup>a</sup>	0.681 <sup>b</sup>	11.69	303.27 <sup>b</sup>
NH <sup>+</sup> <sub>4</sub> -N	9.47 <sup>ab</sup>	0.778 <sup>ab</sup>	10.96	306.47 <sup>b</sup>
NO <sup>-</sup> <sub>3</sub> -N	8.80 <sup>b</sup>	0.802 <sup>a</sup>	10.17	318.69 <sup>a</sup>
Significance	*	*	ns	*

<sup>1</sup>Means separated with different letters in columns differ significantly according to Duncan's multiple range test.

<sup>2</sup>\*, \*\*, \*\*\* statistically significant at P<0.05, P<0.01 and P<0.001 levels, respectively.

content of garden cress leaves was only affected by growing period (P<0.05) (Table 6). The highest total glucosinolate content was obtained in September, followed by October and November, although they did not statistically differ from the other months.

The effect of growing period on nitrate content was statistically significant in both years (P<0.001). Nitrate accumulation was higher during the colder months, and decreased with increasing temperatures. NO<sup>-</sup><sub>3</sub>-N application increased nitrate accumulation in both years, but

the increase in nitrate accumulation when NH<sup>+</sup><sub>4</sub>-N used was not statistically different from farmyard manure. The use of NO<sup>-</sup><sub>3</sub>-N increased the nitrate content of leaves 4 and 5% in comparison to NH<sup>+</sup><sub>4</sub>-N and farmyard manure, respectively.

## DISCUSSION

In our study, we found that the vegetative growth and dry

matter content of garden cress plants were better in colder months compared to warmer months, and yield declined with the increasing temperature. Since garden cress is reported to be a cool season plant and to prefer temperatures below 20°C (Munro and Small, 1997), this is not a surprising result.

Many research work reports that leafy vegetables prefer nitrogen in nitrate form, and the use of  $\text{NO}_3^-$ -N promotes growth, therefore increase yield (Wang and Li, 2003; Stagnari et al., 2007). There are also articles on yield decrease in leafy vegetables with the use of organic fertilizers (usti et al., 2003; Stagnari et al., 2007).

Similarly in our experiment,  $\text{NO}_3^-$ -N application increased yield compared to farmyard manure, both years. Plants with shorter growing cycles like garden cress may benefit more from  $\text{NO}_3^-$ -N fertilization, since nitrogen is readily available. Also during some months of our experiment, low temperature conditions were unfavorable for nitrogen in the soil mineralization, therefore the effect of  $\text{NO}_3^-$ -N was more pronounced. Although the temperatures were higher during September and October, and therefore should increase nitrogen mineralization and the yield of farmyard manure applied plots, these conditions were not suitable for garden cress production, and yield declined considerably.

León et al. (2007) reported that leaf lightness and hue<sup>0</sup> values are strongly correlated with the leaf chlorophyll content in butter head lettuce (*Lactuca sativa* cv. Lores). Madeira et al. (2003) also reported a strong relationship with chlorophyll content and chroma and hue<sup>0</sup> values in sweet pepper (*Capsicum annum* L., cv 'Capistrano') leaves. Both research groups reported that with the increasing concentrations of chlorophyll, lightness and chroma values decreased and hue<sup>0</sup> values increased. Based on our leaf color results, it can be said that garden cress plants grown in January and November had more chlorophyll content, since they had darker colored leaves compared to other months. It seems that when the days are short and light intensity is low, plants had darker colored leaves, which is in accordance with the results of Naidu and DeLucia (1998), who reported that leaves of *Quercus* spp. plants grown in shade conditions had more total chlorophyll.

Our results showed that leaf color parameters, especially hue<sup>0</sup> and chroma values, were affected by nitrogen form. Mineral fertilizers produced darker colored leaves compared to farmyard manure; and although not statistically significant, lightness values showed a similar trend. Leaf nitrogen concentration is directly related to leaf chlorophyll content, therefore to leaf greenness (Chapman and Barreto, 1997), as a result, there are several researches on the prediction of crop nitrogen status via chlorophyll measurements or analysis (Sandoval-Villa et al., 1999; Shaahan et al., 1999; Sandoval-Villa et al., 2002; Westerveld et al., 2004; Liu et

al., 2006). However, there are not many researches about the effect of nitrogen form. Kopsell et al. (2007) reported that kale (*Brassica oleracea* L. var. *acephala* DC) varieties responded differently to the increasing  $\text{NO}_3^-$ -N in terms of chlorophyll a concentrations, and, similar to Sandoval-Villa et al. (2002) results, the highest

chlorophyll a concentration was obtained with 25%  $\text{NH}_4^+$ -

N:75%  $\text{NO}_3^-$ -N. Since our experiment was conducted in soil conditions, it is not possible to make such clear distinctions; but, mineral fertilizers, probably because of the faster uptake of nitrogen, resulted in darker green colored leaves, which might be due to the increased chlorophyll content.

The vitamin C content of garden cress in our study is quite high compared to Zhan et al. (2009), who reported a total vitamin C content of 33.52 mg 100 g<sup>-1</sup> FW. Compared to other salad greens such as lettuce, garden cress is a rich source of vitamin C with its content of 50 to 80 mg 100 g<sup>-1</sup>, which may provide almost half of the recommended dietary allowance (Cahill et al., 2009).

$\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N applications increased the vitamin C content compared to farmyard manure slightly. Similar to yield, plants benefited more from  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N

applications in terms of vitamin C content, probably because of the higher availability of nitrogen. Excessive nitrogen fertilization was reported to decrease vitamin C content in several crops (Lee and Kader, 2000). Although the nitrogen dose used in this experiment is not low (150 kg N ha<sup>-1</sup>), it seems that this level of nitrogen is not very high for garden cress grown in soil conditions. There are conflicting results on the effect of organic fertilizers on vitamin C content. It is reported to increase, decrease or not affect the vitamin C content of vegetables (Worthington, 2001; Magkos et al., 2003; Premuzic et al., 2004; Toor et al., 2006; Wunderlich et al., 2007; Zahradník and Petíková, 2007). In our study, the low vitamin C content of garden cress grown with farmyard manure could be due to the low nitrogen mineralization during colder months, hence lower availability of nitrogen in the soil.

Climatic conditions have been reported to affect the total glucosinolate content of plants, of which temperature and day light are two of the most important (Rosa and Rodrigues, 1998; Ciska et al., 2000; Vallejo et al., 2003; Charron et al., 2005; Radovich et al., 2005; Schonhof et al., 2007). It is reported that plants tend to accumulate more glucosinolates at higher temperatures (Charron et al., 2005; Cartea and Velasco, 2008). In our experiment, high temperatures during September, October and November (Figure 1) resulted an increase in total glucosinolate content. Although the temperatures were quite high for garden cress during September, which caused a decline in yield, being higher than the optimum might be the cause of the increase in total glucosinolate. Since supraoptimal temperatures has been reported to

increase the total GLS content (Ciska et al., 2000; Vallejo et al., 2003; Charron et al., 2005; Radovich et al., 2005), high maximum temperatures in September, as well as longer days, might have increased total GLS content.

Nitrogen and sulfur fertilization are also known to affect the total glucosinolate content. Schonhof et al. (2007) reported that broccoli plants should be supplied with sufficient sulfur and nitrogen in order to obtain greater amounts of glucosinolates. In low nitrogen conditions (1 g N plant<sup>-1</sup>), sulfur fertilization did not have a significant effect on glucosinolate content, but at optimal nitrogen availability (4 g N plant<sup>-1</sup>), additional sulfur application, especially in sulfur deficit soils, increased the total glucosinolate content. Similarly, a linear increase in glucosinolate content with sulfur fertilization was also reported by Kopsell et al. (2003), who compared the effects of sulfur fertilization (4, 8, 16, 32 and 64 mg S L<sup>-1</sup>) on three different kale cultivars (Winterbor, Redbor, Toscano). Our results showed similar tendencies. Although there was no statistically significant difference between ammonium sulfate and calcium nitrate, a small increase in the total glucosinolate content was observed in 2002 when plants were grown with farmyard manure and ammonium sulfate. Since an additional amount of sulfur was applied with farmyard manure (Table 1) and ammonium sulfate, the higher total glucosinolate content can be expected. But the high sulfur content of the soil (Table 1) in the experimental area may be the cause of the slight increase in total glucosinolate content. De Pascale et al. (2007) suggested that if the sulfur content of the soil is high enough to saturate plant requirements, additional sulfur fertilization may not affect the glucosinolate content.

The effect of nitrogen form on nitrate accumulation in different leafy vegetables, such as rocket salad, lettuce, spinach and garden cress, was studied by several authors (Santamaria et al., 1998a, 1998b; Kim et al., 2006; Pavlou et al., 2007; Stagnari et al., 2007; Zhang et al., 2007; Fontana and Nicola, 2008). Highest nitrate accumulation was caused by NO<sub>3</sub><sup>-</sup>-N, followed by NH<sub>4</sub><sup>+</sup>-N, and manure (Wang and Li, 2003; Pavlou et al., 2007; Stagnari et al., 2007), which is in accordance with our results. Faster uptake of nitrogen in NO<sub>3</sub><sup>-</sup>-N fertilizer causes an accumulation in leaves. Our results confirmed that climatic conditions, especially photoperiod, also affects nitrate accumulation. Increased light conditions cause an increase in nitrate reductase activity, and there is a negative correlation between photosynthetic activity and nitrate accumulation (Pavlou et al., 2007). Lowest nitrate content was observed during September and October, when the days were longest.

Garden cress was reported to be a high nitrate accumulating plant, and Fontana and Nicola (2008) reported nitrate contents varying between 119 and 4040 mg·kg<sup>-1</sup> FW for young garden cress grown in soilless culture with different nitrogen rates, forms and

regimes. In our experiment, although both growing period and nitrogen source affected the nitrate accumulation, the highest nitrate content of both years, 382.42 mg·kg<sup>-1</sup> FW, was well below the Acceptable Daily Intake of 3.7 mg nitrate per kg<sup>-1</sup> bodyweight (Scientific Committee on Food, 1995). A balanced nitrogen and sulfur fertilization may prevent leaf nitrate accumulation by increasing the incorporation of nitrogen to organic compounds (De Pascale et al., 2007). The total sulfur content of soil used was 1630.91 mg kg<sup>-1</sup> (Table 1), which is considered very high (Aguilera et al., 2002), and also an extra sulfur was applied with ammonium sulfate. This may be the cause of lower nitrate content in our experiment.

## Conclusion

Our research has shown that January, February and November are the most suitable months of the year to sow garden cress in our climatic conditions, and probably for other regions which have a Mediterranean climate. Warmer months significantly reduced yield and quality. Although garden cress has a potential to accumulate high amounts of nitrate in leaves, plants grown in soil, especially in sulfur sufficient soils, do not hold a threat in terms of nitrate content.

## ACKNOWLEDGMENT

The authors are grateful to The Scientific and Technological Research Council of Turkey (TUBITAK) and Science and Technical Center of Ege University (EBILTEM) for their financial support.

## REFERENCES

- Adams M, Pelletier R, Zive M, Sallis J (2005). Salad Bars and Fruit and Vegetable Consumption in Elementary Schools: A Plate Waste Study. *J. Am. Diet. Assoc.*, 105(11): 1789-1792.
- Addiscott TM (2005). Nitrate, Agriculture and the Environment. Wallingford, Oxfordshire, UK: CABI Publishing.
- Aguilera M, de la Luz Mora M, Borie G, Peirano P, Zunino H (2002). Balance and distribution of sulfur in volcanic ash-derived soils in Chile. *Soil Biol. Biochem.* 34(9): 1355-1361.
- Balks R, Reekers I (1955). Bestimmung des Nitrat und Ammoniakstickstoffs im Boden (Determination of nitrate and ammonium nitrogen in soil). *Landwirt. Forsch.* 8(1): 7-13.
- Cahill L, Corey PN, El -Sohemy A (2009). Vitamin C Deficiency in a Population of Young Canadian Adults. *Am. J. Epidem.* 170(4): 464-471.
- Cartea ME, Velasco P (2008). Glucosinolates in Brassica foods: bioavailability in food and significance for human health. *Phytochem. Rev.* 7(2):213-229.
- Chapman SC, Barreto HJ (1997). Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agron. J.* 89 (3): 557-562.
- Charron CS, Saxton AM, Sams CE (2005). Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J. Sci. Food Agr.* 85(4): 671-681.
- Ciska E, Martyniak -Przybyszewska B, Kozłowska H (2000). Content of glucosinolates in cruciferous vegetables grown at the same site for



- two years under different climatic conditions. *J. Agr. Food Chem.* 48(7): 2862–2867.
- Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, Tubaro A, Menichini F (2009). The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. *Food Chem.* 112(3): 587-594.
- Cox BD, Whichelow MJ, Prevost AT (2000). Seasonal consumption of salad vegetables and fresh fruit in relation to the development of cardiovascular disease and cancer. *Public Health Nutr.* 3(1), 19–29.
- usti M, Poljak M, oga L, osi T, Toth N, Pecina M (2003). The influence of organic and mineral fertilization on nutrient status, nitrate accumulation, and yield of head chicory. *Plant Soil Environ.* 49(5): 218-222.
- De Pascale S, Maggio A, Pernice R, Fogliano V, Barbieri G (2007). Sulfur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. *Eur. J. Agron.* 26(4): 418-424.
- Dufault RJ, Ward B, Hassell RL (2009). Dynamic relationships between field temperatures and romaine lettuce yield and head quality. *Sci. Hortic. Amsterdam* 120(4): 452-459.
- Elia A, Santamaria P, Serio F (1998). Nitrogen nutrition, yield and quality of spinach. *J. Sci. Food Agr.* 76(3): 341-346.
- Fontana E, Nicola, S (2008). Producing garden cress (*Lepidium sativum* L.) for the fresh-cut chain using a soilless culture system. *J. Hortic. Sci. Biotech.* 83(1): 23–32.
- Kassie F, Rabot S, Uhl M, Huber W, Qin HM, Helma C, Schulte-Hermann R, Knasmüller S (2002). Chemoprotective effects of garden cress (*Lepidium sativum*) and its constituents towards 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions. *Carcinogenesis.* 23(7):1155-61.
- Kim SJ, Kawaharada C, Ishii G (2006). Effect of ammonium: nitrate nutrient ratio on nitrate and glucosinolate contents of hydroponically-grown rocket salad (*Eruca sativa* Mill.). *Soil Sci. Plant Nutr.* 52(3): 387–393.
- Kopsell DA, Kopsell DE, Curran-Celentano J (2007). Carotenoid pigments in kale are influenced by nitrogen concentration and form. *J. Sci. Food Agr.* 87(5):900–907.
- Kopsell DE, Kopsell DA, Randle WM, Coolong TW, Sams CE, Curran-Celentano J (2003) Kale Carotenoids Remain Stable while Flavor Compounds Respond to Changes in Sulfur Fertility. *J. Agric. Food Chem.* 51(18): 5319-5325.
- L'hirondel J, L'hirondel J-L (2002). Nitrate and man: Toxic, harmless or beneficial? Wallingford, UK, CABI.
- Lee SK, Kader AA (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Tech.* 20(3): 207–220.
- León AP, Viña SZ, Frezza D, Chaves A, Chiesa A (2007). Estimation of Chlorophyll Contents by Correlations between SPAD-502 Meter and Chroma Meter in Butterhead Lettuce. *Comm. Soil. Sci. Plan.* 38 (19-20): 2877–2885.
- Liu YJ, Tong YP, Zhu YG, Ding H, Smith FA (2006). Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. *J. Plant Nutr.* 29(7): 1207-1217.
- Madeira AC, Ferreira A, Verennes A, Vieira MI (2003). SPAD meter versus tristimulus colorimeter to estimate chlorophyll content and leaf color in sweet pepper. *Comm. Soil Sci. Plan.* 34(17-18):2461-2470.
- Magkos F, Arvaniti F, Zampelas A (2003). Organic food: Nutritious food or food for thought? A review of the evidence. *Int. J. Food Sci. Nutr.* 54(5): 357 - 371.
- Munro DB, Small E (1997). *Vegetables of Canada*. NRC Research Press Scientific Publishing, Canada, pp. 245-247.
- Naidu SL, DeLucia EH (1998). Physiological and morphological acclimation of shade-grown tree seedlings to late-season canopy gap formation. *Plant Ecol.* 138(1): 27-40.
- Pavlou GC, Ehaliotis CD, Kavvadias VA (2007). Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. *Sci. Hortic. Amsterdam* 111(4): 319–325.
- Pearson D (1970). *The Chemical Analysis of Foods*. Auxill, London.
- Powelson DS, Addiscott TM, Benjamin N, Cassman KG, De Kok TM, Van Grinsven H, L'Hirondel J-L, Avery AA, Van Kessel C (2008). When does nitrate become a risk for humans? *J. Environ. Qual.* 37(2): 291-295.
- Premuzic Z, Villela F, Garate A, Bonilla I (2004). Light supply and nitrogen fertilization for the production and quality of butterhead lettuce. *Acta Hortic.* 659: 671-678.
- Radovich TJK, Kleinhenz MD, Streeter JG, Miller AR, Scheerens JC (2005). Planting date affects total glucosinolate concentrations in six commercial cabbage cultivars. *HortScience* 40(1): 106-110.
- Rosa EAS, Rodrigues PMF (1998). The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. *J. Sci. Food Agr.* 78(2): 208-212.
- Sandoval-Villa M, Guertal EA, Wood CW (2002). Tomato leaf chlorophyll meter readings as affected by variety, nitrogen form and nighttime nutrient solution strength. *J. Plant Nutr.* 23(5): 649-661.
- Sandoval-Villa M, Wood CW, Guertal EA (1999). Ammonium concentration in solution affects chlorophyll meter readings in tomato leaves. *J. Plant Nutr* 22(11):1717-1729.
- Santamaria P (2006). Nitrate in vegetables: Toxicity, content, intake and EC regulation. *J. Sci. Food Agr.* 86(1): 10–17.
- Santamaria P, Elia A, Parente A, Serio F (1998a). Fertilization strategies for lowering nitrate content in leafy vegetables: Chicory and rocket salad cases. *J. Plant Nutr.* 21(9): 1791-1803.
- Santamaria P, Elia A, Papa G, Serio F (1998b). Nitrate and ammonium nutrition in chicory and rocket plants. *J. Plant Nutr.* 21(9): 1779-1789.
- Santamaria P, Elia A, Gonnella M, Parente A, Serio F (2001). Ways of reducing rocket salad nitrate content. *Acta Hortic.* 548: 529–537.
- Santamaria P, Elia A, Serio F, Gonnella M, Parente A (1999). Comparison between nitrate and ammonium nutrition in fennel, celery, and swiss chard. *J. Plant Nutr.* 22(7): 1091-1106.
- Schonhof I, Blankenburg D, Müller S, Krumbein A (2007). Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. *J. Plant Nutr. Soil Sci.* 170(1): 65–72.
- Scientific Committee on Food. 1995. Opinion on nitrate and nitrite, Annex 4 to Document III/5611/95, European Commission (ed.), Brussels, p. 20.
- Shaahan MM, El-Sayed AA, Abou El-Nour EAA (1999). Predicting nitrogen, magnesium and iron nutritional status in some perennial crops using a portable chlorophyll meter. *Sci. Hortic. Amsterdam* 82(3–4): 339–348.
- Stagnari F, Di Bitetto V, Pisante M (2007). Effects of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. *Sci. Hortic. Amsterdam* 114(4): 225-233.
- Su LJ, Arab L (2006). Salad and Raw Vegetable Consumption and Nutritional Status in the Adult US Population: Results from the Third National Health and Nutrition Examination Survey. *J. Am. Diet. Assoc.* 106(9): 1394-1404.
- Szmigielska AM, Schoenau JJ, Levers V (2000). Determination of glucosinolates in canola seeds using anion exchange membrane extraction combined with the high-pressure liquid chromatography detection. *J. Agr. Food Chem.* 48(10): 4487-4491.
- Toor RK, Savage GP, Heeb A (2006). Influence of different types of fertilisers on the major antioxidant components of tomatoes. *J. Food Comp. Anal.* 19(1): 20-27.
- Umar AS, Iqbal M (2006). Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. *Agron. Sustain. Dev.* 27(1): 45-57.
- Vallejo F, Tomas-Barberan FA, Gonzalez Benavente-Garcia A, Garcia.Viguera C (2003). Total and individual glucosinolate contents in inflorescences of eight broccoli cultivars grown under various climatic and fertilisation conditions. *J. Sci. Food Agr.* 83(4): 307–313.
- Wang Z-H, Li S-X (2003). Effects of N forms and rates on vegetable growth and nitrate accumulation. *Pedosphere* 13(4): 309-316.
- Wang Z-H, Li S-X, Malhi S (2008). Effects of fertilization and other agronomic measures on nutritional quality of crops. *J. Sci. Food Agr.* 88(1): 7-23.
- Westerveld SM, Mckeown AW, Scott D, McDonald MR (2004). Assessment of chlorophyll and nitrate meters as field tissue nitrogen tests for cabbage, onions, and carrots. *HortTechnology* 14(2): 179–188.
- WHO (2003). Diet, nutrition and the prevention of chronic disease. Report of a Joint WHO/FAO Expert Consultation. World Health

- Organization, Geneva.
- Williams DEM, Wareham NJ, Cox BD, Byrne CD, Hales CN, Day NE (1999). Frequent Salad Vegetable Consumption Is Associated with A Reduction in the Risk of Diabetes Mellitus. *J. Clin. Epidem.* 52(4): 329-335.
- World Cancer Research Fund (1997). *Food Nutrition and the Prevention of Cancer: a Global Perspective*. Washington, American Institute for Cancer Research, p. 442.
- Worthington V (2001). Nutritional quality of organic versus conventional fruits, vegetables, and grains. *J. Altern. Complem. Med.* 7(2): 161–173.
- Wunderlich SM, Feldman C, Kane S, Hazhin T (2007). Nutritional quality of organic, conventional, and seasonally grown broccoli using vitamin C as a marker. *Int. J. Food Sci. Nutr* 59(1): 34-45.
- Zahradník A, Pet íková K (2007). Effect of alternative organic fertilizers on the nutritional value and yield of head cabbage. *Hortic. Sci. (Prague)*. 34(1): 65–71.
- Zhan LJ, Fontana E, Tibaldi G, Nicola S (2009). Qualitative and physiological response of minimally processed garden cress (*Lepidium sativum* L.) to harvest handling and storage conditions. *J. Food Agric. Environ.* 7(3&4): 43-50.
- Zhang F-C, Kang S-Z, Li FS, Zhang J-H (2007). Growth and major nutrient concentrations in *Brassica campestris* supplied with different  $\text{NH}_4^+/\text{NO}_3^-$  ratios. *J. Integr. Plant Biol.* 49(4): 455–462.