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Growth responses of *Lavandula pubescens* to temperature regimes of Tabuk, Saudi Arabia

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Abstract
Tabuk region of Saudi Arabia is characterized by highly variable environmental conditions where temperature goes extremely low to extremely high and affects the morphology, growth, physiology and biochemistry of the plants. In this research, we investigated the growth and stress tolerance in a medicinally important plant, namely *Lavandula pubescens* (family Lamiaceae) in response to temperature dynamics. The plant samples were collected from Tabuk-Jorden road on the 15th day of January, April, July and October 2013. Plant height, fresh and dry matter, total chlorophyll nitrogen (N), phosphorus (P) and potassium (K), leaf relative water contents were determined in terms of growth of the plant. Physiological and biochemical parameters of plants like activity of carbonic anhydrase (CA), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and leaf protein contents were also determined. Plant growth parameters showed a significant increase in height, fresh and dry matter accumulation, total chlorophyll, NPK and leaf relative water contents investigated in the month of April and October 2013. Plant growth was suppressed and an active role of CAT, POD and SOD was observed to cope with the extremely low temperature in January and extremely high temperature in July 2013. Therefore, based on the results it is recommended that during April and October the environmental conditions are best suitable for growth, development and medicinal use of *Lavandula*. However, the plants are also found capable to adapt and tolerate the temperature regimes in different seasons of Tabuk.

Keywords: Antioxidants, Climate Change, Growth, Medicinal Plants

1. Introduction
Any change in climatic condition results in enhanced intensity of solar ultraviolet radiation, seasonal and daily variations in temperature [1] results in accumulation of chemically active molecules and free radicals in plant cells causes alterations in metabolic processes [2]. To cope with such detrimental conditions, plants are equipped with a system of antioxidant enzymes, which inhibit free radical processes [3]. It is shown that under extreme conditions the protective mechanism of antioxidant system is activated. The higher is the antioxidant activity, the more resistant is the species toward the stressor. Tabuk region of Saudi Arabia is characterized by highly variable environmental conditions from extremely low to high temperatures (Table 1). These seasonal variations in temperature significantly affect the medicinal properties of the plants. Therefore, in this research, we investigated the impact of seasonal dynamics of temperature on an important medicinal plant *Lavandula pubescens* in terms of its growth, physiological and biochemical attributes.

Table 1: Temperature regimes in four different seasons of Tabuk, Saudi Arabia, in 2013 (recorded on 15th day of each month).

Temperature regimes (ºC)	January-2013	April-2013	July-2013	October-2013
Average	10.4	23.9	34.6	22.2
Maximum	19.0	32.0	42.0	29.0
Minimum	2.0	17.5	26.0	15.0

2. Materials and Methods
In this research a medicinally important plant of Tabuk namely *Lavandula pubescens* was selected. Plant samples were collected from Tabuk-Jorden road (760 meter above sea level) on the 15th day of January, April, July and October 2013 to evaluate the effect of temperature dynamics of Tabuk on *Lavandula* plants. For fresh weight the plants were uprooted and

washed to remove surface adhered soil particles and wrapped in blotting papers. Dry weight of plants was recorded after drying the plants at 80 °C for 24 hours in hot air oven. The nitrogen and phosphorus contents were determined using the method of Lindner [4] and Fiske and Subba Row [5] respectively. Potassium was determined with a flame photometer (AIMIL). The activity of carbonic anhydrase (E.C. 4.2.1.1) was measured using the method as described by Dwivedi and Randhawa [6]. The enzyme was expressed as µM CO_2 kg-1 leaf FW s-1. Total chlorophyll contents in leaves were estimated using the method of Lichtenthaler and Buschmann [7]. Leaf relative water content (LRWC) measured by adopting the method of Yamasaki and Dillenburg [8] using following formula:

$$LRWC\ (\%) = [(FM-DM)/(TM-DM)] \times 100$$

Leaf protein content was determined according to Bradford [9] using BSA as a standard. For antioxidant enzymes assay leaf tissues were homogenized with three volumes (w/v) of an ice-cold extraction buffer (50 mM Tris–HCl, pH 7.8, 1 mM EDTA, 1 mM MgCl2 and 1.5% (w/w) polyvinyl pyrrolidone). The homogenate was centrifuged at 15,000g for 20 min at 4 °C. The supernatant was used as the crude extract for the assay of enzyme activities. Superoxide dismutase (SOD; E.C. 1.15.1.1) activity was determined according to Beauchamp and Fridovich [10] by following the photo-reduction of nitro blue tetrazolium (NBT). Activity of CAT (E.C. 1.11.1.6) was measured in accord to Cakmak and Marschner [11]. Activity of POD (E.C. 1.11.1.7) was assayed by the method of Upadhyaya [12]. Secondary metabolites were estimated using dry leaf material (1 g) following Zhao and Zeng [13]. Statistical analysis of data obtained from research was done using five replicates of collecting plants for each season. The data was analysed statistically for significance at 5% level of probability using SPSS software for statistical analysis.

3. Results

It is evident from the results that *Lavandula* plants, collected in four different seasons in Tabuk region, exhibited a diverse pattern of growth, physiological and biochemical attributes (Table 2). Plants collected in April 2013 exhibited highest values for plant height (65.68 cm), whereas the significant reduction in plant height was recorded in July 2013 (50.24cm) which was the least value among the four seasons. However, the plants collected in October and April showed at par values for plant height. A highest (55.45g) value for fresh weight per plant was recorded in April 2013, whereas it was lowest (40.76g) in July 2013. The values for fresh weights were at par in January and July 2013.

Plants exhibited maximum (30.37g) dry matter accumulation and minimum (20.81g) during April and July, respectively. However, the plants showed almost similar dry matter accumulation pattern in April-October and January-July 2013. For nitrogen, phosphorus and potassium (NPK) uptake by plants a similar pattern was observed. The results of these growth parameters showed that the plants investigated in January and July gave lower values for the growth parameters as compared to plants collected in April and October. CA enzyme in plants was found highly active in October and least in January 2013. The plants in April and October showed a similar pattern for CA activity.

Table 2 showed that the plants investigated in October gave highest values for leaf chlorophyll contents whereas; plants in July gave lowest values for the same parameter. There was a significant increase in leaf chlorophyll content observed in October 2013. Plants grown in October accumulated highest leaf relative water content as compared to the plants in July 2013 with least value. *Lavandula* has grown in July showed the highest values for SOD enzyme while the plants grown in April gave the least value. However, activity of SOD was at par in plants grown in April and October 2013.

The plants grown in July exhibited maximum POD activity as compared to the plants with lowest values of this enzyme in April. However, the same parameter was at par in plants investigated in January-July and April-October 2013. Catalase (CAT) activity also showed a similar trend as shown by SOD and POD. A significant enhancement in CAT activity in plants was recorded in July and a reduction in April 2013. The data showed that leaf protein content increased in April as compared to the plants observed in January-July 2013. A significant difference in leaf protein contents in plants was observed in different seasons of Tabuk (Table 2).

Table 2: Response of *Lavandula pubescens* to temperature regimes in Tabuk, Saudi Arabia.

Plant Parameters	January 2013	April 2013	July 2013	October 2013	CD at 5%
Height (cm)	55.78±3.10	65.68±2.63	50.24±2.52	60.54±3.01	4.15
Fresh weight (g)	53.77±3.41	55.45±4.31	40.76±1.05	50.87±2.02	6.28
Dry weight (g)	23.28±1.91	30.37±1.53	20.81±1.81	25.22±2.21	2.78
Leaf relative water contents (%) LRWC	63.45±2.61	68.77±0.95	47.56±1.22	73.75+2.52	2.76
Leaf protein (mg/g)	5.10±0.21	6.41±0.13	5.13±0.16	5.42±0.26	0.35
Leaf chlorophyll (mg-1 leaf FW)	1.80±0.01	1.90±0.03	1.67±0.02	2.80±0.02	0.07
Nitrogen (mg 100 mg-1DW)	2.84±0.135	3.26±0.152	2.34±0.173	2.91±0.184	0.24
Phosphorus (mg 100 mg-1DW)	0.580±0.055	0.735±0.048	0.548±0.057	0.696±0.038	0.052
Potassium (mg 100 mg-1DW)	1.02±0.067	1.54±0.056	1.33±0.068	1.34±0.044	0.105
CA (µM CO2 kg-1 leaf FW s-1)	380.76±5.43	420.55±8.12	415.35±6.21	430.93±4.82	6.84
CAT (Units g-1 leaf FW)	22.40±0.88	15.36±0.95	26.22±1.07	18.21±1.10	1.29
POD (Units g-1 leaf FW)	53.35±2.52	33.92±1.02	55.86±0.98	36.65±2.21	2.78
SOD (Units g-1 leaf FW)	103.74±3.13	98.87±3.20	115.56±2.45	100.67±2.33	3.54

Data = Mean±SD, CD = Critical Difference at 5% probability.

4. Discussion

In this research, we studied growth, morphological, physiological and biochemical attributes of an important medicinal plant *Lavandula* in response to varying temperature conditions of Tabuk, Saudi Arabia. The plant species investigated in April and October 2013 showed optimum growth (plant height, fresh weight, dry weight accumulation and uptake of NPK) whereas the same parameters showed a significant decrease in July and January 2013. Prakash *et al*. [14] observed an increase in morphological characteristics of plants in suitable environmental conditions and a decrease beyond a certain limit. Sudden and extreme increase in temperature is accompanied with more stressful conditions which affect the growth and development of plant species.

Temperature is an important determinant of morphology, physiology and biochemistry of plants. Growth patterns of *Lavandula* plants, fresh and dry matter accumulation and nutrient contents (NPK) were studied as they indicate primary productivity in response to temperature regimes [15]. Temperature not only acts as an important limiting factor for enzymatic activities and metabolism of plants, but also regulates cell division, translocation of food and photosynthesis. A temperature of 30 ºC is optimal for most biochemical processes [16] in plants. Effect of temperature also pronounced when the duration of treatment exceeded [17].

The reduced growth characteristic in the studied plants at higher temperature can also be explained on the basis of the fact that plants grown in July are exposed to greater irradiance, large diurnal fluctuations of temperature, increased rate of transpiration due to high wind velocity, reduced partial pressure of gases, limited water and nutrient supply and a narrow time window for growth and development of plants [18] [19, 20]. Moreover, induced expression of antioxidants was also recorded at higher temperature during July 2013, which might have protected the plants from extreme temperature reflected in the form of some improvement of growth and physiological parameters in the *Lavandula* plants.

Regarding physiological and biochemical parameter, CA activity was recorded higher in plant species grown in April and October 2013 and it was lower in plants investigated in July and January 2013. CA is the enzyme which catalyzes the reversible hydration of CO_2 and maintained its constant supply to rubisco resulted in an optimum rate of photosynthesis and thus more dry matter accumulation. Therefore, increased CA activity might have been one of the reasons for improved growth performance of plants at optimum temperature in April and October 2013. It was also reported that while transpiration rates increased with lower atmospheric pressure at higher temperature, the corresponding increase in CO_2 uptake was relatively higher than expected [21], thus there is a possibility of greater non-stomatal capability for CO_2 uptake in plants at higher temperature [22].

Leaf chlorophyll content is another important parameter in determining the growth and yield performance of a plant. An increase in chlorophyll content was recorded in October. However, a significant decrease was noted at the highest temperature during the month of July 2013. The reason behind decrease in chlorophyll content at higher temperatures may be due to the photo-oxidation of pigments in the presence of high radiations [23]. A significant decrease in leaf relative water contents was recorded with a concomitant increase in temperature. The decrease in leaf relative water contents at higher temperature in July is due to high wind velocity, large diurnal fluctuations in temperature and limited supply of water and nutrients [21]. This results from the higher total radiation absorbed by leaves, increase in the diffusion coefficient of water vapor in air and the intern increase in transpiration in reduced barometric pressure and the increased density gradient of H_2O vapor from the leaf to the ambient air [21, 24].

An extreme increase and decrease in temperature causes generation of several detrimental effects and set the plants under stress leading to the generation of reactive oxygen species (ROS). However, to cope with such stressful conditions, plants are equipped with antioxidant enzymes such as SOD, POD, and CAT. These enzymes prevent or alleviate the damage caused by ROS and set the plants to perform normally even under stressful conditions. The results exhibit that SOD, POD and CAT show a parallel increase in the activities with increasing temperature giving highest values. Thus, increased antioxidant enzyme activities protected the plants from stressful environment which is reflected in terms of improved growth and dry matter and protein accumulation, CA activity and leaf Chl content in the plants grown in July and January 2013. On the other hand, plants grown in April and October showed a decline in antioxidant enzyme activities and enhanced growth, physiological and biochemical parameters including protein accumulation. The inhibition in the activities of antioxidant enzymes during optimum temperature was recorded by Wang *et al.* [25].

Xu *et al*. [26] also reported that genes function differently in response to temperature, osmotic and salt stress. A stressful condition such as low temperature, UV-B radiations causes reduction in biomass accumulation but presence of light absorbing phenyl compounds and other metabolites protect plants from UV-B radiations [27]. The antioxidant activities can selectively resist the free radicals generated by UV-B irradiation [28, 29]. Sridevi and Girdhar [30] and several authors suggested the dependence of secondary metabolite production on the temperature. A decrease in temperature has been shown to induce more accumulation of these compounds.

Any changes in climatic conditions affect growth and development processes in plants as they are depend on many factors, including retention time, season, temperature, pH, diversity of species, nutrients availability, hydraulic regimes, plant harvesting and light intensity etc [31, 32, 33, 34].

5. Conclusions

Based on the results of this research it is recommended that during April and October the environmental conditions are best suitable for growth, development and medicinal use of *Lavandula pubescens.*

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