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Interaction Effect of CO_2 Enrichment and Nutritional Conditions on Physiological Characteristics, Essential Oil and Yield of Lemon Balm (*Melissa officinalis* L.)

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Abstract

Carbon dioxide enrichment and nutritional improvement can increase photosynthesis and growth of different crops. The aim of the present study was to assess interaction effects of CO_2 enrichment and fertilizer on physiological characteristics and lemon balm essential oil. Experimental units were composed of CO_2 at 380, 700, and 1050 ppm with and without manure and N fertilizer application. A continuous increasing trend of individual plant leaf area, total dry weight accumulation and relative growth ratio were recorded with CO_2 enrichment. When CO_2 was elevated from 380 to 1050 ppm, the values of height (24.3%), SPAD reading (2.7%), essential oil yield (26.3%) and final yield (65.3%) were increased, unlike, stomatal conductance (35.2%) and essential oil percentage (53%) were decreased. The highest and the lowest values (except for oil percentage) were obtained under N and no fertilizer application, respectively. Except for SPAD, interaction between CO_2 enrichment and each fertilizer on all measured characteristics had a significant effect, so that CO_2 effect was intensified by applying each fertilizer. Therefore, it can be concluded that when temperature increase caused by rising CO_2 is not considered or there is not a limitation for resources, CO_2 enrichment will improve lemon balm biomass and essential oil yield.

Keywords: climate change, essential oil yield, medicinal plant, stomatal conductance

Introduction

Industrial revolution caused a careless consumption in the fossil fuels and subsequently led to a remarkable increase in the atmospheric CO_2 concentration. A further accelerating increase of CO_2 concentration is one of the most certain aspects of climate change over the coming decades. As recent reports indicated, CO_2 is rising in the atmosphere faster than previously forecasted (IPCC, 2007). Although the atmospheric CO_2 concentration increased approximately from 280 to 380 ppm during two recent centuries, it is now predicted that the amount of two ppm is added yearly (IPCC, 2007). Therefore, it is expected that it will reach to 700-1000 ppm until 2100 (Cheng *et al.*, 2009; Mavrogianopoulos *et al.*, 1999). This process lead to an increase in temperature and a change in rainfall patterns, which it will influence agroecosystems function in different regions of the world. These effects can either increase or reduce biomass of plant production. Therefore, the final effect of climate change on process of growth and development in plants depends on the interactions between these factors (Asseng *et al.*, 2009). Atmospheric CO_2 is fundamental to growth and product of the terrestrial vegetation, as well as it is critical for the irradiation properties of the atmosphere and hence climate. Therefore, the changing atmospheric concentration of CO_2 will have far-reaching effects on agricultural production (Asseng *et al.*, 2009).

Nowadays, global food production depends more or less on the useful usage of resources. The issues such as climate change, soil carbon sequestration and their long-term impact on food security and environmental sustainability have been debated, having a great importance (Andarzian *et al.*, 2008). Therefore, because of present concern, various experiments have been implemented in controlled environments to forecast agro-ecosystems function in the future. Although these experiments provide much information about the impacts of temperature and atmospheric CO_2 on plant growth processes, it is in need of technical equipment and high cost. Moreover, complexity and intricacy in doing this type of studies decelerates the implementation of researches on the various crops especially medicinal plants.

Carbon dioxide enrichment has two main influences on plant growth. It increases the intercellular CO_2 concentration, leads to an increase in net photosynthesis rate, and at the same time decreases stomatal conductance, resulting in reduced transpiration (Farquhar *et al.*, 1978). Many researches have showed that the higher CO_2, the higher biomass and subsequently the higher crop yield. Hogy and Fangmeier (2009) reported that the yield and market-friendly of potato had improved with CO_2 enrichment. They also stated that the partitioning of photosynthesis compounds into tubers was increased. Cheng *et al.* (2009) stated that the yield and biomass of rice were increased

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with CO_2 enrichment. Moreover, the production of blanket flower (*Gailerdia pulchella*), floss flower (*Ageratum conicoides*) and marigold (*Tagetes* spp) was improved when CO_2 concentration was increased (Shoor *et al*., 2008). The influence of CO_2 enrichment on growth and development in plants depends on water and nutrient availability. Low nutrient availability can decrease the advantage of CO_2 enrichment (Yang *et al*., 2006). Therefore, a main indirect influence of higher atmospheric CO_2 is the reduction of plant nutrient contents, resulting in the reduction of biological yield quality (Yang *et al*., 2007).

A plant that in one or some of its organs the active ingredient is contained, having medicinal properties, is called as a medicinal plant. Medicinal plant extracts and essential oils are increasingly of interest as antimicrobial and antiviral agents and have been widely used in traditional medicine (Schnitzler *et al*., 2008). Extracts from plants of the *Lamiaceae* family have been described for antifungal (Darouche *et al*., 2006), immune modulatory (Fang *et al*., 2005) and antioxidant (Bozin *et al*., 2007) activities. Among Lamiaceae's plants, lemon balm is a perennial and aromatic herb species that is native to the Mediterranean region (Guginski *et al*., 2009). For years, physicians have prescribed lemon balm to cure the nervous problems. Moreover, it is a beneficial salve for curing the emphysema, calming the nerves and reinforcing the stomach and heart (Ibarra *et al*., 2010). The active ingredient of lemon balm in various forms such as essential oil, ointment, and the brewed is used. For example, the essential oil of this plant is more useful to cure the bellyaches with nerve origin (Awad *et al*., 2009). The leaf of lemon balm is a super medicine to cure those who become afflicted with stomach cramp when they are anxious (Allaverdiyev *et al*., 2004; Dastmalchi *et al*., 2008).

There has been little information about elevated CO_2 on lemon balm. Therefore, in the present study, growth and yield of lemon balm cultivated at ambient and elevated CO_2 were assayed. The objectives were (*i*) to determine the elevated CO_2 effect; (*ii*) the different fertilizer on the physiological indices, stomatal conductance, essential oil and final yield, and (*iii*) how interaction between atmospheric CO_2 concentration and nutrimental condition affect them.

Material and methods

Experimental design

This study was carried out in three large-scale climate chambers at the Research Greenhouse of Ferdowsi University of Mashhad, Iran, during 2010. Internal chamber dimensions were 3 m (length) × 1.5 m (width) × 2 m (height). The chambers have a circulating base plate, which draws ambient air into the chamber bottom and distributes evenly inside the chamber. A fully automated system was used to inject the gas. A photocell did the on/off of system at day and night, respectively. Gas injection was performed using capsules containing 50 kg of CO_2, with electrical valves. A timer was placed in the path to control the amount of gas injection. Using a portable CO_2 sensor, the measurements of CO_2 concentrations was done during the day. The medium temperature varied from 25°C during the day to 18°C at night. Medium relative humidity was 65%. The photoperiod was a daily cycle of 14 h light and 10 h dark.

The seeds (Khorasan Razavi native, Iran) were planted in pots with 30 cm diameter and 40 cm depth that was filled with a mixture of sand, clay loam soil, and peat (1:1:1; v/v/v). The seedlings were thinned to ten per pot at 5-7 cm height stage and then were exposed to CO_2 enrichment. To achieve the fertilizer treatment, animal manure and N fertilizer were added at 40 ton ha^{-1} and 100 kg ha^{-1}, respectively. They were applied at the time of filling pots and at four-leaf stage, respectively.

Data collection

First sampling was done 15 day after emergence (DAE) and continued at 15 day intervals. In each sampling, two plants in each pot were randomly selected and then leaf area and dry weight were assayed. The values of leaf area (LA) were determined using Leaf Area Meter (LICOW model). The samplings were oven-dried at 70°C for 48 h, then weighed. The changes trend daily of LA was extrapolated by following equation (Koocheki *et al*., 2008):

$$LA = \frac{a + b \times 4.\exp(\frac{-(x-c)}{d})}{1 + \exp(\frac{-(x-c)}{d})^2} \quad (1)$$

where *a* is the intercept, *b* is x value for maximum LA (estimated from data), *c* is maximum LA, *d* is the inflection point of LA, and *x* is the time (DAE). The total dry weight (TDW) accumulation was extrapolated by following equation (Koocheki *et al*., 2008):

$$TDW = \frac{a}{1 + b.\exp(-c.x))} \quad (2)$$

where *a* is the maximum dry weight accumulation, *b* is equation parameter, *c* is relative growth ratio (g g day^{-1}), and *x* is the time (DAE). The value of relative growth ratio (RGR) was computed using the following equation (Gardner *et al*., 1985):

$$RGR = \frac{\ln w_2 - \ln w_1}{t_2 - t_1} \quad (3)$$

where w_1 and w_2 are the dry weight at t_1 and t_2, respectively, and *t* is the sampling time (DAE). Leaf stomatal conductance was measured using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) at 60 DAE. Newly fully expanded leaves (the second leaf from the top) were selected for the measurement of stomatal conductance (Li *et al*., 2004). The SPAD reading was taken using a chlorophyll meter (SPAD-502, Minolta, Japan) and recorded as an average of ten measurements for each individual leaf (Young *et al*., 2007) at 8:30-11:00 a.m.

(Huan *et al*., 2010). The final yield and height per plant were measured before the appearance of first flower.

Isolation of essential oil

Twenty gram from aerial parts were crushed by electric grinder and suspended in 750 ml distillated water from each pot. Groundmass was subjected to hydro-distillation using Clevenger's apparatus (Clevenger, 1928). After four hours, the essential oil were collected and dehydrated with sodium sulphate (Na_2SO_4) using the method of Guenther (1961). Then, yield and percentage of essential oil were measured.

Data analysis

The experiment was analyzed as a factorial experiment based on completely randomized design with CO_2 and fertilizers as treatment variables. The experimental treatments are composed of three CO_2 concentrations (380, 700, and 1050 ppm) applied with and without the fertilizers of animal manure and nitrogen. Analysis of variance (ANOVA) was performed using the general linear model-univariate procedure from SAS software (version 9.1.2). ANOVAs were done with CO_2, animal manure and N fertilizer as the main effects and including two-way interactions.

Results and discussion

Leaf area

Regardless of the treatments, LA-value of lemon balm plant has shown a similar trend during the growing season. It was increased slowly at the beginning of growth period and followed by a linear steady state up to 60 DAE in which it culminated to maximum, following a steady state thereafter (Fig. 1). The LA-value was significantly increased with an increase in concentration of CO_2 from 380 to 1050 ppm (Fig. 1). This increase was due to an improvement in net photosynthesis rate and subsequently an increase in partitioning of dry matter to produce more leaves. This result was consistent with findings reported by Shoor *et al*. (2008) working with some ornamental plants. In the C_3 plants such as lemon balm, more partitioning of dry matter is not far-fetched to produce and expands leaves due to atmospheric CO_2 enrichment.

The LA of lemon balm was enhanced when nutritional condition was improved with the application of each of the fertilizers (Fig. 1). In all concentrations of CO_2, the highest LA-value was recorded in N fertilizer. Under N fertilizer application, the LA-value maximum was increased from 9.3% to 17.8% when CO_2 was increased from 380 to 1050 ppm. The LA-value at 380 ppm was recorded 1600.5, 1663.2, and 1764.9 cm^2 per plant under the conditions of without and with the animal manure and N fertilizers, respectively. These values were increased to 2098.2, 2243.2, and 2552.3 cm^2 per plant at 1050 ppm. Therefore, with rising CO_2 from 380 to 1050 ppm, the LA-value increased 23.7, 25.9, and 30.9% under the conditions free from fertilizers and the application of animal manure and N fertilizers, respectively. Namely, the effects of nutritional condition were intensified with CO_2 enrichment. Therefore, it seems that atmospheric CO_2 enrichment will have a great-

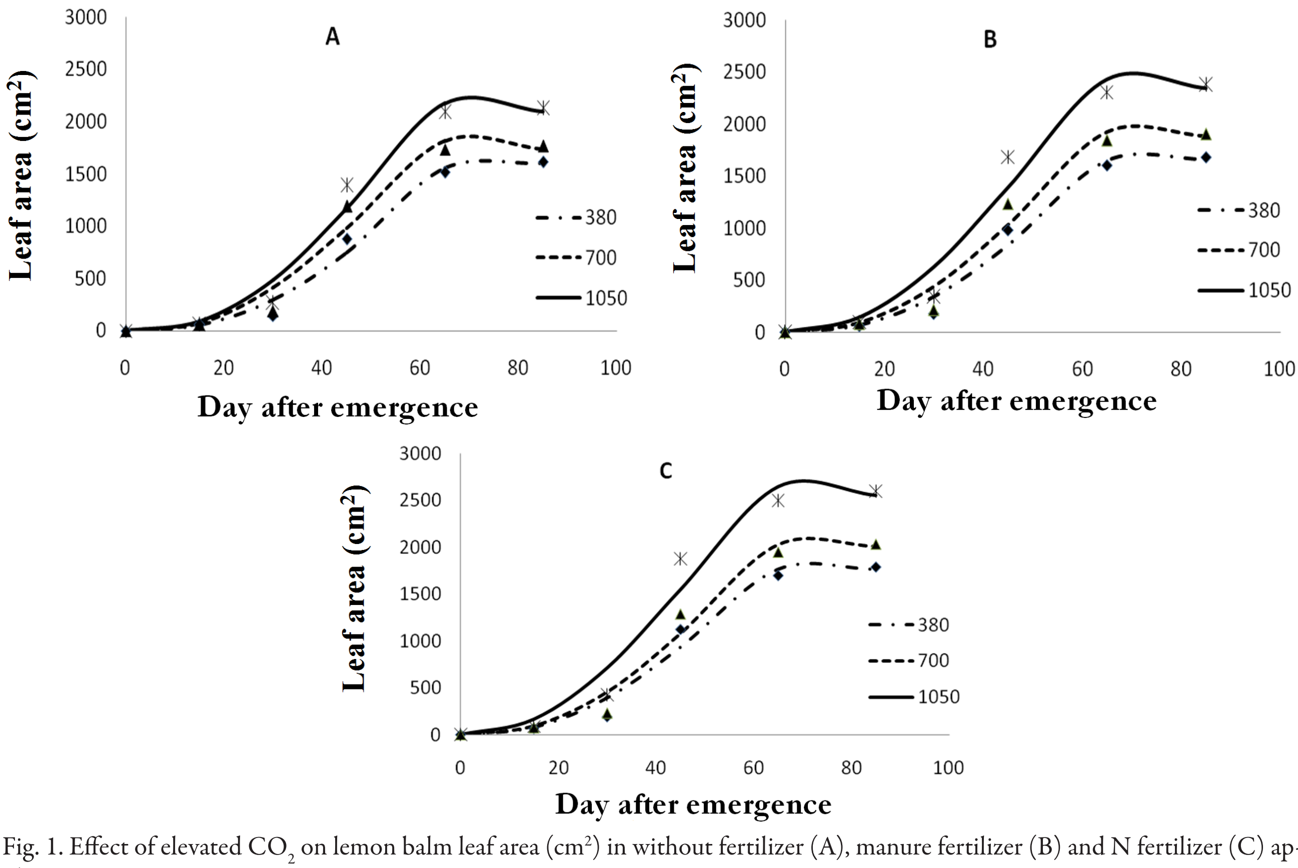

Fig. 1. Effect of elevated CO_2 on lemon balm leaf area (cm^2) in without fertilizer (A), manure fertilizer (B) and N fertilizer (C) application

er effect to increase LA if plant does not confront with the limitations of nutrient elements because when a plant can enjoy the availability of environmental conditions properly (such as light, water and nutrients) that all those factors be optimum. Since LA is a effective factor to receive light and produce dry matter, thus large LA leads to more light absorption to improve photosynthesis process and it will increase plant production ultimately (Shoor *et al*., 2008). Croonenborghs *et al*. (2009) reported that leaf area index of *Guzmania hilda* was increased up to 34% when CO_2 concentration was increased from 380 to 750 ppm. They stated that specific leaf weight increased due to an increase for carbohydrate. Other study have shown that atmospheric CO_2 enrichment increased the leaf area index of wheat at a period from closing canopy until starting canopy senescence (Schahczenski and Hill, 2009).

Plant height

Lemon balm plant height was increased with an increase in concentration of atmospheric CO_2 (Tab. 1). It was recorded 37.4 cm and 46.5 cm at concentrations of 380 and 1050 ppm, respectively. In the other words, it was increased up to 24.3%. This result may attribute to improve dry matter production and thus it causes the share increase of photosynthetic material dedicated to grow shoot. Plant height is considered as an effective trait in leaf area distribution and subsequently light absorption in the canopy profile. It can increase the competitive ability of crop to obtain light against weeds. Reddy *et al*. (1998) also reported that the stem height of cotton increased at 700 ppm as compared with 380 ppm. Moreover, lemon balm height was increased when each of the fertilizers was applied merely (Tab. 2) so that it was increased up to 9.2% and 17.6% with application of the manure and N fertilizers, respectively. Interaction between the fertilizer conditions and atmospheric CO_2 enrichment on the height showed a significant effect (Tab. 3). The effect of elevated CO_2 under the fertilizer treatments was more than of without fertilizer treatment. Among all of the treatments, the highest height (52.5 cm) was recorded at 1050 ppm plus the application of N fertilizer while the lowest height (35.6 cm) was recorded at 380 ppm without fertilizer. Therefore, the treatments in which nutritional conditions were more favorable, CO_2 enrichment led to a greater effect in increasing the lemon balm height (Tab. 3). It seems that N fertilizer increases plant height by improving the processes involved in division and elongation of the stem cell. In such circumstances, when supply and demand for water and nutrients is out of balance, increasing a source such as CO_2, the performance will not be enough. This finding was in agreement with the results of Kima *et al*. (2003) who reported that the rice height has increased by atmospheric CO_2 enrichment and N fertilizer application.

Stomatal conductance

The results indicated that stomatal conductance was reacted and decreased with an increase in atmospheric CO_2 concentration (Tab. 1). It was recorded 1073.3 and 695.9 m s^{-1} at 380 and 1050 ppm, respectively. In other words, it was reduced up to 35.2%. Medlyn *et al*. (2001) reported that a doubling of atmospheric CO_2 concentration decrease stomatal conductance, which was in agreement with the present result. It is known that CO_2 enrichment causes partial stomatal closure and reduces stomatal conductance (Medlyn *et al*., 2001). With elevation of CO_2 concentration, stomata do not appear to limit photosynthesis process any more than they do at normal ambient CO_2 concentration, even though stomatal conductance usually decreases under these conditions (Drake and Gonzàlez-Meler, 1997). In most species, it has been reported that elevated CO_2 reduces the stomatal conductance by 33-50% (Kimball *et al*., 2002; Medlyn *et al*., 2001).

Lemon balm stomatal conductance was not reacted when each of the fertilizers was applied (Tab. 2). Interac-

Tab. 1. Effect of elevated CO_2 on height, final yield, stomata conductive, SPAD reading, essential oil percentage and yield in lemon balm plant

CO_2 concentration (ppm)	Height (cm)	Final yield (g $plant^{-1}$)	Stomatal conductance (m s^{-1})	SPAD	Essential Oil percentage (%)	Essential oil yield (mg $plant^{-1}$)
380	37.4	4.3	1073.3	28.9	1.7	73.1
700	41.2	6.2	879.5	29.1	1.2	74.4
1050	46.5	12.4	695.9	29.7	0.8	99.2
LSD	3.7	0.8	37.7	3.6	0.2	10.2

Tab. 2. Effect of fertilizer on height, final yield, stomata conductive, SPAD reading, essential oil percentage and yield in lemon balm plant

Fertilizer	Height (cm)	Final yield (g $plant^{-1}$)	Stomatal conductance (m s^{-1})	SPAD	Essential Oil percentage (%)	Essential oil yield (mg $plant^{-1}$)
No fertilizer	37.3	6.2	884.3	22.8	1.6	99.2
Manure	41.3	7.3	869.5	28.3	1.4	102.2
Nitrogen	45.1	9.5	895.1	36.2	1.1	104.5
LSD	3.7	0.8	37.7	3.6	0.2	10.2

Tab. 3. Interactive elevated CO_2 and fertilizer on height, final yield, stomata conductive, SPAD reading, essential oil percentage and yield in lemon balm plant

CO_2 concentration + Fertilizer	Height (cm)	Final yield (g plant^{-1})	Stomatal conductance (m s^{-1})	SPAD	Essential Oil percentage (%)	Essential oil yield (mg plant^{-1})
380 + No fertilizer	35.6	3.8	1121.0	23.8	1.9	72.2
380 + Manure	37.3	4.1	1024.2	28.3	1.7	69.7
380 + Nitrogen	39.3	4.9	1076.7	34.6	1.2	58.8
700 + No fertilizer	37.0	5.2	862.3	21.8	1.5	78
700 + Manure	41.4	6.1	882.5	27.5	1.2	73.2
700 + Nitrogen	45.8	7.7	893.8	37.8	1	77
1050 + No fertilizer	40.5	9.5	669.8	22.7	1.2	114
1050 + Manure	46.6	11.8	702.0	29.1	1	118
1050 + Nitrogen	52.5	15.9	716.1	36.3	0.8	127.2
LSD	6.4	1.31	65.3	17.3	0.3	6.3

tion between the application of each fertilizer and CO_2 enrichment on the stomatal conductance showed a significant effect (Tab. 3). The effect of elevated CO_2 on the stomatal conductance under the fertilizer treatments was more than that of no fertilizer treatment. The highest (1121.0 m s^{-1}) and the lowest (669.8 m s^{-1}) stomatal conductance were recorded at 380 and 1050 ppm when fertilizer was not applied, respectively. An indirect effect of elevated CO_2 is temperature increase in canopy via reducing stomatal conductance (Asseng *et al.*, 2009). There is evidence in both wheat and cotton that selection for improved grain yields in breeding programs has been associated with selection for high stomatal conductance, resulting in heat avoidance through evaporative cooling in tropical climates (Amani *et al.*, 1996; Lu *et al.*, 1994). Therefore, reduced stomatal conductance due to CO_2 enrichment can have an additional effect on plant growth and development similar to an increase in temperature (Asseng *et al.*, 2009). Grant *et al.* (2001) using Ecosys-model predicted that at low nitrogen condition, evapotranspiration of wheat reduces due to a reduction in Rubisco activity and concentration, which compels a greater reduction in stomatal conductance in order to maintain a constant ratio of the inside leaf CO_2 concentration to that of outside air.

SPAD readings

A lemon balm plant SPAD reading was not reacted with an increase in concentration of atmospheric CO_2 (Tab. 1). In another investigation on wheat, leaf SPAD readings was not affected by the CO_2 enrichment at anthesis stage but it accelerated chlorophyll breakdown during grain filling (Ommen *et al.*, 1999). A SPAD reading was increased with application of each fertilizer (Tab. 2). So that, when the manure and N fertilizers were applied, it increased by 19.4% and 37.1%, respectively. Interaction between the fertilizers and atmospheric CO_2 enrichment on SPAD readings showed a no significant effect (Tab. 3). Nitrogen is one of the elements to form chlorophyll. Therefore, enhanced leaf N content via applying fertilizer led to an increase in SPAD readings. Huan *et al.* (2010) reported that the SPAD readings were significantly different across N rates, and with the increase of N application rate, the SPAD readings appeared in an ascent trend.

Relative growth ratio

The RGR of lemon balm was high at the beginning of the growth period. Then, it gently started to decline after 25 DAE and it reached to 0.007 g g^{-1} day^{-1} at the end of experiment (Fig. 2). This result, undoubtedly, can be related to that increasing of plant weight will be required to an increase in plant maintenance costs. Regardless of the fertilizer treatments, the RGR maximum was increased with elevated CO_2 concentration from 380 to 1050 ppm (Fig. 2). The RGR value is an effective characteristic in absorption of surrounding resources by plant, especially at the beginning of growing season. In other words, at this stage, the more RGR value, the higher resources will be captured and subsequently the more dominance of species in ecosystems will occur. As it was mentioned above, it seems that the elevated CO_2 led to an increase in RGR of lemon balm plant via improving net photosynthesis, followed by increasing dry matter production at the beginning of the growing season (Fig. 2). Beerling and Kelly (1997) also reported that the rate and speed of plant growth, especially underground organ, had improved with increasing of CO_2 concentration.

The RGR maximum was improved when N fertilizer and manure were applied (Fig. 2). It seems that the higher RGR can be attributed to more amount of N availability. Moreover, under all of the atmospheric CO_2 enrichment treatments, the lowest RGR value was obtained from the treatment of no fertilizer and the highest RGR value was obtained from the treatment of N fertilizer. The RGR value was increased with applying N fertilizer by 5.4% at 380 ppm and by 28.5% at 1050 ppm compared to no fertilizer treatment. The RGR maximum at 380 ppm was 0.087, 0.088, and 0.092 g g^{-1} day^{-1} under the conditions of no fertilizer, manure fertilizer and N fertilizer, respectively. The RGR value at 1050 ppm was 0.094, 0.11 and 0.13 g g^{-1} day^{-1} in no fertilizer, manure and N fertilizer treatments, respectively. Therefore, with increasing of CO_2 concentration from 380 to 1050 ppm, the maximum of RGR was improved up to 8.1%, 17.4%, and 30.7% in no fertilizer, manure fertilizer and N fertilizer treatments, respectively. It can be concluded that if there is not a limiting factor for plant growth and development, atmospheric CO_2 enrichment will increase individual plant RGR, leading to an improvement in resources capture. Lambers *et al*. (1996) reported that under elevated CO_2 condition, the amount of photosynthetic compound increased. They concluded that this action improved the flow of carbohydrates from sources toward sinks. Besides, when plant is placed in adjacent with high CO_2, especially when there is not water and nutrient restriction, it will grow faster and, the underground organs will accumulate more carbohydrates.

Total dry weight accumulation

Among treatments, there was not any significant difference in trend of total dry weight accumulation at the beginning of growth period. This was due to small size of lemon balm plant (Fig. 3). Total dry weight (TDW) was increased linearly from 25 to 65 DAE in which it reached to a maximum, following a steady state thereafter (Fig. 3). The results indicated that TDW maximum was improved with an increase in atmospheric CO_2 concentration (Fig. 3). Moreover, at high CO_2, TDW entered to the linear phase of growth that it could be related to higher RGR (Fig. 2). Biomass accumulation can be caused by three factors, which are composed of (i) length of growing season; (ii) leaf area index and (iii) photosynthetic efficiency. Generally, atmospheric CO_2 enrichment has a positive impact on photosynthetic efficiency and leaf area (Schahczenski and Hill, 2009). Based on available information, the leaf area of C_3 plants is less affected with elevated CO_2 as compared with its biomass. Hence, atmospheric CO_2 enrichment will reduce the ratio of leaf area to biomass. Jeffery *et al*. (2006) showed that atmospheric CO_2 enrichment not only caused an increase in potato yield but also caused a speedup in tubering. Moreover, it caused an augmentation in the number of tubers and market-friendly of potato (Craigon *et al*., 2002).

The values of TDW was affected and varied by different fertilizer conditions (Fig. 3). The highest and the lowest TDW were observed when the treatments of N fertilizer and no fertilizer were exerted, respectively. When N fertilizer was applied plus CO_2 at 380 and 1050 ppm, TDW maximum was increased by 23.1% and 39.9% compared to no fertilizer, respectively. The TDW maximum at 380

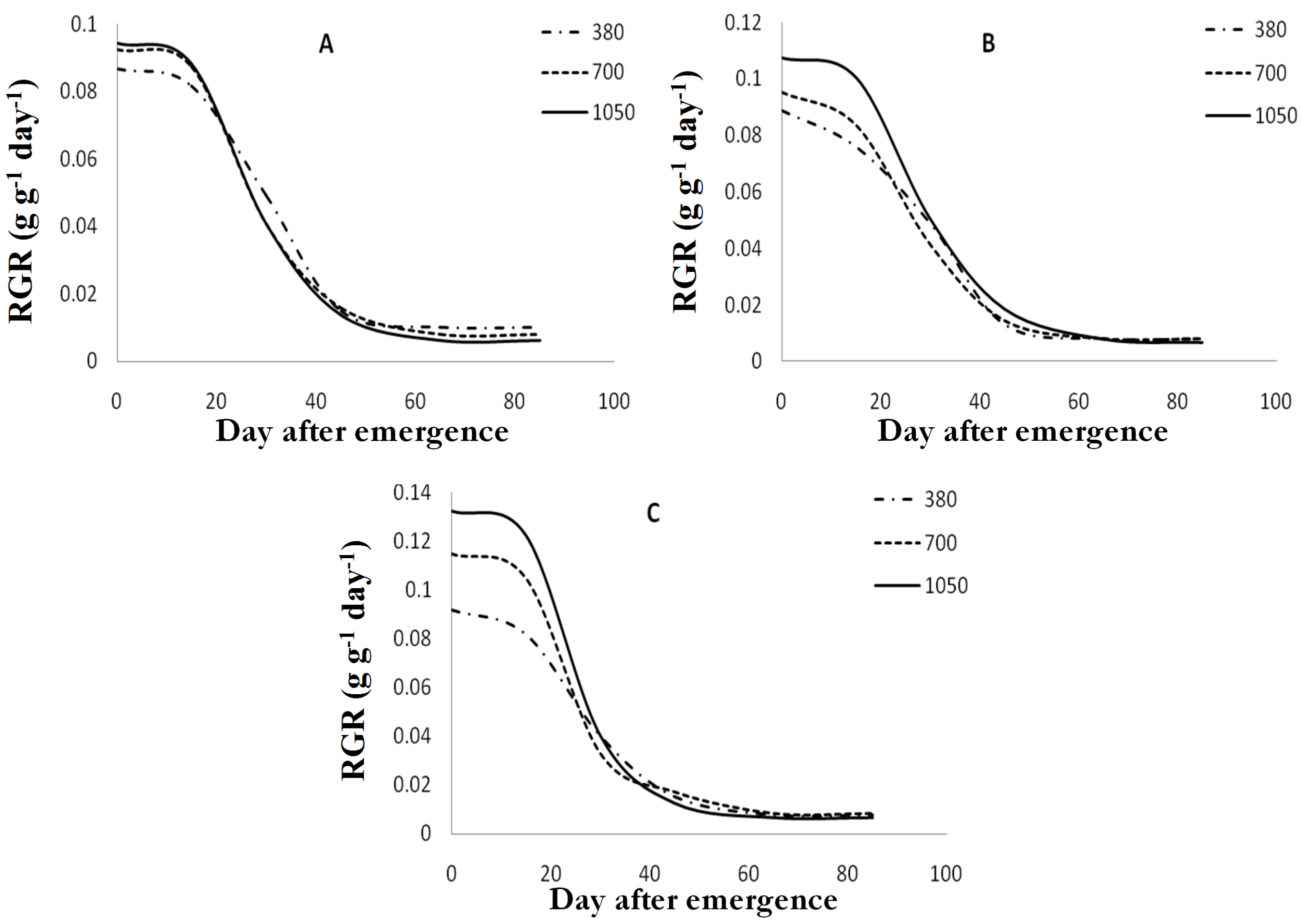

Fig. 2. Effect of elevated CO_2 on lemon balm RGR (g g^{-1} day^{-1}) in without fertilizer (A), manure fertilizer (B) and N fertilizer (C) application

ppm was obtained 3.7, 4.0, and 4.8 g plant^{-1} in no fertilizer, manure fertilizer and N fertilizer treatments, respectively. At 1050 ppm, the TDW maximum was recorded 9.2, 11.5, and 15.2 g plant^{-1} in the same treatments, respectively. Namely, with elevated CO_2 from 380 to 1050 ppm, the TDW maximum increased by 58.8, 64.5, and 67.8%, respectively. It seems that fertilizer effects were intensified with high CO_2 concentration. This result can be related to more nutrient supplement in response to the demand created, resulting in improvement of net photosynthesis under elevated CO_2 condition. Yang *et al*. (2006) indicated that the impact of CO_2 enrichment on rice biomass is reduced when nitrogen availability is low.

Final yield

Elevated CO_2 concentrations had a significant impact on final yield of lemon balm so that the lowest (4.3 g plant^{-1}) and the highest (12.4 g plant^{-1}) final yield were obtained at 380 and 1050 ppm, respectively (Tab. 1). With increasing atmospheric CO_2 concentration from 380 to 1050 ppm, the final yield was improved by 65.3%. It seems that CO_2 enrichment lead to an improvement in net photosynthesis rate and an increase in dry matter production and in final yield ultimately. Previous studies showed that the enrichment of some plants such as rice and soybean with CO_2 led to an increase in their growth and yield because net photosynthesis rate was increased (Croonenborghs *et al*., 2009; Guginski *et al*., 2009; Van-Labeke and Dambre, 1998). Increasing of net pphotosynthesis by reason of atmospheric CO_2 enrichment, is due to a reduction in ribulose-1,5-bisphosphate carboxylase (*Rubisco*) enzyme inhibitory activity in photosynthesis process. Moreover, high CO_2 can indirectly increase the optimum temperature for growth processes. A reduction in the respiration was also documented at high CO_2 concentration (Chunyan *et al*., 2008; Das, 2003).

Regardless of the elevated CO_2, the various fertilizers had a significant impact on final yield of lemon balm (Tab. 2). The lowest (6.2 g plant^{-1}) and the highest (9.5 g plant^{-1}) final yield were obtained under condition of no fertilizer and the application of N fertilizer, respectively (Tab. 2). N fertilizer treatment was affected by 34.7% on final yield compared to no fertilizer treatment. Moreover, the effect of elevated CO_2 on final yield was intensified when the fertilizers were applied (Tab. 3). The highest final yield (15.9 g plant^{-1}) was recorded at 1050 ppm plus the application of N fertilizer. Unlike, the lowest height (3.8 g plant^{-1}) was recorded at 380 ppm plus no fertilizer treatments. Kima *et*

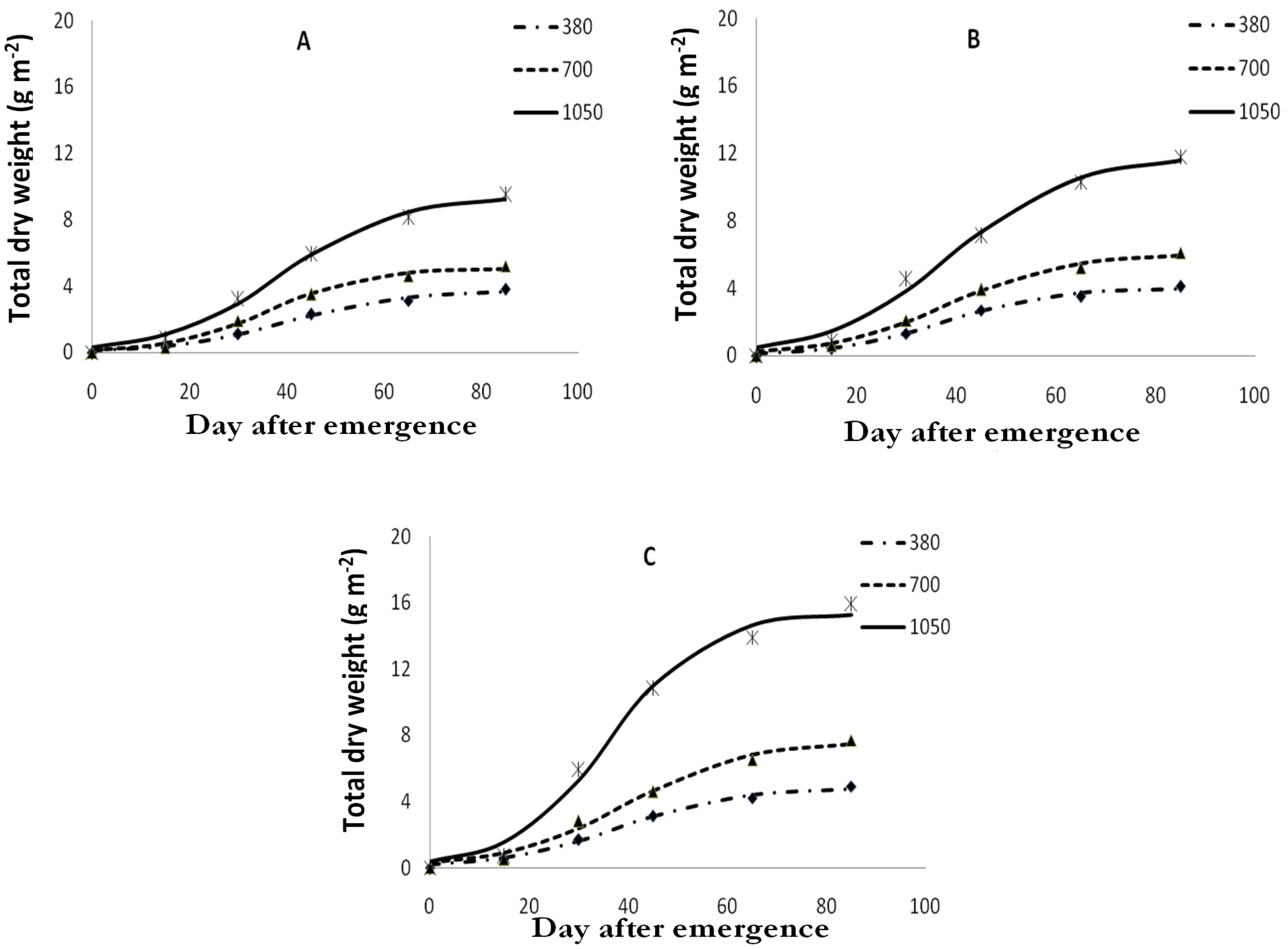

Fig. 3. Effect of elevated CO_2 on lemon balm total dry weight accumulation (g m^{-2}) in without fertilizer (A), manure fertilizer (B) and N fertilizer (C) application

al. (2003) investigated the interaction between CO_2 and N fertilizer on rice and reported that the elevated CO_2 can improve yield and growth of shoots under application of N fertilizer. They stated that atmospheric CO_2 enrichment increased the request of rice photosynthetic organs for obtaining more nutrient elements, which this request was eliminated with applying N fertilizer. At another experiment was identified that with application N fertilizer, the yield of rye grass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) were increased from 8 to 30% and from 11 to 20% by CO_2 enrichment after a period 3 years, respectively (Hebeisen *et al*., 1997). Moreover, Daepp *et al*. (2000) reported that at low N and elevated CO_2, rye grass yield was increased up to 10%, while at high N and elevated CO_2; it was increased up to 25% during a period of 6 years.

Essential oil percentage and yield

The results showed that essential oil percentage of lemon balm was decreased with elevated CO_2 (Tab. 1). It was recorded 1.7% and 0.8% at 380 and 1050 ppm, respectively. In the other words, it was decreased by 53%. It seems that CO_2 enrichment due to increasing of net photosynthesis rate and subsequently increasing of structural and nonstructural carbohydrates led to a decrease in concentration of secondary component. This impact results in a reduction of essential oil percentage. Moreover, the essential oil yield was increased with CO_2 enrichment (Tab. 1). It was recorded 73.1 and 99.2 mg plant^{-1} at 380 and 1050 ppm, respectively. In other words, it increased up to 26.3%. Although the elevated CO_2 reduced percentage of oil, this consequence was compensated by an increase in final yield (Tab. 1). Moreover, essential oil percentage was decreased with application of each fertilizer (Tab. 2). So that, the application of manure and N fertilizers decreased essential oil percentage by 12.5% and 31.2%, respectively. The essential oil yield was not reacted with application of each fertilizer (Tab. 2). The response of lemon balm to manure fertilizer was due to the ability of manure to develop water holding capacity of the soil, thus augmenting water supply to plant, and the nutrients supplying capacity of manure which might have at least moderately satisfied the well nutrients condition. Moreover, nitrogen being a nutrient element that promotes vegetative growth thereby increased the biomass yield of lemon balm, which in turn increased the essential oil yield. These consequences were in agreement with findings of Rajeswara Rao (2001) who reported that application of manure fertilizer at 15 t ha^{-1} increased the total biomass yield of Palmarosa (*Cymbopogon martinii*) by 10.7% and total essential oil yield by 10.3% as compared with when the manure fertilizer was not applied. In addition, the application of N fertilizer at 80 kg ha^{-1} enhanced the total biomass yield by 57.6% and total essential oil yield by 60.3% as compared with when the N fertilizer was not applied. In the other investigation, applying fertilizer increased essential oil of *Chrysanthemum* sp., while, essential oil content decreased (Alvarez-Castellanos and Pascual-Villalobos, 2003).

The results indicated that the interaction between CO_2 enrichment and each fertilizer on essential oil percentage showed a significant effect (Tab. 3). The effect of elevated CO_2 on essential oil percentage under the fertilizer treatments was lower than the no fertilizer treatment. The highest essential oil percentage (1.9%) was recorded at 380 ppm plus no fertilizer treatment. Unlike, the lowest essential oil percentage (0.8%) was recorded at 1050 ppm plus N fertilizer application. Moreover, interaction between CO_2 enrichment and fertilizer treatments on essential oil yield illustrated a significant effect differently (Tab. 3). The highest essential oil yield (127.2 mg plant^{-1}) was recorded at 1050 ppm plus N fertilizer and the lowest essential oil yield (58.8 mg plant^{-1}) was recorded at 350 ppm plus N fertilizer. Although N fertilizer increased final yield, it seems that it did not affect the essential oil yield due to a reduction in essential oil percentage (Tab. 1).

Conclusions

Certainly, plant will show various reactions in response to CO_2 enrichment. These reactions will correlate to yield change, growth characteristics, and the ratio of shoot to root. Moreover, better growth resulting from elevated CO_2 under climate change will cause an increase in need for minerals in the future. It seems that climate change will increase mineral necessity and therefore plant will compete more in agroecosystems to obtain minerals. Therefore, it can be conclude that if the temperature increase caused by rising CO_2 is not considered, and if there is not a limitation for water and nutrients, the CO_2 enrichment will lead to an improvement in lemon balm biomass and essential oil yield under climate change in the future.

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References

Allaverdiyev A, Duran N, Ozguven M, Koltas S (2004). Antiviral activity of the volatile oils of *Melissa officinalis* L. against Herpes simplex virus type-2. Phytomed 11:657-661.

Alvarez-Castellanos PP, Pascual-Villalobos MJ (2003). Effect of fertilizer on yield and composition of flowerhead essential oil of *Chrysanthemum coronarium* (*Asteraceae*) cultivated in Spain. Ind Crop Prod 17:77-81.

Amani I, Fischer RA, Reynolds MP (1996). Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. Z Acker Pflanzenbau 176:119-129.

Andarzian B, Bakhshandeh AM, Bannayan M, Emam Y (2008).

Evaluation of the CERES-wheat model in Ahvaz condition. Iran J Field Crop Res 6:22-29.

Asseng S, Cao W, Zhang W, Ludwig F (2009). Crop Physiology, Modelling and Climate Change: Impact and adaptation strategies. Crop Physiol 511-543 p.

Awad R, Muhammad A, Durst T, Trudeau VL, Arnason JT (2009). Bioassay-guided fractionation of lemon balm (*Melissa officinalis* L.) using an *in vitro* measure of GABA transaminase activity. Phytother Res 23:1075-1081.

Beerling DJ, Kelly CK (1997). Stomatal density responses of temperate woodland plants over the past seven decades of CO_2 increase: a comparison of Salisbury (1927) with contemporary data. Am J Bot 84:1572-1583.

Bozin B, Mimica-Dukic N, Samojlik I, Jovin E (2007). Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. ans *Salvia officinalis* L., Lamiaceae) essential oils. J Agr Food Chem 55:7879-7885.

Cheng W, Sakai H, Yagi K, Hasegawa T (2009). Interactions of elevated CO_2 and night temperature on rice growth and yield. Agr Forest Meteorol 149:51-58.

Chunyan W, Maosong L, Jiqing S, Yonggang C, Xiufen W, Yongfeng W (2008). Differences in stomatal and photosynthetic characteristics of five diploidy wheat species. Acta Ecol Sin 28:3277-3283.

Clevenger JH (1928). Apparatus for the determination of volatile oil. J Am Pharm Assoc 17:346-349.

Craigon J, Fangmeier A, Jones M, Donnelly A, Bindi M, De-Temmerman L, Persson K, Ojanpera K (2002). Growth and marketable-yield responses of potato to increased CO_2 and ozone. Eur J Agron 17:273-289.

Croonenborghs S, Ceusters J, Londers E, De-Proft MP (2009). Effects of elevated CO_2 on growth and morphological characteristics of ornamental bromeliads. Sci Hortic 121:192-198.

Daepp M, Suter D, Ameida JPF, Isopp H, Hartwig UA, Frehner M, Blum H, Nosberger J, Luscher A (2000). Yield response of *Lolium perenne* swards to free air CO_2 enrichment increased over six years in a high N input system on fertile soil. Glob Change Biol 6:805-816.

Darouche RO, Mansouri MD, Kojic EM (2006). Antifungal activity of antimicrobial-impregnated devices. Clin Microbiol Infec 12:397-399.

Das R (2003). Characterization of response of *Brassica* cultivars to elevated carbon dioxide under moisture stresses. Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi.

Dastmalchi K, Dorman HJD, Oinonen PP, Darwis Y, Laakso I, Hiltunen R (2008). Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. J Food Sci Tech 41:391-400.

Drake BG, Gonzàlez-Meler MA (1997). More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Phys 48:609-639.

Fang X, Chang RC, Yuen WH, Zee SY (2005). Immune modulatory effects of *Prunella vulgaris*. Int J Mol Med 15:491-496.

Farquhar GD, Dubbe DR, Raschke K (1978). Gain of the feedback loop involving carbon dioxide and stomata. Plant Physiol 62:406-412.

Gardner FP, Pearce RB, Mitchell RL (1985). Physiology of Crop Plants. Iowa State University Press, USA, 186-208 p.

Grant RF, Kimball BA, Brooks TJ, Wall GW, Printer PJ, Hunsaker DJ, Adamsen FJ, LaMorte RL, Leavitt SW, Thompson TL, Matthias AD (2001). Interactions among CO_2, and climate on energy exchange of wheat: model theory and testing with a free-air CO_2 enrichment (FACE) experiment. Agron J 93:638-649.

Guenther E (1961). The Essential Oils. D. von Nostrand Comp. Press, New York.

Guginski G, Luiz AP, Silva MD, Massaro M, Martins DF, Chaves J, Mattos RW, Silveira D, Ferreira VMM, Calixto JB, Santos ARS (2009). Mechanisms involved in the antinociception caused by ethanolic extract obtained from the leaves of *Melissa officinalis* (lemon balm) in mice. Pharmacol Biochem Be 93:10-16.

Hebeisen T, Luscher A, Zanetti S, Fischer BU, Harwig UA, Frehner M, Hendrey GR, Blum H, Nosberger J (1997). The different response of *Trifolium repens* L. and *Lolium perenne* L. grassland to free air CO_2 enrichment and management. Glob Change Biol 3:149-160.

Hogy P, Fangmeier A (2009). Atmospheric CO_2 enrichment affects potatoes: 1. Aboveground biomass production and tuber yield. Eur J Agron 30:78-84.

Huan Y, Hua-Song W, Zhi-Jie W (2010). Evaluation of SPAD and Dualex for In-Season Corn Nitrogen Status Estimation. Acta Agron Sin 36:840-847.

Ibarra A, Feuillere N, Roller M, Lesburgere E, Beracochea D (2010). Effects of chronic administration of *Melissa officinalis* L. extraction anxiety-like reactivity and on circadian and exploratory activities in mice. Phytomed 17:397-403.

IPCC (2007). Climate Change. Cambridge University Press, New York.

Jeffery S, Verlan C, Cochran L (2006). Response of potato (*Solanum tuberosum* L.) to elevated atmospheric CO_2 in the North American Subarctic. Agr Ecosyst Environ 112:49-57.

Kima HY, Lieffering M, Kobayashic K, Okadad M, Mitchelle MW, Gumpertze M (2003). Effects of free-air CO_2 enrichment and nitrogen supply on the yield of temperate paddy rice crops. Field Crop Res 83:261-270.

Kimball BA, Kobayashi K, Bindi M (2002). Responses of agricultural crops to free-air CO_2 enrichment. Adv Agron 70:293-368.

Koocheki A, Nassiri-Mahallati M, Mondani F, Feizi H, Amirmoradi S (2008). Evaluation of radiation interception and use efficiency by maize and been intercropping canopy. Agroecol 1:23-31.

Lambers H, Stulen I, Werf A (1996). Carbon use in root respiration as affected by elevated atmospheric CO_2. Plant Soil 187:251-263.

Li F, Kang S, Zhang J (2004). Interactive effects of elevated CO_2, nitrogen and drought on leaf area, stomatal conductance, and evapotranspiration of wheat. Agr Water Manage 67:221-233.

Lu Z, Radin JW, Turcotte EL, Percy R, Zeiger E (1994). High yields in advanced lines of 'Pima' cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. Plant Physiol 92:266-272.

Mavrogianopoulos GN, Spanakis J, Tsikalas P (1999). Effect of CO_2 enrichment and salinity on photosynthesis and yield in melon. Sci Hortic 79:51-63.

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De-Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E, Rey A, Roberntz P, Sigurdsson BD, Strassemeyer J, Wang K, Curtis PS, Jarvis PG (2001). Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. New Phytol 149:247-264.

Ommen OE, Donnelly A, Vanhoutvin S, Van-Oijen M, Manderscheid R (1999). Chlorophyll content of spring wheat flag leaves grown under elevated CO_2 concentrations and other environmental stresses within the 'ESPACE-wheat' project. Eur J Agron 10:197-203.

Rajeswara-Rao BR (2001). Biomass and essential oil yields of rainfed palmarosa (*Cymbopogon martinii* (Roxb.) Wats. var. motia Burk.) supplied with different levels of organic manure and fertilizer nitrogen in semi-arid tropical climate. Ind Crop Prod 14:171-178.

Reddy KR, Robana RR, Hodges HF, Liu XJ, McKinion JM (1998). Interactions of CO_2 enrichment and temperature on cotton growth and leaf characteristics. Environ Exp Bot 39:117-129.

Schahczenski J, Hill H (2009). Agriculture, Climate Change and Carbon Sequestration. A Publication of ATTRA-National Sustainable Agriculture Information Service. www.attra.ncat.org.

Schnitzler P, Schuhmacher A, Astani A, Reichling J (2008). Melissa officinal is oil affects infectivity of enveloped herpesviruses. Phytomed 15:734-740.

Shoor M, Goldani M, Mondani F (2008). Effect of CO_2 enrichment on morphophysiologycal traits in Tagets, *Ageratum* and *Gauilardia* in greenhouse condition. Agroecol 2:108-114.

Van-Labeke MC, Dambre P (1998). Effect of supplementary lighting and CO_2 enrichment on yield and flower stem quality of *Alstroemeria* cultivars. Sci Hortic 74:269-278.

Yang L, Huang J, Yang H, Zhu J, Liu H, Dong G, Liu G, Han Y, Wang YL (2006). The impact of free-air CO_2 enrichment (FACE) and N supply on yield formation of rice crops with large panicle. Field Crop Res 98:141-150.

Yang L, Wang Y, Dong G, Gu H, Huang J, Zhu J, Hongjian Y, Liu G, Han Y (2007). The impact of free-air CO_2 enrichment (FACE) and nitrogen supply on grain quality of rice. Field Crop Res 102:128-140.

Young YC, Sungbong O, Myoung MO, Jung ES (2007). Estimation of individual leaf area, fresh weight, and dry weight of hydroponically grown cucumbers (*Cucumis sativus* L.) using leaf length, width, and SPAD value. Sci Hortic 111:330-334.