

INFLUENCE OF DIVERSE CLIMATIC AND SOIL CONDITIONS ON THE PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES AND ANTIOXIDANT ACTIVITIES OF WILD MILK THISTLE (*SILYBUM MARIANUM* L. GAERT.)

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(Received 13th Jan 2020; accepted 6th May 2020)

Abstract. Wild Milk thistle (*Silybum marianum* L. Gaert.) plants were collected from ten different locations of Punjab, Pakistan during the years 2018 and 2019. These locations have variations for soil characteristics and climatic conditions. Significant variations were noted in all the parameters from location to location as well as year to year. Antioxidant activities i.e. catalases (CAT), peroxidase dismutase (POD) and superoxide dismutase (SOD) changed significantly under fluctuating climatic conditions. Changes in antioxidant activities altered the defense mechanism of milk thistle and caused better balance in plant metabolism to survive. Ionic concentrations (NPK) in stem, leaves and roots also changed significantly. Location Dina showed the best results for physiological, antioxidant activities and ionic contents among all the locations. The soil of Dina location was sandy loam with 0.65 dS/m EC, 0.65% organic matter and 47% soil saturation. Location Dina had a maximum temperature of 25 °C, average temperature of 21 °C and high amounts of rainfall (838 mm) with 84% humidity. It was concluded that milk thistle required an optimum soil and climatic conditions similar to location Dina for its best growth and metabolism. These physiological and biochemical indicators can be used to evaluate the milk thistle response towards diverse climatic conditions.

Keywords: *temperature, plant growth, ion contents, silymarin, rainfall*

Introduction

Silybum marianum (L.) Gaert. generally called as milk thistle that is a yearly/biennial plant belongs to Asteraceae family, Mediterranean territory native and now growing as well as cultivated throughout the world (Bijak, 2017; Abenavoli et al., 2018). Purpose for the cultivation of this plant is to produced silymarin contents (Alemardan et al., 2013), while the other use of this plant is generation of bioenergy as well as oil from seeds and entire plant biomass (Cui et al., 2015; Dominguez et al., 2017). Silymarin complex typically comprises 36.3% of silybin, 15.7% silychristin, 5.9% of silydianin, and 5.1% of isosilybin that is a liver tonic (Sersen et al., 2006).

Studies related to milk thistle revealed that the growth and crop yield strictly depend upon climatic and environmental conditions and optimum crop cultivation should, therefore, be in accordance with rainfall and temperature regime (Karkanis et al., 2011). The environmental factors effect on seed maturation and agronomic quality of Milk thistle. Accelerated aging of seeds is different between various plant species and inside one seed clone but it induced by several days of exposure to high temperature and high humidity (Wong et al., 1986). Faster aging likewise brought about enhanced peroxidation of lipid, diminished antioxidant levels and also decreased action of a few chemicals associated with peroxides and free radicals scavenging (Bailly et al., 1998; Carlsson et al., 2003).

Distinctive medicinal species of plant indicated a well-marked variation in dynamic ingredients during various seasons; these have been generally credited to variation in different ecological factors, for example, temperature and rainfall (Ghimire et al., 2006; Kumar et al., 2007; Ahmad et al., 2008, 2009). Impacts of regular changes on biological systems are impinging hugely and progress in climate conditions, temperature variety, patterns of rainfall and corresponding mechanisms are related to natural changes (Root et al., 2003). Analysts opine that synthetic constituent and perseverance of medicinal plants are influenced significantly by climatic changes. Regularly plants under pressure conditions can gather increasingly secondary metabolites because of growth inhibition and fixed carbon diversion in the biosynthesis of phenolics and glycosidic mixes rather than photosynthesis (Gairola et al., 2010).

Contingent on natural variations, photosynthesis vary among species and decline relentlessly with enhancement of leaf age (Herath and Ormrod, 1979). Numerous studies have exhibited that ascent in temperature upgraded the production of secondary metabolites (Litvak et al., 2002), in spite of the fact that others believe that these metabolites declined (Snow et al., 2003). Tholl et al. (2006) also described that increment in temperature just increase the flowing of synthetic production in most plants and the impacts of high temperature are still getting attention (Wahid et al., 2007). Activities of enzymes (CAT, POD and SOD) are a significant index to foretell the plant responses to the changing environments (Sen and Mukherji, 2009). In plants, antioxidants activities may act like a defense line for many troublesome conditions. (Lohrmann et al., 2004). In plants specialized mechanisms for antioxidant defense contains many types of enzymes, for example enlistment of glutathione reductase, POD, CAT, SOD (Keles and Oncel, 2002). Research results show that the yield of milk thistle fruit (Andrzejewska and Sadowska, 2008) is determined by various agro-technological factors, including NPK fertilization.

In the light of above-mentioned literature, the main objectives of this study was to find out the effects of various agro climatic conditions on physio-chemical attributes of milk thistle and to determine the optimum soil and climatic conditions required for its better growth and metabolism.

Materials and methods

Wild Milk thistle (*Silybum marianum* L.) plants were collected from ten different locations of Punjab, Pakistan (Jalalpur Jattan, Gujrat, Kharian, Sarai Alamgir, Mandi Bauhudin, Dina, Sohawa, Gujar Khan, Rawalpindi and Islamabad) during the years of 2018 and 2019 as described in *Figure A1* in the *Appendix*. Five plant samples were collected randomly from each location between 9:00 am to 2:00 pm. Samples were collected from the away of road side that have no influence of automobile and industries pollutants. The meteorological data of these locations were noted with the help of Pakistan Meteorological Department (PMD), Pakistan. Soil samples were also collected from these locations for the study of soil characteristics with the help of Punjab Agriculture Soil Testing Labs, Pakistan. Photosynthetic pigments like chlorophyll *a*, *b* and total chlorophyll and carotenoid contents were measured using the method given by Arnon (1949). Gas exchange parameters such as photosynthetic rate, stomatal conductance, transpiration rate and sub stomatal CO₂ concentration were measured from fully expanded young leaves using LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England. Model C1-340) along with

climatic factors described in *Table 1*. All the measurements were taken between 10:00 am - 02:00 pm as described by Khalid et al. (2017). The adjustments/specifications of IRGA were: leaf surface area 11.35 cm², temperature of leaf chamber (T_{ch}) varied from 29.2 to 37.5 °C, ambient CO₂ concentration (C_{ref}) 349.12 μmol mol⁻¹, ambient temperature ranged from 31-36 °C, leaf chamber volume gas flow rate (v) 397 ml min⁻¹, water vapor pressure in chamber ranged from 6-9.0 m bar, molar flow of air per unit leaf area (U_s) 401.06 mol m⁻² s⁻¹, ambient pressure (P) 99.95 KPa, PAR (Q leaf) at leaf surface was up to 1515 μmolm⁻².

Table 1. Means of 2018 and 2019 Meteorological data of various locations

Locations	Average rainfall (mm)	Average temperature (°C)	Minimum average temperature (°C)	Maximum average temperature (°C)	Humidity (%)
L1(Jalalpur Jattan)	802	20	4	23.9	49
L2(Gujrat)	706	18	4	19.0	76
L3(Kharian)	809	18	4	23.7	78
L4(Sarai Alamgir)	726	18	4	17.7	77
L5(Mandi Bauhudin)	576	10	4	23.9	74
L6(Dina)	838	21	4	25.5	84
L7(Sohawa)	779	13	4	22.9	75
L8(Gujar Khan)	747	18	9	22.5	75
L9(Rawalpindi)	941	15	2.5	17.0	67
L10(Islamabad)	809	17	3.6	23.0	60

Whole plant water use efficiency (WUE) were measured from a mature and intact fully expanded upper leaves by following formula (Guo et al., 2006; Bacon, 2009):

$$\text{WUE (g/L)} = (\text{dry weight of final biomass} - \text{dry weight of initial biomass}) / \text{total water consumed} / \text{rainfall}$$

Estimation of CAT, POD and SOD activities were determined by the procedure of Chance and Maehly (1955). Determination of nitrogen contents according to Kjeldhal procedure as described by Bremner (1965) method and Phosphorous contents was measured with the help of Jackson (1962) method. Contents of potassium were detected with the help of Wolf method (1982).

Data were analyzed statistically using analysis of variance (ANOVA) technique using Ministate-C software. Data were presented as mean ± SE of five replicates and subjected to one-way analysis of variance (ANOVA) and mean separation was done at $P \leq 0.05$ by using Tukey's test (Silverman, 2018).

Results

There were following results from these experiments:

Meteorological characteristics

Mean data related to meteorology of two years is given in *Table 1*. Data was collected for those months (October, November, December, January, February, March and April) in which milk thistle completed its life cycle. Data was complied with the help Pakistan Meteorological Department (PMD) from ten different locations which

were under considerations. All the locations had varied rainfall, average, minimum and maximum temperature and humidity. Maximum rainfall (838 mm) was noted in location Dina and minimum (576 mm) rainfall was noted in Mandi Bauhudin. Maximum average temperature (21 °C) was in location Dina and minimum in location Sohawa (13 °C). In Location Dina maximum temperature was noted upto 25.5 °C. Location Dina had also maximum humidity (84%).

Soil properties

Physiochemical soil properties were studied of all the locations from which wild plants of milk thistle were collected (*Table 2*). There were clay loamy soils in all the locations except Rawalpindi and Islamabad. Soil collected from location Dina was best due to low EC, normal pH, high organic matter, nitrogen and potassium contents. There was high soil saturation % in location Dina that was 46 while other locations had up to 40% soil saturation percentage.

Table 2. Physiochemical properties of the soil collected from various locations

Soil characteristics	Soil texture	EC (dS/m)	pH	Organic matter (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Saturation %age
L1(Jalalpur Jattan)	Clay loam	0.98	7.1	0.45	0.44	3	112	40
L2(Gujrat)	Clay loam	0.88	7.6	0.77	0.88	5	102	40
L3(Kharian)	Clay loam	0.88	7.3	0.69	0.31	5	104	40
L4(Saraialamgeer)	Clay loam	0.75	7.5	0.66	0.22	5	98	40
L5(Mandi Bauhudin)	Clay loam	0.74	7.0	0.57	0.16	5	98	38
L6(Dina)	Clay loam	0.65	7.2	0.85	0.87	5	100	46
L7(Sohawa)	Clay loam	0.75	7.1	0.62	0.12	7	98	42
L8(Gujarkhan)	Clay loam	0.85	7.2	0.50	0.32	3	102	40
L9(Rawalpindi)	Sandy clay loam	1.07	7.6	0.55	0.74	6	98	42
L10(Islamabad)	Sandy clay loam	1.02	7.5	0.57	0.76	3	114	40

Photosynthetic pigments

Results from analysis of variance (ANOVA) indicated that the effect of different climatic conditions on chlorophyll a, b, total chlorophyll and carotenoids contents were highly significant among different locations, years and their interaction in milk thistle collected from various locations (*Table 3*). *Figure 1A-D* showed that maximum amount of chlorophyll a, b total and carotenoids contents were recorded at L6 (Dina) in 2019 and minimum contents at L8 (Gujar Khan) in 2018. Location Dina was also best for soil and climatic conditions. Overall, there were high contents of photosynthetic pigments in the plants collected 2019 as compared to 2018.

Photosynthetic rate

Data revealed that maximum rate of photosynthesis was measured in the plants collected from L6 (Dina) during 2019 (*Fig. 1E*). ANOVA related to photosynthetic rate was highly significant for locations, years and its interaction (*Table 3*). Photosynthetic rate was significantly varied under different locations by the influence of ecological conditions. There was low photosynthetic rate during the year 2018 that might be due to changes in climatic conditions observed in both the years.

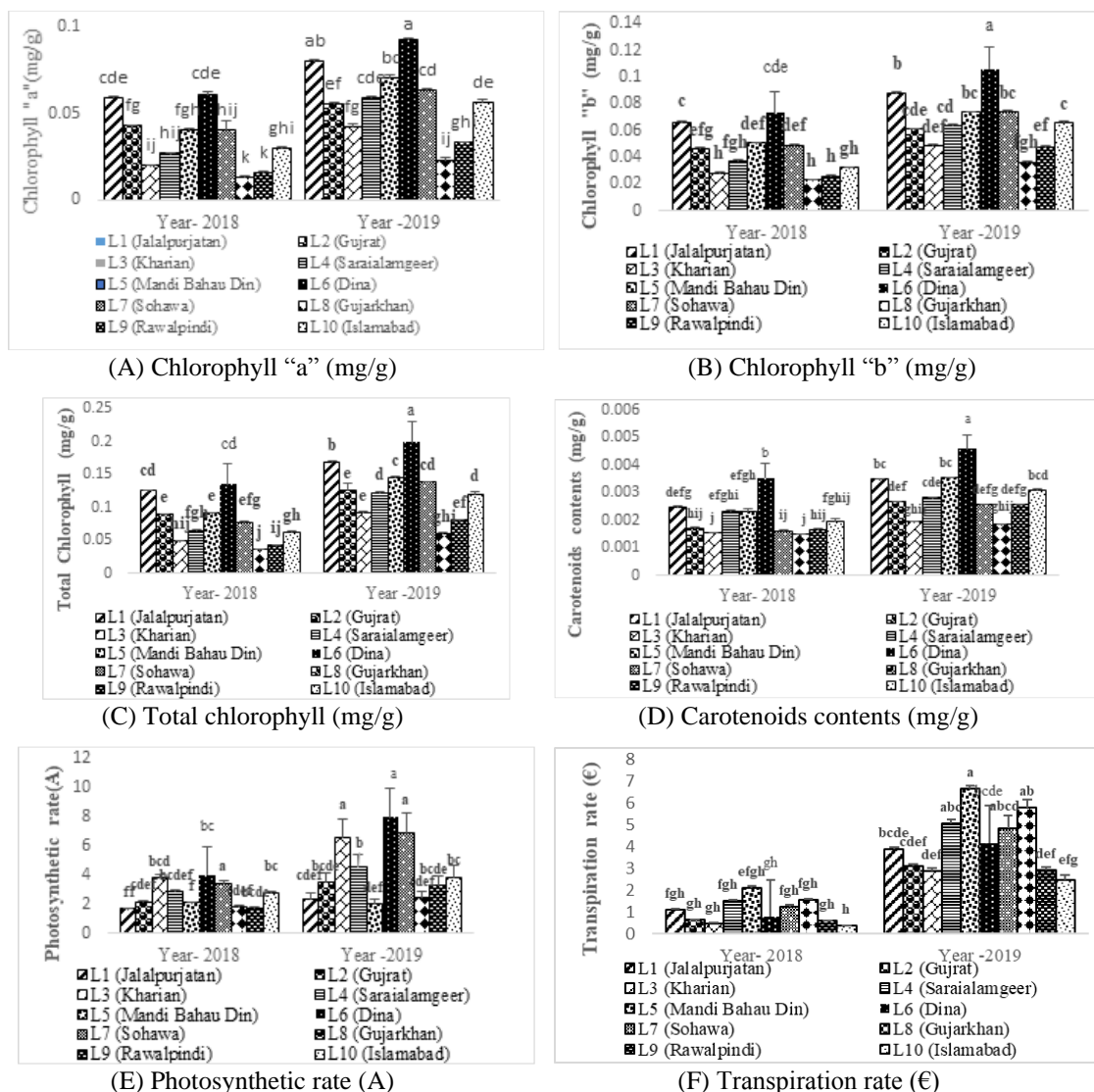


Figure 1. Influence of climatic conditions on photosynthetic and physiological attributes of milk thistle (*Silybum marianum*) at various ecological locations

Transpiration rate

Table 3 demonstrated that the impact of environmental factors on transpiration rate of milk thistle was highly significant among all the location. Variations among years and its interaction were also highly significant. High transpiration rate of milk thistle was noted in 2019 as compared to 2018 (Fig. 1E). Highest transpiration rate was noted in 2019 at Location-5 (Mandi Bahaudin) and minimum at Location-10 (Islamabad) in 2018. Results also indicated that effect of factors on transpiration rate was highly observed at Location-5 (Mandi Bahaudin) during 2018 as well as in 2019. High temperature resulted high rate of transpiration in milk thistle.

Stomatal conductance

Effect of various ecological factors (temperature, soil and rain fall) had significant results stomatal conductance. Variations between years were significant while,

interaction between locations and years was non-significant results (Table 3). Maximum stomatal conductance was observed in 2018 and minimum in 2019 (Fig. 2A). Data narrated that high stomatal conductance was noted at L8 (Gujar Khan) in 2018 and low conductance was in 2019 at L2 (Gujrat). During 2018, there was less variations in the data related to stomatal conductance. These changes were due to variations in climatic conditions in the both the years.

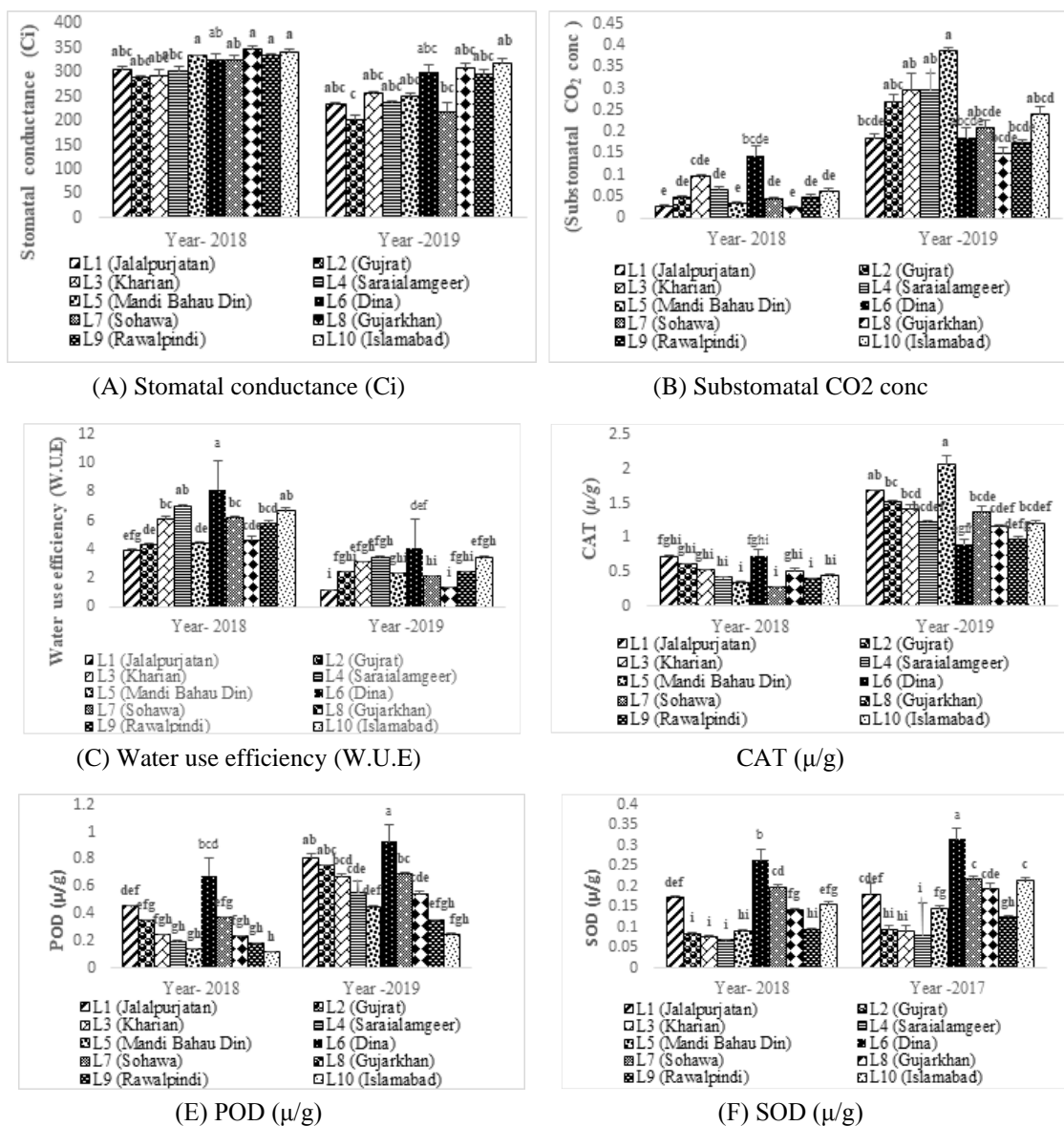


Figure 2. Influence of climatic conditions on physiological attributes and antioxidant activities of milk thistle (*Silybum marianum*) at various ecological locations

Substomatal CO₂ concentrations

Results present in Table 3 indicated that effect of different environmental conditions was significant on concentration of substomatal CO₂. Response of years and its interaction with locations was also highly significant. Concentration of substomatal CO₂

was high during 2019 as compared to 2018 (*Fig. 2B*). *Figure 2B* illustrated that high concentration of substomatal CO₂ was detected in 2019 at L5 (Mandi Bahaudin) and low values were noted in 2018 at L8 (Gujar Khan).

Water use efficiency (WUE)

Effect of climatic conditions was highly significant on whole water use efficiency of milk thistle (*Table 3*). High water use efficiency was noted at L6 (Dina) in 2018 and low water use efficiency was noted in 2019 at L1 (Jalalpur Jattan) due to climatic temperature and rainfall variations among locations and years (*Fig. 2C*). Water use efficiency was high during 2018 as compared to 2019. Location-6 (Dina) showed highest values on water use efficiency in both years as it has much varied climatic conditions from other location.

Catalase activity (CAT)

Effect of ecological factors on CAT was highly significant. Year response was also highly significant with its interaction (*Table 4*). Results from *Figure 2D* narrated that best antioxidant activities of CAT were noted at L5 (Mandi Bahaudin) in 2019 and low activity was in 2018 at L7 (Sohawa). Results also indicated that highest Catalase activities were observed in 2019 at all locations. CAT activities can be good indicators to show the environmental variation.

Peroxidase activity (POD)

Table 4 demonstrated that impact of various ecological factors was highly significant for POD activities with yearly and their interaction. *Figure 2E* indicated that high POD activities were obtained at Location-6 (Dina) in 2019 and minimum in 2018 at L10 (Islamabad). POD activities were high during at Dina during both the years 2018 and 2019. It was also clear from the results that in 2019 highest POD activities were obtained in comparison with 2018.

Superoxide-dismutase activity (SOD)

ANOVA results showed that SOD activities were highly significant in milk thistle under varied climatic conditions (*Table 4*). *Figure 2F* indicated that maximum activities of SOD were observed at L6 (Dina) in 2019 and Minimum activities was detected at L4 (Sarai Alamgir) in 2018. Results also revealed that the effect of factors on superoxide-dismutase activities was highly detected in all location of 2019 as compare to 2018. Changes in SOD was due to variations in climatic conditions.

Nitrogen (N) contents (mg/g)

Means square from ANOVA (*Table 5*) showed effect of ecological factors on nitrogen contents in root, stem, leaves and seeds were highly significant. Results indicated that effect of temperature, rain fall, soil and pH on nitrogen contents was highly significant among different variation and years. While the interaction between location and years showed non-significant results. *Figure 3A* showed that maximum contents of nitrogen were noted in stem part and minimum contents of nitrogen was noted in seeds at both 2018 and 2019. However, L6 (Dina) showed highest contents of nitrogen on all plants part at both the years.

Table 3. Means squares (MS) from the Analysis of variance (ANOVA) for photosynthetic and physiological attributes of milk thistle (*Silybum marianum*) collected from various ecological locations

Source	df	MS of chlorophyll a	MS of chlorophyll b	MS of total chlorophyll	MS of total carotenoids	MS of photosynthetic rate	MS of transpiration rate	MS of stomatal conductance	MS of substomatal CO ₂ conc.	MS of water use efficiency (W.U.E)
Location	9	0.03422***	0.003419***	0.013743***	0.000005***	29.4768***	9.599***	8406***	0.016912**	12.535***
Year	1	0.013512***	0.013366***	0.048146***	0.000018***	30.008***	248.252***	81693***	0.808201***	244.844***
Location × year	9	0.000238***	0.000107**	0.001104***	0.000001*	4.5499***	1.810**	2155 ns	0.016710**	1.326**
Error	80	0.000033	0.000042	0.000065	0.000002	0.6175	0.803	2375	0.007338	0.552
Total	99	0.049095	0.048460	0.187002	0.000073	385.65	415.20	366761	1.6979	413.73

Table 4. Means squares (MS) from the analysis of variance (ANOVA) for antioxidant activities of milk thistle (*Silybum marianum*) collected from various ecological locations

Source	df	MS of catalase activity (CAT)	MS of peroxidase activity (POD)	MS of superoxide-dismutase activity (SOD)
Location	9	0.3210***	0.35089***	0.046313***
Year	1	18.0889***	2.33356***	0.023525***
Location × year	9	0.4030***	0.02295**	0.001117***
Error	80	0.0468	0.01031	0.000272
Total	99	28.345	6.5234	0.47212

Table 5. Means squares (MS) from the analysis of variance (ANOVA) for ion concentrations in milk thistle (*Silybum marianum*) collected from various ecological locations

Source	df	MS of N-contents in root	MS of N-contents in stem	MS of N-contents in leaves	MS of N-contents in seeds	MS of K-contents in root	MS of K-contents in stem	MS of K-contents in leaves	MS of K-contents in seeds	MS of P-contents in root	MS of P-contents in stem	MS of P-contents in leaves	MS of P contents in seeds
Location	9	77.480***	75.969***	91.247***	53.470***	94.388***	82.112***	108.027***	94.804***	0.0026530***	0.016790***	0.002950***	0.000515***
Year	1	137.124***	85.748***	427.662***	165.894***	90.250***	327.610***	33.640***	17.640***	0.001600***	0.027275***	0.005127***	0.001190***
Location × year	9	0.932ns	1.824ns	2.310ns	1.546ns	2.406ns	2.032ns	2.262ns	1.440ns	0.000083ns	0.000772ns	0.000012ns	0.000010ns
Error	80	2.322	2.032	4.072	1.536	3.150	2.770	2.795	2.100	0.000222	0.000633	0.000025	0.000014
Total	99	185.74	948.44	1595.46	783.95	1213.39	136.51	1249.84	1051.84	0.226950	0.048253	0.33789	0.007005

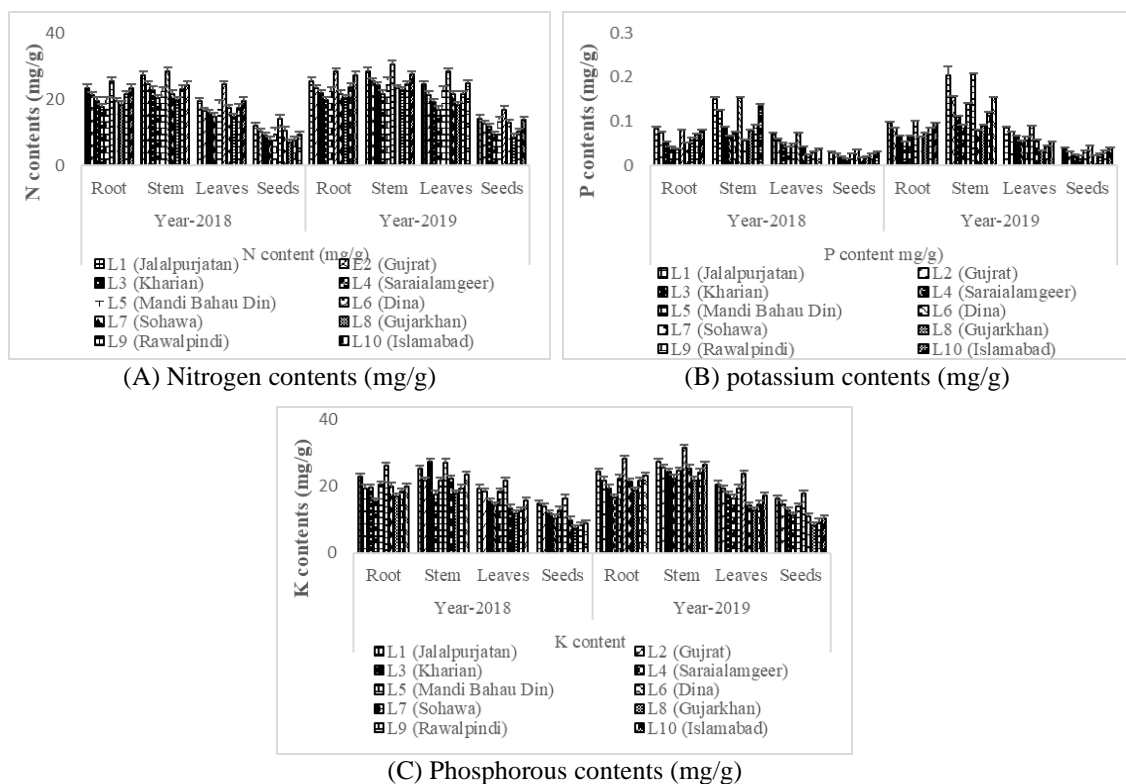


Figure 3. Influence of climatic conditions on ions concentrations of milk thistle (*Silybum marianum*) at various ecological locations

Potassium (K) contents (mg/g)

Maximum potassium contents were noted in stem and minimum in seeds during both the years (Fig. 3B). ANOVA results showed that Location Dina showed maximum potassium contents on various parts of the plant (Table 5). Diverse climate (temperature, rain fall and soil) had highly significant results on different locations as well as years for K contents. While the interaction between years and location showed non-significant results. It was observed that maximum K contents were measured during 2019 from all locations as compared to 2018.

Phosphorous (P) contents (mg/g)

Table 3 showed the impacts of environmental factors on phosphorous contents on root, stem, leaves and seeds. There were highly significant among different locations and years. However, its interaction was non-significant (Table 5). Figure 3C showed that maximum phosphorous contents were present in stem and seeds during 2018 and 2019. Maximum phosphorous contents were measured in Location-1 (Jalalpur Jattan) and Location-6 (Dina) during both the years (2018 and 2019).

Discussion

It is observed that changes occurring in the environment create negative effects on milk thistle. These effects create an impression on the physiology of this plant and change it in various respects. Researches carried out to notice such type of changes have

shown reductions in chlorophyll pigment in numerous plants as *Eucalyptus citriodora*, *Mangifera indica*, *Shorea robusta*, *Tectona grandis* (Joshi and Sawami, 2007), *Duranta repens* (Raina and Bala, 2011), *Prosopis juliflora* (Seyyednejad and Koochak, 2013) and *Artemisia maritima* (Laghari et al., 2015) as a result of the effects of environmental changes. It is, therefore, argued that a decrease within the pigment contents that results from the impact of various factors in the environment creates a degradation of pigments and destruction of plastid in cells (Satija et al., 2009). Swaisgood et al. (2018) also reported in earlier study that variations in the environment cause a decrease in photosynthetic pigments of plants.

The photosynthetic machinery of plants is sensitive to temperature especially biosynthesis of chlorophyll, activity of enzyme Rubisco, PS- II and net rate of photosynthesis are the main targets of heat damage in plants (Sinsawat et al., 2004). Heat stress damage the process of photosynthesis in plants which lead to photo-inhibition. As a result reduced electron acceptors accumulates and reactive oxygen species form (hydrogen peroxide) hence cause oxidative damage (Cui et al., 2006). Heat stress causes the accumulation ROS in excess amount at cellular level leading to lipid peroxidation and damage nucleic acid, proteins and especially photosynthetic pigments (Wang et al., 2014). Due to climatic conditions related to temperature stress, 15-20% of maize production has been lost throughout the world (Chen et al., 2012). The C4 plants like maize are more adapted to temperature stress but net rate of photosynthesis drops down when temperature of leaves of this plant increases up to 38°C (Crafts-Brandner and Salvucci, 2002; Coskun, 2011).

Due to global warming, air vapor pressure deficit of leaves will increase (Kirschbaum, 2000; Way et al., 2013). So the rate of transpiration may also increase in high temperature (Rawson et al., 1977; Kirschbaum, 2004). However, plants may close their stomata to cope with high temperature so that water loss may be prevented in arid ecosystem (Larcher, 2003). After that the rate of transpiration can decrease under warming climate. Previous studies showed that under supplemental precipitation, the rate of transpiration in plants was also high (Wullschleger and Hanson, 2006; Patrick et al., 2007). According to Starck et al. (1993) stomatal conductance decrease in tomato when temperature increases. Von Caemmerer and Farquhar (1981) reported that net changes in the rate of CO₂ show fluctuations in both conductance of stomata and capacity of mesophyll cells for the process of photosynthesis. According to Camejo et al. (2006), reduced CO₂ assimilation was seen in heat shocked Amalia plants could be due to decreased stomatal conductance, but fluorescence emission of chlorophyll in the genotype of Amalia was also altered because of heat shock, showing to modifications in mesophyll content of leaf.

High temperature (above 33°C) cause oxidative stress that degrade the proteins and enzyme activity which in turns damage the cell membrane (Bavita et al., 2012). The rate of respiration in plants increase in high temperature that enhance antioxidant response to accumulation of reactive oxygen species (ROS) in mitochondria. According to Dizengremel (2001), increased NADH synthesis enhance SOD formation under high rate of respiration. Respiration rate and SOD-manganese enzyme activity increase due to heat stress in *Nicotiana plumbagifolia* (Bowler et al., 1992). Alterations in evapotranspiration and ambient temperature were highly significant among environmental fluctuations. Osmotic strain over the leaves and induction enzymatic antioxidants caused by these fluctuations are well reported in abiotic stresses such as heat stress (Wahid et al., 2007), cold stress (Wu et al., 1999; McKersie et al., 1999),

high light intensity (Gupta et al., 1993) and drought stress (Farooq et al., 2009). Various other studies have reported that different conditions of agriculture can influence concentration of bioactive compounds like silymarin in milk thistle. Hammouda et al. (1993) reported that individual silymarin constituents and silymarin levels were influenced by levels of nitrogen and moisture availability. In the plants grown at 60% field capacity, highest level of silymarin (63.1% Silymarin in ethyl acetate extract) was observed. At this water level, the levels of silybin, silychristin and isosilybin were also highest.

Conclusion and recommendations

It was concluded that milk thistle plants were flourishing best at location Dina that had an optimum soil characteristics i.e. Sandy loam, 0.65 dS/m EC, 0.65% organic matter and 47% soil saturation) and climatic conditions (a maximum temperature of 25 °C, average temperature of 21 °C and high amounts of rainfall (838 mm) with 84% humidity). So, it is proposed that such categories of soil and climatic conditions are best for the cultivation of milk thistle at commercial scale. These physiological and biochemical indicators can be used to evaluate the milk thistle response towards diverse climatic conditions.

It is recommended to grow the milk thistle at an average temperature of 25 °C, rainfall amount 838 mm and humidity 84%. Further studies are required to evaluate its active constituents at varied climatic conditions for quality medicines.

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APPENDIX

Figure A1. Map showing the different locations where study was conducted

