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Effects Of Temperature DIF And Drop On The Growth, Quality, Total Phenolic Content And Antioxidant Activity Of Herbs

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Abstract*—Basil (*Ocimum basilicum* L.), lemon balm (*Melissa officinalis* L.) and coriander (Coriandrum sativum) were grown in growth chambers to investigate the effect of different day/night temperature differentials (DIF) and drop on growth, antioxidant activity and total phenolic content. The day/night temperature combinations with same average temperature were 20/20ºC (0 DIF), 32/8ºC (+24 DIF), 30/10ºC (+20 DIF), 20/20ºC with 12 ºC (-12ºC drop) and 6ºC drop (-6ºC drop) at the end of night for 2 h. The response to positive DIF on growth (shoot length, fresh and dry weight of shoot) varied among the species and was greatest at +24 DIF than in all other treatments. Effect of +24 DIF on shoot length was opposite in lemon balm to that in basil. In lemon balm, shoot length was shortest at +24 DIF while in basil it was longest. Under positive DIF treatments (+20 and +24 DIF) dry weight of shoot (mg cm^{-1} shoot length) was higher in lemon balm but lower in basil. In general, there was little effect of temperature DIF and drop on growth of coriander. There was a pronounced effect on postharvest life of plants grown at +24 DIF compared to those grown at constant temperature, but the effect varied among the species. In basil, postharvest life was reduced in plants grown under +24 DIF, while the opposite result was found for lemon balm. Antioxidant activity and total phenolic contents were 2-3 times higher in lemon balm than in basil, and higher in samples collected at the end of day to those start of the day. Samples from +24 DIF and -12ºC drop both had lower antioxidant activity and total phenolic content than the control (0 DIF); the lowest were at +24 DIF. The results indicate that, although +24 DIF can reduce the shoot length and improve the shoot quality in lemon balm, it reduces total phenolic content and antioxidant activity thus reducing quality.

Keywords—Shoot length; Antioxidant activity; Phenolic content; Ocimum basilicum L.; Melissa officinalis L.; Postharvest life

I. INTRODUCTION

Air temperature is one of the major factors affecting the growth, productivity and quality of herbs. Manukyan and Schnitzler [9] found that air temperatures had substantial influence on the productivity of medicinal plants grown under controlled environment conditions. Temperature affects chemical reactions and the physical properties of plants, and this influence occurs both at cellular and plant level. According to Dorais et al. [5] temperature is also the most important climatic factor influencing sink strength and consequently photo-assimilate partitioning between plant organs. Control of stem elongation is the major problems in commercial production of herbs [19]. Basil (*Ocimum basilicum),* lemon balm (*Melissa officinalis)* and coriander (*Coriandrum sativum*) are used for human consumption, and the use of any chemical growth retardants during growth of these crops is not allowed in Norway. Control of shoot length through regulation of greenhouse climates is a good alternative to chemical growth retardants. High day and low night temperatures (positive DIF) enhance the elongation of herbs, produce weaker shoots and thus reduced the quality, whereas negative DIF reduced the elongation and improved the quality [19]. Negative DIF conditions require more energy than positive DIF. The reduction of energy per unit production area is an important issue today, as is maintaining the quality of herbs grown. Natural light and/or artificial light increase the heat of the greenhouse during day time while night time temperature is lower (i.e. positive DIF). Temperature drop during night may diminish the effect of high day temperature and improve the quality of the plants. A complex investigation of temperature drop is therefore important [16].

Scientists, manufacturers, producers, and consumers have gained interest on phytochemicals and antioxidant constituents in plant material [10]. There has been an increased interest in aromatic and medicinal herbs as sources of natural antioxidants. Herbs have been identified as sources of various phytochemicals, many of which possess important antioxidant activity [20, 8]. Polyphenols, a group of chemical substances found in plants, may have antioxidant characteristics with potential health benefits. Fresh herbs exhibited stronger antioxidant activity and contained significantly higher phenolic contents compared to common vegetables and fruits [2].

Basil which contains many antioxidants components is one of the most popular fresh herbs grown in Europe. The potency of basil as a natural antioxidant is due to the high content of phenolic compounds**.** Rosmaric acid is the main active component in basil, and its superior antioxidant activity confirms the importance of basil as a culinary herb [6].

Lemon balm grows widely in central and southern Europe and in Asia Minor. Phenolic and polyphenolic substances present in lemon balm have proved to possess antioxidant activity; therefore, it is likely that they are contributing to the antioxidant properties of the extract [3].

To understand how the climatic factors affect the growth, total phenolic content and antioxidant activity of herbs is thus important. The aim of this study is to investigate the effect of positive DIF and intensity of temperature drop on growth of selected herbs, and also their antioxidant activity and total phenolic contents.

II. MATERIALS AND METHODS

A. Plant material and general growing conditions

Three different species of herbs; basil (*Ocimum basilicum),* lemon balm (*Melissa officinalis)* and coriander (*Coriandrum sativum*) were sown in growth chambers providing 200 (±10) µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) artificial light for 16 h d^{-1} photoperiod at 75% relative humidity (RH) at the Norwegian University of Life Sciences. The light was measured at pot height level with a quantum sensor (model LI-250, LI-COR, Lincoln, Nebr.). Dry and wet sensors were placed close to the plant canopy to monitor the temperature and relative air humidity. A PRIVA greenhouse computer was used for recording, control and storage of climate data. The seeds were sown directly on 12 cm pots in peat medium**.** Depending on the seed size 25 to 50 seeds were sown in each pot. The herbs were grown under different day/night temperature differentials (DIF) and temperature drops at the end of night for 2 h. The day/night temperature combinations were 20/20ºC (0 DIF), 32/8ºC (+24 DIF), 30/10ºC (+20 DIF), 20/20º with 12ºC (-12ºC drop) and 6ºC drop (-6ºC drop) with same average diurnal temperature (Fig. 1). After seeding the seeds were covered with vermiculite and 15 pots from each of the species were placed under each treatment. For about 1 week, the temperature in all chambers was kept at 20°C to provide equal germination conditions. After germination, thinning was done and 15 basil, 20 coriander, and 30 lemon balm plants per pot were grown until harvest. Initially, the pots were placed close to each other giving no space in between the pots, and after 1 week the pots were placed at 5 cm X 5cm spacing until the final harvest. The size of each growth chamber was 1.5 m X 1.0 m.

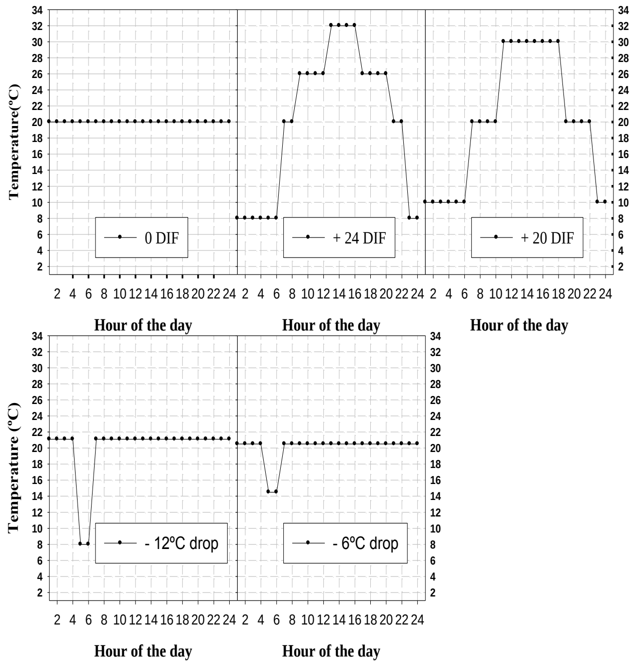

Fig. 1. Set point for different temperature DIF and drop treatments with the same average diurnal temperature of 20ºC (Day: 7.00-23.00; night: 23.00-7.00).

B. Measurement of growth data

For the growth data (shoot length, fresh and dry weight of shoot) 8 pots per treatment of each species were harvested. Basil and coriander was harvested 30 days after seeding (DAS) and lemon balm after 35 DAS. The fresh herbs were dried at 70°C for 3-5 days. Plants with shorter shoot length and higher shoot dry weight were considered as good quality.

C. Leaf sample collection for analysis

Analysis was carried out to determine if there is any change of total phenolic content and antioxidant activity due to different day/night temperature regimes. Samples were collected 35 DAS from basil and lemon balm, and at 10.30 pm (end of day; 30 minutes before the light turned off) and at 07.15 am (at day start; 15 minutes after the light turned on in

the morning). The upper fully expanded leaf pair was collected from 5 plants. Immediate after detachment from the plants the leaf samples were submerged in liquid nitrogen, ground to a coarse powder in a porcelain mortar with liquid nitrogen, and then stored in plastic tubes at -70ºC for analysis.
A given amount of the ground material was dissolved in 30 ml of methanol. The bottles were flushed with nitrogen before being capped; the samples were then mixed and sonicated in a water-bath at 0°C for 15 min. The extracts were stored at -20ºC until analyzed.

D. Postharvest

At the time of harvest 5 pots of each species and each treatment were placed in a test room. In the test room, florescent tubes were used for 12 h d^{-1} photoperiod to provide 15 µ mol m^{-2} s^{-1} PPFD at pot height level, with 40% RH and a temperature of 19 (±1)°C. Before placing in the test room the pots were kept well watered and placed in 'polyethylene bags'. For postharvest water loss from the whole plants, initial weights of the pots were taken and measured at every 24 h for 5-7 days. The postharvest test was ended when plants showed wilting symptom. No water was added until wilting and/or for the first 7 days. The plants with longer postharvest life during this test were considered to be of good quality.

E. Antioxidant activity and total phenolic content

The antioxidant activity was measured by Ferric reducing activity power (FRAP) assay [1] carried out using a Konelab 30i (Thermo Electron Crop., Vantaa, Finland). In brief, a 200 µl of FRAP reagents (3.0 mM acetatebuffer, 10 mM 2,4,6-tri (2pyridyl)-s-triazine in 40 mM HCl, 20 mM $FeCl_3$,$6H_2O$, ratio 10:1:1) were automatically pipetted separately and mixed in the cuvettes, 8 µl of sample were added, mixed and incubated at 37°C for 10 min, before the absorbance was measured at 595 nm. Trolox (Vit. E analogue) were used as control. The sample replicated twice and each replication repeated three times in Konelab 30i analysis. The results are expressed as µmol g^{-1} fresh weight (FW).

Total phenolic content was estimated as gallic acid equivalants (GAE; mg gallic acid equivalents per 100g FW) using a Konelab 30i as described by Singleton et al. [15]. In brief, a 20 µl sample were added to 100 µl Folin-Ciocalteu reagent (diluted 1:10 with distilled water), mixed and incubated at 37°C. After 1 min, 80 µl 7.5% (w/v) Na_2CO_3 was added and were mixed and incubated at 37°C for 15 min. The sample replicated twice and each replication repeated six times in Konelab 30i analysis. The absorbance was measured at 765 nm and assessed against a gallic acid calibration curve. The plants with higher total phenolic contents and higher antioxidant activity were considered to be of good quality.

F. Statistical analysis

Data were subjected to an analysis of variance (ANOVA) with individual pots as replicates. Data are expressed as the mean values of samples and ±SE indicated. Means within treatments were separated by the least significant difference (LSD) at $P<0.05$ probability level.

III. RESULTS

A. Measurement of growth data

The herb species responded differently to the different day/night temperature (Fig. 1) regimes. For example, the effect of +24 DIF was opposite in lemon balm to that in basil. In lemon balm, shoot length was shortest at +24 DIF and longest at 0 DIF. On the other hand in basil, the longest shoots were recorded under +24 DIF while the shortest were at 0 DIF. The temperature drop treatment didn't have any significant effect on the shoot length of herbs (Fig. 2). In coriander there was little effect of positive DIF on increasing shoot length.

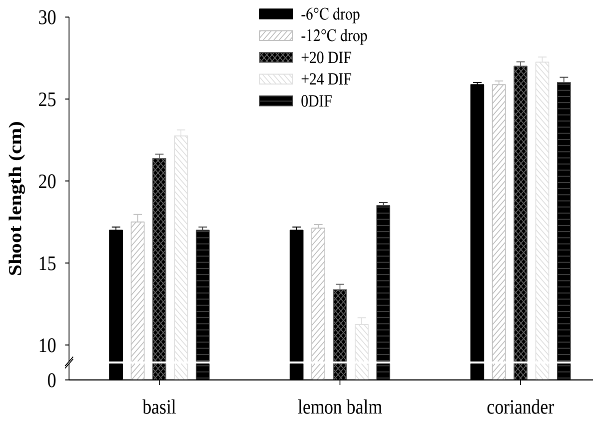

Fig. 2. Effect of temperature DIF and drop on the shoot length of basil, lemon balm and coriander (the bars indicate ± SE).

Differences in shoot weights (fresh and dry weight) were recorded in different treatments but the effects varied among the species. Fresh weight basil increased under +20 and +24 DIF treatments while in lemon balm and coriander it was reduced under +24 DIF (Fig. 3 A). In respect to species and treatments there was little effect on percent shoot dry weight except in basil where it was reduced under positive DIFs (Fig. 3 B). The dry weight (mg cm^{-1} shoot length) was higher in positive DIF treatments (+20 and +24 DIF) only in lemon balm, while in basil it was reduced. In lemon balm, highest (278 mg cm^{-1} shoot length) at +24 DIF and lowest (180 mg cm^{-1} shoot length) at 0 DIF was recorded, and in basil highest (184 mg cm^{-1} shoot length) was in 0 DIF and lowest (144 mg cm^{-1} shoot length) was recorded at +24 DIF (Fig. 3 C).

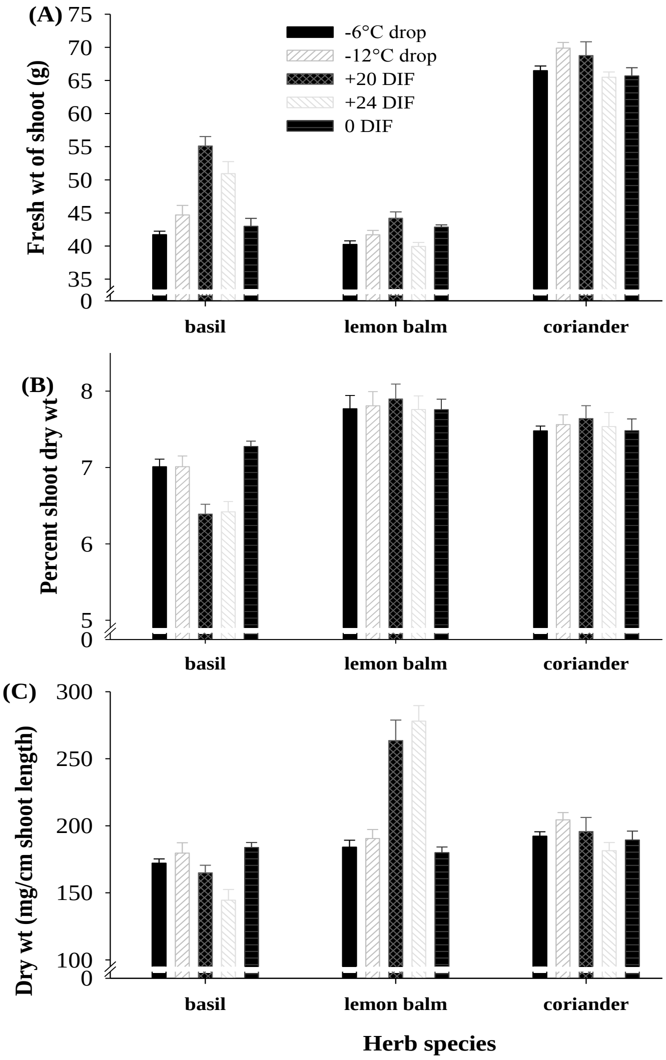

Fig. 3. Effect of temperature DIF and drop on fresh wt of shoot (A), percent shoot dry wt (B) and dry wt (mg/cm shoot length) (C) of basil, lemon balm and coriander (the bars indicate ± SE).

B. Postharvest

Positive DIF treatments affected postharvest life of all herbs, but the effect varied among species. Postharvest life of basil and coriander was the shortest in plants grown under +24 DIF, while in lemon balm it was longest (Fig. 4). In basil, the postharvest life increased slightly in plants grown under 0 DIF, while in coriander the longest was recorded in plants grown under -12°C drop treatment. In lemon balm, the rate of water loss from the whole plants during postharvest period was the lowest in plants grown under +24 DIF compared to other treatments (data not presented). In lemon balm, the longest (11 days) postharvest life was recorded in plants from +24 DIF while the shortest (8.4 days) was recorded in -6°C drop treatment. In basil, positive DIF treatments reduced postharvest life, and shortest (6 days) was recorded at plants grown under +24 DIF while longest (8.2 days) was at 0 DIF (Fig. 4).

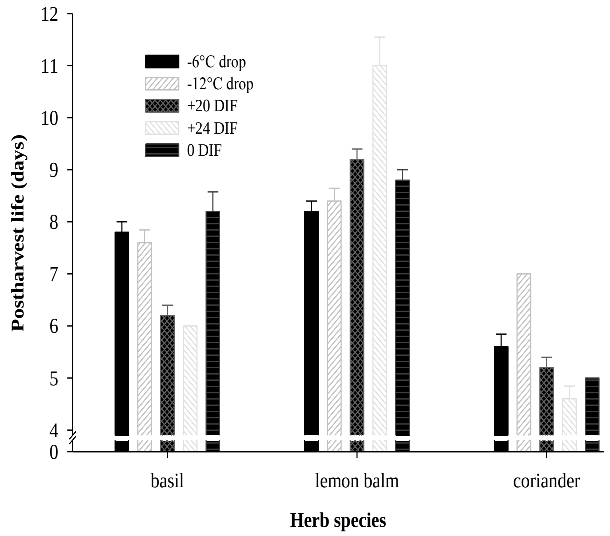

Fig. 4. Effect of temperature DIF and drop on the postharvest lifeof basil, lemon balm and coriander (the bars indicate ± SE).

Antioxidant activity and total phenolic content

The antioxidant activity was much higher in lemon balm than in basil. The amount was higher in the 10.30 pm samples than in the 07.15 am samples. In lemon balm, the 10.30 pm sample of control treatments (0 DIF) gave the highest activity (188 µmol g^{-1} fresh weight), while the lowest activity was in the 07.15 am samples at +24 DIF (78 µmol g^{-1} fresh weight). In basil, the highest (53 µmol g^{-1} fresh weight) was recorded in the 10.30 pm sample of 0 DIF, and the lowest (33 µmol g^{-1} fresh weight) in the 07.15 am sample of +24 DIF (Fig. 5 A).

The total phenolic content of the leaves was higher in lemon balm than in basil, being higher in the 10.30 pm samples than in the 07.15 am samples in both the species (Fig. 5 B). In lemon balm, 10.30 pm sample from control treatments (0 DIF) had the highest content (986 mg GAE per 100 g fresh weight) while the lowest was recorded in the +24 DIF treatment (440 mg GAE per 100 g fresh weight). The corresponding values for basil were 281 and 196 GAE per 100 g fresh weight (Fig. 5 B).

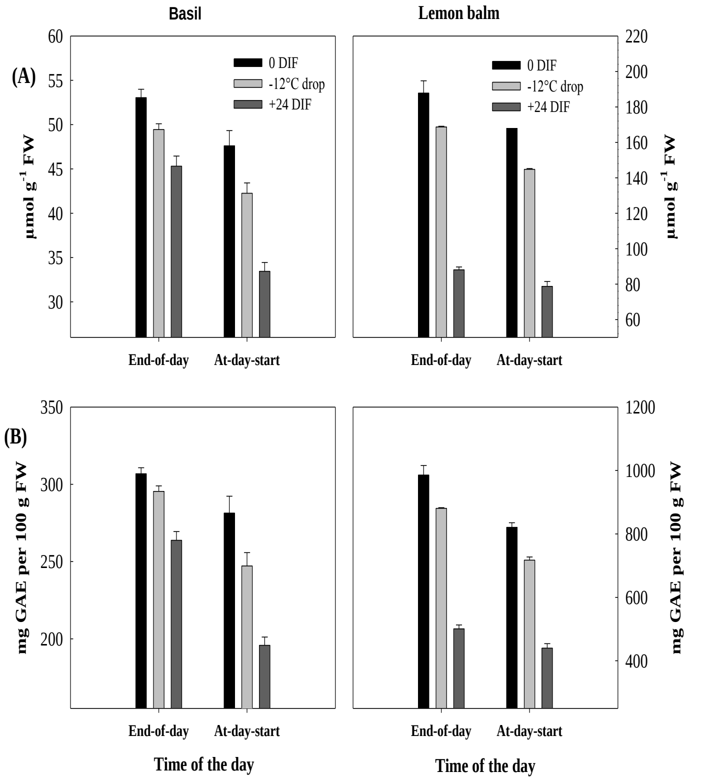

Fig. 5. Effect of temperature DIF and drop, and sampling time on the antioxident activity (A) and the total phenolic contents (GAE; mg gallic acid equivalents/100g fresh weight (FW)) (B) in the leaf samples of basil and lemon balm (the bars indicate ± SE).

DISCUSSION

Plants grown under +24 DIF produced shortest stems in lemon balm with higher dry weight (mg cm^{-1} shoot length) and the opposite result was in basil. The different effect of DIF on basil and lemon balm may be related to climate of their geographical origins. Basil originated from tropical Asia and the Pacific Islands and lemon balm is native to southern Europe and northern Africa and grows in mild temperate zones. Thus, basil grown with higher positive DIF may grow faster, and lemon balm may be connected with nonspecific effects of stress. The positive DIF can be used as a way of controlling plant size in lemon balm. Effect of DIF on the stem elongation may elicit responses through affecting the concentration of endogenous gibberellins [11]. A short temperature drop to 4°C in the morning reduced internodes elongation and shoot length in some bedding plants [7]. However, temperature drops for 2 h in our study had little or no effect on growth and quality. Probably, 2 h drop is not long enough time to have a strong effect as positive DIF. In cucumber, temperature drop duration for 2 h had no effect but drop for 4 and 6 h had an accelerated effect on growth [18]. The role of thermoresistance on whole plants to a temperature drop treatment is not clear [17]. To use higher day temperature (above 32ºC) and for long periods, care would be needed, as this may cause excessive shortening in lemon balm and excessive elongation in basil. The effect of +24 DIF in increasing shoot length of basil is in agreement with the results of Putievsky [14]; where increasing day temperature in the range 21°C to 30°C increased shoot length and dry weight. However, to avoid stem strength problems it would require high irradiance with such use of positive DIF [4]. Increasing postharvest life in lemon balm and decreasing postharvest life of basil in plants grown under +24 DIF is related to rate of water loss during postharvest period. The different rate of water loss in different temperature treatments may be related to stomata malfunctioning during postharvest. However, daily variation of RH can be an effective measure to avoid the malfunction of stomata, as it ensures the development of normal stomata functioning in roses [13].

Total phenolic content and antioxidant activity were affected by the DIF and drop (+24 DIF and -12°C drop) treatments where both the amounts were reduced compared to control (0 DIF). The affects were similar for both phenolic content and antioxidant activity. Thus, it is worth remembering that manipulation of shoot length through temperature manipulation (positive DIF) may reduce the content of phenolic compounds and antioxidants in herbs. The plants grown under +24 DIF had the lowest total phenolic content and antioxidant activity. Our result is in agreement with [12], whose studies reported a high correlation between antioxidant activity and total polyphenols. In lemon balm 25°C gave high content and yield of essential oil [9] whereas 32°C temperature during day may be too high temperature; that may account for the reduce contents that we recorded. The composition of herbs' essential oil depended on air temperature [9]. Total volatile oil content in fresh leaves was three times higher when grown at 25°C than those grown at 15°C. Our results indicate that constant temperature is the best treatment if all the species need to be grown under the same greenhouse condition.

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