Impact of Exogenous Application of Salicylic Acid and Hydrogen Peroxide on Essential Nutrients and Silymarin in Silybum Marianum (L.) Gaertn Grown at Two Different Altitudes of Balochistan Under Cadmium Stress

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Abstract

Cadmium (Cd^{+2}) is a potentially toxic element that inhibits growth and development of several species of plants along with *Silybum marianum* (L.) Gaertn which is an essential medicinal plant belonging to family Asteraceae. The exogenous application with 0.25μM Salicylic acid (SA) and 10μM hydrogen peroxide (H_2O_2) to ameliorate harmful effects of cadmium (500 μM) on milk thistle were studied that were grown at two different ecological zones of Balochistan province of Pakistan i.e. Quetta (Qta) and Turbat (Tbt). The design of experiment was Randomized Complete Block Design (RCBD) with three replicates. Application of SA and H_2O_2, priming (P), Foliar spray (FS) and combinational treatments (P+FS) were highly helpful in alleviating the negative role of cadmium toxicity. The essential nutrients i.e. nitrate (NO_3^-), calcium (Ca^{2+}) and potassium (K) were affected by Cd^{+2} induced toxicity however, the substantial role of SA and H_2O_2 widely helped to reduce the cadmium stress and boosted up the plant nutrients content. In a nutshell, exogenous treatments of SA and H_2O_2 enhanced the yield potential along with highest silymarin contents in milk thistle seeds which is of prime significance for its medicinal importance in treatment of liver diseases. The data obtained in this study highly recommend the priming and foliar spray of SA and H_2O_2 on milk thistle plants, as the best solution to alleviative the cadmium toxicity which will ultimately leads to better growth and yield of the plants.

1 Introduction

Variations in climate effect in various ways which directly represents a constant threat to the existing flora and fauna of that area. Industrialization, urbanization and other major developments discharge a considerable concentration of toxic materials and trace elements in the environment which leads to increase in the rate of pollution and thus, degradation of soil occurs that results in economy loss of different crops (Kapahi & Sachdeva, 2019; Ng et al., 2020; Mohy El-Din & Abdel-Kareem, 2020). The growth of plant is largely affected by some geographical factors i.e. altitude, latitude and longitude. The alpine plants which grow in low altitude with shorter life duration grew like the colonial carpets which show less concentration of nutrient contents in various parts (Galland, 1986).

Cadmium (Cd^{+2}) is widely recognized as heavy metal pollutant and very toxic that is present throughout the environment of the waste produced by the rapid industrialization process, uncontrolled utilization of fertilizers and urban activities and the sludge of sewage (Liu et al., 2007). It is also one of the major factors that is involved in the stress conditions in plants remain highly toxic and harmful as well at very low amount due to its non-essential role in the plants (White and Brown, 2010). once cadmium is observed by the plants, it directly reached out to plants edible organs (Fang et al., 2014) while passing through various steps of food chain that ultimately would become harmful for human diet that leads to severe health issues in human being (Recatala et al., 2010). Numerous negative effects of cadmium are reported at physiological, molecular and also biochemical level in plants leading to alterations in the ultra-structures of photosynthetic apparatus, reducing the rate of photosynthesis etc. (Shi and Cai, 2008;

Xue et al., 2013). Cadmium causes chlorosis and necrosis of leaf by suppressing chlorophyll content and influencing/hindering distribution of essential minerals (Liu et al., 2011).

Medicinal plants are very significant at providing the community health care and prevention from diseases. Rapid increasing in the population and pharmaceutical industry needs the plants which are medicinally important of active ingredients of them is the cause to cultivation and production of medicinal plant (Abdullaev and & Espinosa-Aguirre, 2004). Among the important plants, Milk thistle is widely recognized as an important herb that is playing its antioxidant role. Milk thistle is scientifically known as *Silybum marianum* (L.) Gaertn which is member of family Asteraceae is recommended and widely recognized as a medicinal plant that is origin to Mediterranean Basin. This crops also grow as a weed in the agriculture practices and plantations that occurs in mostly European countries such as North Africa, North and South America, Western and central Asia along with southern Australia (Chiavari et al., 1991; Morazzoni & Bombardelli, 1995; Carrier et al., 2002).It has also been used to strongly protect the liver against snake bites poisoning and remove bile from gallbladder (Ross, 2008). Compounds of pharmaceutical properties derived from milk thistle fruits, are achenes (*Fructus silybin mariani*). However, many times this silymarin content ranges from 1.0% – 3.0 % dry matter of achene that also increase up to 4.0 % (Chiavari et al., 1991; Kozlowski & Holynska, 1985). Several methods were attempted to extract silymarin contents of milk thistle with methods of biotechnology but its obtained results are not that much promising that it should be able to alter the field crops through its initiative source as raw material for pharmaceutical in near future (Cacho et al., 1999; Alikaridis et al., 2000; Saanchez-Sampedro et al., 2008). Silymarin is obtained from seeds, leaves and fruits of Milk thistle but maximum silymarin concentration is present in the seed part of plant. In the fruit, silymarin content rely on the varieties *Silybum marianum* (L.) Gaertn, climate along with geographic changes in the places it grows (Ghahreman, 1999).

Silymarin is obtained from milk thistle seeds which is lipophilic and is composed of three isomers i.e. (Silybin, flavonolignans, silydianin & silychristin) which was initially isolated by Wagner et al., (1968). The protective and medicinal significance of silymarin for the cure of liver is widely documented medically (Flora et al., 1998). The greatest biological activity of Silybin component comprises of 50–70% of silymarin. One of major silymarin constituent is the Silybin by having effective effects of therapeutic properties among various others flavonolignans (Bijak, 2017; Dixit et al., 2007). The silymarin oral bioavailability is low because of the less solubility in water. While some special formulations drawn to increase their absorption and solubility (Bijak, 2017). Silymarin, for its hepatoprotective effects has highly been recognized to cure especially liver diseases (Vargas-Mendoza et al., 2014). Some effective and positive role for silymarin were reported on non-alcoholic fatty liver disease, non-alcoholic steatohepatitis along with fibrosis experiment to hepatocytes of human beings. Silybin also is a barrier that interferes the oxidative stress, fact accumulation in the liver, resistance to insulin (Federico et al., 2017; Marin et al., 2017). Milk thistle seeds contain a very low concentration of flavonoids called taxifolin which contains about 20–35 % of fatty acids with some other compounds of polyphenolic (Ramasamy & Agarwal, 2008). Different other flavonolignans also have been recognized in seeds that includes dehydrosilybin, isosilybin, desoxysilydianin, desoxysilycristin, silybinome, silandrin, neosilyhermin and silyhermin (Kvasnicka et al., 2003).

Foliar feeding of plants with wide range of nutrients with minerals has been utilized as a mean to overcome unavailability of nutrients under stress and it is a common practice of agriculture in order to supplement the nutrient that are deprived from the soil. This technique of fertilization has also been recommended and also integrated in the production of various plants due to its role for being environmentally friendly thus, providing the possibility by gaining maximum quality along with productivity based good yield (Wojcik, 2004).

Seed priming with hydrogen peroxide (H_2O_2) capable to accelerate resistance to drought, chilling, heat and salinity stress (Uchida et al., 2002). H_2O_2 is recognized as one of the best chemicals by having properties of inducing stress plants under abiotic and biotic conditions. Such issues related to environmental stresses are recognized to have been inducing H_2O_2 and other types of toxic oxygen species production in the cellular compartments thus, resulting in the enhancement of senescence in leaf by through peroxidation of lipid and other oxidative damages that brings changes to redox status of such cells surrounding it which starts antioxidative response by acting as signal of oxidative stress (Sairam & Srivastava, 2000) hydrogen peroxide also play a double role in plants which in minimum concentration acts like a messenger being involved in process of signaling and trigger the tolerance potential against different stresses of abiotic conditions while at maximum concentration it hydrogen peroxide causes oxidative damage leading to loss in the function of proteins, programmed cell death and membrane integrity (Asada, 1996). It was reported that the priming with H_2O_2 can be highly recommended for reducing the salinity effect even under maximum concentration of salt (Hemalatha et al., 2017). However, the indicated results described that effect of H_2O_2 priming that is reliable on soybean line along with treatment, while some lines which respond to immersion however, the other priming has inhibitory effects that cause an important reduction in the germination process (Zlatica et al., 2018).

2 Materials And Methods

2.1 Description of research area and experimental design

Considering the effects of altitudes on Milk thistle nutrient profile and silymarin content, a study was planned in two areas of Balochistan, Pakistan that vary in altitude i.e. Quetta (1,679 m a.s.l) and Turbat (129 m a.s.l). A Randomized Complete Block Design (RCBD) experiment was designed in which the field was divided to two major plots; Control and Cadmium. Each plot further contained ten treatments with three replicates. Seeds were sown at 1 inch depth, row to row and plant to plant distance was 1 ft. In the field of cadmium, a black sheet was placed at 5 feet depth in order to avoid its leaching. Both areas have diverse environmental regimes with varying maximum and minimum temperature around the year.
(Fig. 1)

2.2 Collection and preparation of seeds

Seeds of milk thistle were collected from Balochistan Agriculture Research and Development Centre (BARDC), Quetta for experiment. Seeds were primed with 0.25μM Salicylic acid (SA), 10μM hydrogen peroxide (H_2O_2) and with distilled water (H_2O) for 8 hours and then seeds were sown. After 15 days of germination cadmium (Cd^{+2}) was applied to both altitudinal fields at a concentration of 500 μM. After 15 days of cadmium treatment the foliar spray was applied using the same level of SA and H_2O_2 treatment as used for the priming of seeds. To study the nutrient status and silymarin content in seeds the plants were harvested at yield stage.

Dry matter yield (DMY) of plant sample and chemical composition

Harvested plant samples were dried at $65^{O}C$ for 48 hours. 0.1 grams of root and shoot samples were weighted and digested with Nitric acid.

2.3 Determination of Nitrate, calcium, potassium and cadmium

For the determination of nitrate, the following method developed by Kowalenko & Lowe, (1973) was used. In which 3ml of the digested samples added 7ml of CTA (Chromotropic acid) and left for 20 minutes. The absorbance was noted at 430nm.

However, calcium, potassium and cadmium concentration were determined by using flame photometer and atomic absorption spectrophotometer (Yoshida et al., 1976).

2.4 Weight of 100 Seeds/plant

Weight of 100 seeds/ treatment as noted at both experimental fields.

2.5 Silymarin extraction and determination

Silymarin was determined using Soxhlet extraction method, the protocol developed by Saleh et al., (2017). For this purpose, 10 g seed sample placed in thimble holder, and positioned in Soxhlet apparatus. The extraction was carried out by using 200 mL of 80% methanol (prepared in dH_2O) in the reservoir over 6 hours. The absorbance of extracted solution was recorded at 530 nm by using the method of Rahman et al., (2004). For this protocol, 80% methanol was used as blank. Calibration curve was constructed by dissolving silymarin (1 mg/mL) in methanol. After that, 0.18–0.50 mL of standard silymarin solution was pipetted into series of 10 mL volumetric flasks. In each flask, 0.003M potassium permanganate solution was added. Then, solution was diluted to volume with dH_2O. After that mixture was immediately transferred to a spectrophotometer and decrease in absorbance was recorded for 20 min at 530 nm.

2.5 Statistical Analysis

The data was statistically analyzed by using “STATISTIX9”. The graphs and mean, standard deviation was calculated by using MS. EXCEL.

3 Results

3.1 Calcium (Ca^{2+})

Data observed for calcium root and shoot of *Silybum marianum* (L.) Gaertn, grown at two different altitudes i.e. Qta & Tbt under Cd^{+2} (500µM) toxicity statistically showed significant ($P < 0.05$) and non-significant ($P > 0.05$) respectively. The obtained results revealed that highest calcium content in root was significant ($P < 0.05$) and reported in H_2O_2 spray under cadmium stress at high altitude Qta while the lowest calcium content was observed in H_2O priming under control conditions at Qta (Fig. 2).

However, the order of changes in Root calcium content at Qta was observed as: H_2O_2 spray Cd^{+2}> SA P + FS Cd^{+2}>H_2O_2 P + FS Cd^{+2}>H_2O P + FS Cd^{+2}> H_2O spray Cd^{+2} >SA priming Cd^{+2}> H_2O_2 priming control > H_2O_2 priming control > H_2O_2 spray control > SA priming control > SA spray Cd^{+2}>Control Cd^{+2}>SA P + FS control > H_2O priming Cd^{+2}>H_2O spray control > SA spray control > Control of control > H_2O_2 P + FS control > H_2O P + FS control > H_2O priming control.

While the order of improvement at Tbt was recorded as follows: H_2O_2 P + FS Cd^{+2}> H_2O_2 priming Cd^{+2}> SA priming control > SA P + FS control > SA priming Cd^{+2}>SA P + FS Cd^{+2}> SA spray control > H_2O priming control > H_2O P + FS control > H_2O spray control > SA spray Cd^{+2}>H_2O spray Cd^{+2}> H_2O priming Cd^{+2}> H_2O_2 P + FS control > H_2O_2 spray Cd^{+2}> Control Cd^{+2}>H_2O P + FS Cd^{+2}> H_2O_2 priming control > H_2O_2 spray control.

Furthermore, the results for shoot calcium content revealed non-significant ($P > 0.05$) results by showing SA Foliar spray under cadmium stress to be the best treatment in enhancing calcium content in shoot of Milk thistle while the lowest content of calcium in shoot was observed in Control under control conditions at high altitude Qta (Fig. 2).

The order of changes in shoot calcium content at Qta was observed as: SA spray Cd^{+2}> SA priming Cd^{+2}> H_2O spray Cd^{+2}> SA P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}>H_2O_2 P + FS Cd^{+2}>H_2O_2 priming Cd^{+2}>H_2O priming Cd^{+2}> SA priming control > H_2O spray control > SA P + FS control > H_2O_2 spray control > H_2O P + FS Cd^{+2}> Control Cd^{+2}> H2O2 priming control > H_2O_2 P + FS control > SA spray control > H_2O priming control > H_2O P + FS control > Control of control.

While at low altitude Tbt, the order of improvement was observed as: SA spray control > SA priming Cd^{+2}> SA priming control > H_2O P + FS Cd^{+2}> H_2O priming control > H_2O P + FS control > SA P + FS control > Control Cd^{+2}>H_2O_2 P + FS control > H_2O_2 P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}> H_2O spray Cd^{+2}> SA P + FS Cd^{+2}> SA spray Cd^{+2}> H_2O_2 priming Cd^{+2}>H_2O_2 priming control > H_2O spray control > H_2O_2 spray control > H_2O priming Cd^{+2}> Control of control.

In a nutshell, results revealed that the treatment of plants with 10 μM Hydrogen peroxide (H_2O_2) enhanced the root calcium content under heavy metal toxicity (Cd^{+2}) however, the calcium content in shoot reported to have increased by the application of 0.25 μM Salicylic acid (SA) under cadmium stress which illustrates a positive sign of plant signaling molecules to have been playing key role in plant growth and development mechanism under stress conditions (Fig. 2).

3.2 Potassium (K)

Data obtained for Potassium (K) content of root and shoot of Milk thistle which was grown at two varying altitudes i.e. (Qta & Tbt) under heavy metal cadmium (500μM) toxicity showed statistically significant ($P < 0.05$) results. Data further suggested that in root of milk thistle the best treatment for the potassium was observed as H_2O P + FS under cadmium stress at low altitude (Tbt) while the treatment for low potassium content was observed in H_2O spray under control conditions at high altitude (Qta). Resultantly, the potassium content in Milk thistle root under both cadmium and control conditions of H_2O priming and foliar spray enhanced at both altitudes (Fig. 2).

Hence, the order of changes in potassium content of root at high altitude Qta was observed as: SA spray control > H_2O_2 priming control > SA priming Cd^{+2}>H_2O_2 priming Cd^{+2}>SA spray Cd^{+2}>H2O P + FS control > SA P + FS control > H_2O P + FS Cd^{+2}> H_2O_2 P + FS Cd^{+2}> SA priming control > H_2O spray Cd^{+2}> H_2O_2 P + FS control > SA P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}>H_2O priming Cd^{+2}> Control Cd^{+2}> H_2O priming control > H_2O_2 spray control > Control of control > H_2O spray control.

While, the order of improvement at Tbt observed as: H_2O P + FS Cd^{+2}> H_2O spray Cd^{+2}> H_2O_2 P + FS Cd^{+2}> SA P + FS Cd^{+2}> H_2O P + FS control > H_2O_2 spray Cd^{+2}>SA priming control > SA P + FS Cd^{+2}>H_2O_2 priming Cd^{+2}> SA spray control > H_2O_2 P + FS control > H_2O_2 spray control > H_2O priming Cd^{+2}> H_2O spray control > SA P + FS control > Control Cd^{+2}> H_2O priming control > H_2O_2 priming Cd^{+2}> Control of control > SA spray Cd^{+2}.

Apart from significant results of root potassium content, Milk thistle showed statistically significant ($P < 0.05$) results in shoot as well as by illustrating that the treatment H_2O_2 priming under Cd^{+2} stress at Qta was the best while the least treatment with potassium content was recognized as H_2O priming under control conditions at the same altitude (Qta) (Fig. 2).

The order of changes in shoot potassium content at high altitude Qta was observed as: H_2O_2 priming Cd^{+2}> H_2O_2 priming control > H_2O_2 P + FS control > SA P + FS control > SA P + FS Cd^{+2}> H_2O_2 P + FS Cd^{+2}> H_2O P + FS control > H_2O_2 spray Cd^{+2}> SA priming Cd^{+2}> H_2O spray control > H_2O_2 spray control > SA spray control > H_2O spray Cd^{+2}> H_2O P + FS control > H_2O priming Cd^{+2}> SA priming control > SA spray Cd^{+2}> Control of control > Control Cd^{+2}> H_2O priming control.

While the order of improvement at Tbt was observed as: SA P + FS Cd^{+2}> SA priming Cd^{+2}> H_2O P + FS Cd^{+2}> H_2O spray Cd^{+2}> SA P + FS control > SA priming control > H_2O_2 P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}> H_2O P + FS control > SA spray Cd^{+2}>H_2O_2 priming Cd^{+2}>H_2O_2 spray control > H_2O_2 spray control > H_2O_2 P + FS control > H_2O priming Cd^{+2}> H_2O_2 priming Cd^{+2}> Control Cd^{+2}> SA spray control > Control of control > H_2O priming control.

Considering overall results of Milk thistle potassium content, it has been observed that plant perform well at different altitudinal variations because of their properties and different sort of adaptation. It has been reported that potassium content in Milk thistle at both altitudinal fields i.e. (Qta & Tbt) reported to have been increased due to the plant signaling molecules such as H_2O_2 which reported to be the best source of enhancement of plant physiological and biochemical attributes under stress of heavy metal cadmium. Thus, potassium content increase under control conditions but significantly enhanced due to the SA along with H_2O_2 by suppressing the negative and harmful role of cadmium (Cd^{+2}) (Fig. 2).

3.3 Nitrate (NO_{3-})

Data reported for nitrate content in the root and shoot of Milk thistle under a concentration of 500µM Cd^{+2} stress at two varying altitudes i.e. (Qta & Tbt) statistically showed significant ($P < 0.05$) results. The data further revealed that in root, maximum nitrate content was observed in SA P + FS under cadmium stress at high altitude Qta while the minimum nitrate accumulation was observed in Control of control conditions at low altitude Tbt (Fig. 2).

However, the order of changes within root nitrate content at Qta was recorded as: SA P + FS Cd^{+2}> SA spray Cd^{+2}> SA P + FS control > H_2O_2 spray Cd^{+2}> H_2O_2 P + FS Cd^{+2}> H_2O_2 priming control > SA priming Cd^{+2}> H_2O_2 P + FS control > H_2O_2 spray control > SA priming control > SA spray control > H_2O spray Cd^{+2}> H_2O_2 priming control > H_2O P + FS control > H_2O P + FS Cd^{+2}> H_2O priming Cd^{+2}> H_2O priming control > Control Cd^{+2}> Control of control > H_2O spray control.

While the order of improvement at Tbt was observed as: H_2O_2 priming Cd^{+2}> H_2O priming Cd^{+2}> SA priming Cd^{+2}> H_2O_2 spray Cd^{+2}> SA spray Cd^{+2}> SA P + FS Cd^{+2}> H_2O_2 P + FS control > SA spray control > H_2O_2 P + FS Cd^{+2}> H_2O P + FS Cd^{+2}> H_2O_2 priming control > H_2O spray control > H_2O priming Cd^{+2}> SA P + FS control > Control Cd^{+2}> H_2O_2 spray control > H_2O priming control > H_2O P + FS control > SA priming control > Control of control.

Data for shoot nitrate content in *Silybum marianum* (L.) Gaertn also showed statistically significant ($P < 0.05$) results that showed the maximum nitrate accumulation in SA P + FS under control conditions at high altitude Qta while the minimum nitrate content was reported in Control under control conditions at low altitude Tbt.

The order of improvement in shoot nitrate content in Milk thistle at high altitude Qta was observed as: SA P + FS control > SA P + FS Cd^{+2}> SA spray Cd^{+2}> SA spray control > H_2O_2 spray control > H_2O_2 P + FS Cd^{+2}> SA priming Cd^{+2}>H_2O_2 P + FS control > SA priming control > H_2O spray Cd^{+2}> H_2O P + FS Cd^{+2}> H_2O_2 priming Cd^{+2}> H_2O_2 spray Cd^{+2}>H_2O P + FS control > H_2O spray control > H_2O_2 priming control > H_2O priming Cd^{+2}> Control Cd^{+2}> H_2O priming control > Control of control.

While the trend for shoot nitrate at low altitude Tbt was reported as: SA P + FS control > H_2O_2 P + FS Cd^{+2}> SA spray Cd^{+2}>H_2O_2 P + FS control > H_2O P + FS Cd^{+2}> SA P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}> H_2O_2 spray Cd^{+2}> SA spray control > H_2O_2 priming Cd^{+2}> SA priming Cd^{+2}> H_2O_2 spray control > H_2O P + FS control > H_2O priming Cd^{+2}>H_2O_2 spray control > H_2O_2 priming control > SA priming control > H_2O priming control > Control Cd^{+2}> Control of control.

In a nutshell, it has been observed that the combination treatment of 0.25 µM salicylic acid (SA) (priming and foliar spray) was the best treatment in increasing the nitrate accumulation in root and shoot of milk thistle. (Fig. 2)

3.4 Cadmium (Cd^{+2})

Data obtained for cadmium content in both root and shoot of Milk thistle at two varying altitudes i.e. (Qta & Tbt) under the toxicity of cadmium at a concentration of 500µM that showed statistically significant ($P < 0.05$) and non-significant ($P > 0.05$) results respectively (Fig. 3). The data further revealed that Cd^{+2} concentration in root was significant ($P < 0.05$) by illustrating that the maximum cadmium content was observed in H_2O_2 Priming treatment under cadmium toxicity at high altitude Qta while the minimum content of Cd^{+2} was observed in H_2O_2 P + FS under control conditions at Tbt (Fig. 3).

However, the order of cadmium content in root at Qta was observed as: H_2O_2 priming Cd^{+2}>SA spray Cd^{+2}> H_2O_2 spray Cd^{+2}> H_2O P + FS Cd^{+2}> SA priming Cd^{+2}>H_2O_2 P + FS Cd^{+2}>SA spray control > SA P + FS Cd^{+2}> H_2O spray Cd^{+2}> H_2O_2 priming control > H2O spray control > H_2O priming Cd^{+2}>H_2O P + FS control > SA priming control > H_2O priming control > H_2O_2 P + FS control > H_2O_2 spray control > Control Cd^{+2}> SA P + FS control > Control of control.

While the trend for root cadmium content at Tbt was recorded as: Control Cd^{+2}> H_2O priming Cd^{+2}> H_2O P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}> SA spray Cd^{+2}>H_2O spray Cd^{+2}> SA priming Cd^{+2}>H_2O_2 priming Cd^{+2}> SA P + FS Cd^{+2}> SA spray control > SA P + FS control > H_2O_2 P + FS Cd^{+2}> H_2O_2 spray control > SA priming control > H_2O P + FS control > Control of control > H_2O spray control > H_2O_2 priming control > H_2O_2 P + FS control.

Furthermore, the data obtained for shoot cadmium content reported to have non-significant ($P > 0.05$) results by showing the H_2O_2 priming treatment under cadmium concentration at Qta having maximum

Cd^{+2} concentration while the lowest content of cadmium was reported in SA priming under control conditions at Tbt (Fig. 3).

The trend observed for shoot cadmium concentration at Qta was recorded as: H_2O_2 priming Cd^{+2}>SA spray Cd^{+2}> SA P + FS Cd^{+2}> H_2O_2 P + FS Cd^{+2}> SA P + FS control > SA priming Cd^{+2}> H_2O spray Cd^{+2}> H_2O spray control > H_2O_2 priming control > SA priming control > SA spray control > H_2O P + FS Cd^{+2}> H_2O_2 P + FS control > H_2O_2 spray Cd^{+2}>H_2O priming Cd^{+2}> H_2O_2 spray control > Control Cd^{+2}> H_2O P + FS control > Control of control > H_2O priming control.

While the trend observed for shoot Cd^{+2} at Tbt was reported as: SA spray Cd^{+2}> SA spray control > H_2O_2 P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}>H_2O P + FS Cd^{+2}> H_2O P + FS control > H_2O_2 spray control > H_2O_2 priming Cd^{+2}> Control Cd^{+2}>H_2O_2 P + FS control > H_2O priming control > H_2O priming Cd^{+2}>Control of control > H_2O_2 priming control > H_2O spray Cd^{+2}> H_2O spray control > SA P + FS Cd^{+2}> SA priming Cd^{+2}> SA P + FS control > SA priming control.

In a nutshell, it has been reported that both root and shoot, the concentration of cadmium toxicity was overall high but in shoot as compared to root. Cd^{+2} was high in the respective treatments that were applied with cadmium concentration of 500µM. However, the cadmium treated plants that were applied with plant signaling molecules such as H_2O_2 and SA reported to decrease the level of cadmium in the respective treatments at both the varying altitudes i.e. (Qta & Tbt). Overall, the concentration of cadmium by successive application of signaling molecules effectively decreased the cadmium toxicity in milk thistle plants (Fig. 3).

3.5 Weight of 100 seeds/plant

Data recorded for hundred seeds/plant of Milk thistle grown at two different altitudes i.e. (Qta, Tbt) under cadmium (500µM) toxicity showed statistically non-significant ($P > 0.05$) results. Data further revealed that the maximum weight of hundred seeds was observed in H_2O_2 priming in control of Tbt while the minimum seeds were recorded in H_2O_2 P + FS in control conditions at Qta which illustrates that priming and Foliar spray of the treatments with H_2O_2 boosted up the weight of seeds (Fig. 4).

The order of changes under control and cadmium conditions at high altitude Qta was observed as follows: H_2O_2 priming Cd^{+2}> H_2O_2 P + FS control > H_2O P + FS Cd^{+2}> H_2O P + FS control > H_2O priming Cd^{+2}> SA P + FS Cd^{+2}> H_2O_2 spray Cd^{+2}> SA priming Cd^{+2}> SA priming control > Control Cd^{+2}>SA P + FS control > H_2O_2 spray control > H_2O spray Cd^{+2}> Control of control > H_2O_2 spray control > H_2O priming control > H_2O_2 P + FS Cd^{+2}> H_2O_2 priming Cd^{+2}> SA spray control > SA spray Cd^{+2}.

While the order for seeds weight at low altitude Tbt was observed as: SA spray control > SA spray Cd^{+2}> H_2O priming Cd^{+2}> SA priming Cd^{+2}> Control of control > SA P + FS control > H_2O_2 priming control > H_2O spray Cd^{+2}> SA P + FS Cd^{+2}> H_2O spray control > H_2O_2 spray control > H_2O_2 spray Cd^{+2}>H_2O_2 priming

Cd^{+2}>H_2O P + FS Cd^{+2}> SA priming control > H_2O priming control > H_2O P + FS control > H_2O_2 P + FS Cd^{+2}> Control Cd^{+2}> H_2O_2 P + FS control.

In a nutshell, it has been observed that hundred seeds weight was maximum for those treatments which were primed with plant signaling molecules i.e. (H_2O_2 and SA) that enhanced the weight of seeds but interestingly combinational treatment (Priming and Foliar spray) of H_2O_2 yielded healthier seeds. However, it has been observed form the results that the weight of seeds at Tbt was higher as compared to the Qta milk thistle plants. Apart from the weight of seeds, Milk thistle also reported to have the highest yield from those treatments that were applied with SA and H_2O_2 at both altitudes (Fig. 4).

3.6 Silymarin

Data obtained for silymarin content of seeds of Milk thistle at two different altitudes (Qta & Tbt) under Cd^{+2} (500µM) stress gave statistically significant ($P < 0.05$) results (Fig. 4). Further the data revealed that the highest silymarin contents was recorded in H_2O_2 P + FS under cadmium stress at low altitude Tbt while H_2O_2 Priming at low altitude Tbt showed the minimum contents of silymarin in Milk thistle seeds under control conditions (Fig. 5).

The order of improvement for silymarin contents in the seeds of Milk thistle at Qta was observed as: SA P + FS Cd^{+2}>H_2O_2 P + FS Cd^{+2}>Control Cd^{+2}>H2O priming Cd^{+2}>H_2O_2 priming Cd^{+2}> H_2O_2 priming control > H_2O P + FS Cd^{+2}> SA P + FS control > H_2O_2 spray Cd^{+2}> Control of control > SA spray Cd^{+2}> H_2O_2 P + FS control > SA spray control > SA priming Cd^{+2}> H_2O priming control > H_2O_2 spray control > H_2O P + FS control > SA spray control > H_2O spray Cd^{+2}> H2O spray control.

While the order of changes at low altitude Tbt was recorded as follows: H_2O_2 P + FS Cd^{+2}> SA P + FS Cd^{+2}>SA spray Cd^{+2}> SA P + FS control > H_2O_2 P + FS control > H_2O_2 spray Cd^{+2}>H_2O P + FS Cd^{+2}> SA spray control > H_2O priming Cd^{+2}> H_2O spray Cd^{+2}> H_2O_2 spray control > Control of control > SA priming Cd^{+2}> SA priming Cd^{+2}>H_2O priming control > Control Cd^{+2}>H_2O P + FS control > H_2O_2 priming Cd^{+2}>H_2Ospray control > H_2O_2 priming control.

Silymarin is the best component of *Silybum marianum* (L.) Gaertn which is obtained from the seeds and reported to have widely been used for its medicinal properties for liver diseases. In the following results, considering overall silymarin contents of milk thistle at both varying altitudes, it has been revealed that the highest silymarin content was observed in the respective treatments which were applied with 10 µM Hydrogen peroxide (H_2O_2) due to its role in alleviating the negative role of heavy metal (Cd^{+2}) toxicity (Fig. 5).

4 Discussion

There are studies and information available for foliar spray and priming of Milk thistle with signaling molecules such as H_2O_2 and SA but no such information is available for the role of plant signaling molecules under cadmium stress at varying altitudes such as Quetta (1,679m) and Turbat (129m) above sea level. The present study was carried out to elucidate and record the response of Milk thistle nutrients status and silymarin content by the application of SA and H_2O_2 under heavy metal toxicity (Cd^{+2}) to attenuate the negative role of cadmium because *Silybum marianum* (L.) Gaertn is an important medical plant of Mediterranean environments that has been widely used for its major component silymarin as a cure for liver disease.

There are factors of stress which are highly menace to various species of plants that hinder them from attaining maturity, limiting the productivity of crops (Mahajan & Tuteja, 2005). A record of comparison between yields along with the yields average of different crops indicated that mainly crops attain just 20% of their potential as genetic for the yield because of varying stress factors of biotic and abiotic conditions. In fact, stress of abiotic conditions is the main cause of crops loss worldwide, decreasing yield average for different major crops by almost 50–70% (Boyer, 1982; Mahajan & Tuteja, 2005; Khan et al., 2006). The imbalances of nutrients drawn by the application of cadmium introduced negative impacts on numerous physiological and biochemical mechanisms in various plants. In the present study an increase in the nutrient content i.e in Potassium and calcium was observed under cadmium stress. It was reported by (Seregin et al., 2001; Kim et al., 2002) that calcium inhibits the uptake of Cd^{+2} by roots in diverse species of plants, while higher concentration of calcium promoted the uptake of cadmium by the roots of *Phaseolus coccineus* (Skorzynska-Polit et al., 1998). However, Kurtyka et al., (2008) results suggested that the cadmium accumulation was higher in roots as compared to shoots. A significant study was proposed by Drazic and Mihailovic (2005) that reported that the decrease of root potassium content may be because of the minimum uptake of potassium, the leakage of K in a nutrient medium or its enhancement by the transport of K into shoots is reported to induce by the cadmium concentration (Kurtyka et al., 2008).

The effects of cadmium on important mineral concentration of shoots occurs because of the decreased translocation and uptake level that validates with the early findings of Gill et al., (2011). It was observed that the application of plant signaling molecule (SA) optimized the status of nutrients and reported to increase the concentration of mineral nutrient in barley plants exposed to cadmium toxicity (Metwally et al., 2003). The findings of this study on nutrients profile of the medicinal plant milk thistle reported to increase at both altitudes i.e. (Qta & Tbt). Three of the nutrients i.e. calcium (Ca), potassium (K) and nitrate (NO_3^-) obtained from root and shoot of milk thistle reported significant ($P < 0.05$) results but only shoot Ca content reported non-significant ($P > 0.05$) results (Fig. 2). However, overall nutrients accumulation described less degree of differentiation in root and shoot along with varying altitudes. As a result, the main plant growth regulators such as SA and H_2O_2 maintained good nutrient status in both root and shoot under control conditions but highly enhanced the nutrient accumulation under cadmium stress thus alleviating the impact of cadmium toxicity under varying altitudes (Fig. 2).

Zaid et al., (2020) study reported that toxicity of heavy metal in both roots and shoots under exposure of cadmium (Cd^{+2}) enhanced significantly in the cultivars in comparison to control plants (unstressed). It was also reported that the concentration of cadmium in roots enhanced by 31.21fold in kosi cultivar as compared to the plants of control conditions. Plant growth regulators (PGRs) reportedly decreased the concentration of Cd^{+2} in roots of cultivars however, Salicylic acid decreased the concentration of cadmium by 67.73% in Kosi and around 58.63% in Kushal cultivar that exposed to cadmium. They also reported that the cadmium concentration in leaves also increased by 20.71 fold in kosi cultivar and 17.33 fold in the Kushal cultivar in control condition plants. It was suggested that among the three plant growth regulators, SA reported to decrease the concentration of cadmium in leaves by 72.41% in kosi along with 71.63% significantly as compared to the cultivars which were applied with cadmium. Hence, Kushal cultivar reported to have accumulated more cadmium in roots but low in the leaves and found the most responsive to treatment of salicylic acid. Likewise our results are in good agreement with the study of Zaid et al., (2020) that plant regulators especially SA decrease the concentration of Cd^{+2} in leaves along with roots by playing their defensive and stress reducing role in milk thistle (Fig. 3).

Cadmium toxicity is reported to be the key concerns that are restricting the production, crop yield of plants worldwide. It causes disturbances of various Morpho-physiological, molecular and biochemical features by restricting the growth of plants, also disrupts the biosynthesis of chlorophyll, photosynthesis while negatively affects the antioxidant gene expression of defense system in the plants (Imran et al., 2020; Cao et al., 2014). Their results showed that the hormonal homeostasis regulated the growth of the plant along with their physio biochemical performance under cadmium toxicity (Taie et al., 2019; Alharby et al., 2020; Sytar et al., 2019). Alharby et al., (2021) reported impressive outcomes of maize grain extract (MEg) that considerably enhanced maize plant defense system and performance along with gene expression that was related to the antioxidant enzymes. Alharby et al., (2021) reported that MEg is rich source of phytohormones and antioxidants that improves growth of plant, the production of dry matter, physiological and biochemical attributes along with the defense system of antioxidant in maize plants under cadmium stress enhancing GSH and AsA contents. Furthermore, the study suggested that increasing the concentration of hormones and maintaining homeostasis proved effective mechanism which enabled plants to withstand the effects of cadmium toxicity. No doubt, cadmium is the toxic heavy metals which is present in soil has a negative role in suppressing the plant growth and development by reducing in growth, physiological and biochemical attributes. However, plant applied with signaling molecules, help plants to response positively under stressed conditions by alleviating the defensive role under Cd^{+2} stress (Fig. 3). Milk thistle plants were exposed to 500µM concentration of cadmium in both altitudinal fields. The obtained data reported that in both the fields, cadmium was observed high under control conditions and also in its original cadmium filed due to its concentration. Roots were first exposed to Cd^{+2} stress and then reached through aerial parts of the milk thistle plant. Thus, it has been shown that Milk thistle plants applied with signaling molecules i.e (SA and H_2O_2) performed well under heavy metal stress cadmium (Fig. 3).

Yield and yield related attributes get severely effected under cadmium toxicity. Wahid and Ghani, (2008) documented significant decrease in pods number per plant with seeds per pod, weight of 100 seeds, seed yield along with harvest index of *Vigna radiata* plants genotypes due to the toxicity of heavy metal cadmium. Some reports further suggest that accumulation of cadmium is toxic to tissues of mesophyll, that interfere with significant nutrients uptake, thereby decreasing the growth and yield potential of plants at different stages (Khan et al., 2006, 2007). Our findings on the yield potential i.e weight of seeds of milk thistle gave non-significant ($P > 0.05$) results but demonstrating that at high altitude Qta, the weight of hundred seeds decreased as compared to low altitude Tbt which is the true indication of environmental changes and latitudinal locations impact on the plants. H_2O_2 and SA exogenous application improved and alleviated the cadmium toxicity in milk thistle (Fig. 4).

Silymarin (Sm) is a significant free radical scavenger (Soto et al., 2010) which obtained from seeds and leaves of *Silybum marianum* (L.) Gaertn during its various stages of growth and development (Omar et al., 2012).The role of silymarin as a secondary metabolite which is a combination of six flavonolignans i.e. (isosilybin A, B, Silybin A, B, Silychristin and Silydianin) have not been reported before but Sm is vital for improving the productivity of various plant since it widely accumulates plants in stressed conditions that increase the defense mechanism (Ghavami et al., 2008; Afshar et al., 2015). A significant study was reported by Alharbey et al., (2021) which resulted a considerable role of Sm a strong antioxidant, and have a great role in enhancing the resistance of plant to stressed conditions which is attributed as an antioxidant. However, the present study also documents its significant accumulation under stress that eventually enhance under the exogenous application of 0.25μM Salicylic acid (SA), 10μM hydrogen peroxide (H_2O_2) that were exposed to cadmium stress at two varying altitudes i.e. (Qta & Tbt). It was reported form the study that Milk thistle seeds were enriched in silymarin content and showed significant ($P < 0.05$) results at both experimental sites. (Fig. 5).

Conclusion

It has been the key concerns of researchers to develop methods and strategies to reduce the negative impacts of heavy metal cadmium (Cd^{+2}) in order to maintain and improve growth and yield of plants. Keeping in mind the raised question, it has been well established that plant growth regulators such as hydrogen peroxide (H_2O_2) and salicylic acid (SA) plays a significant role by performing signaling and defensive role thereby improving growth, physiological and biochemical attributes of milk thistle by alleviating the cadmium toxicity. Essential nutrients (calcium, potassium and nitrate) and Silymarin; the major component of milk thistle seed that aid to its medicinal potential improved under the exogenous application of SA and H_2O_2.

Declarations

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Conflict of interest

The authors declare no conflict of interest associated with this work.

Authors' contributions

All authors contributed to the study conception and design. The research work and preparation of first draft of this manuscript was performed by Mereen Nizar. Supervision of this research work was carried out by Kanval Shaukat. Noreen Zahra and Mohammad Bilal Hafeez provided facilities related to the chemical analysis of plant samples. Abdul Samad contributed in field and laboratory work. Shamim Gul contributed in manuscript editing.

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References

1. Abdullaev F, Espinosa-Aguirre JJ (2004) Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detect Prev 28(6):426–432
2. Afshar RK, Chaichi MR, Jovini MA, Jahanzad E, Hashemi M (2015) Accumulation of silymarin in Milk thistle seeds under drought stress. Planta 242:539–543
3. Alharby HF, Al-zahrani HS, Hakeem KR, Alsamadany H, Desoky EM, Rady MM (2021) Silymarin-enriched biostimulant foliar application minimizes the toxicity of cadmium in maize by suppressing oxidative stress and elevating antioxidant gene expression
4. Alharby HF, Alzahrani YN, Rady MM (2020) Seeds pre-treatment with zeatins or maize grain-derived organic biostim-ulant improved hormonal contents, polyamine gene expression, and salinity and drought tolerance of wheat. Int J Agric Biol 24:714–724
5. Alikaridis F, Papadakis D, Pantelia K, Kephalas T (2000) Flavonolignan production from *Silybum marianum* transformed and untransformed root cultures. Fitoterapia 71:379–384
6. Asada K (1996) Radical Production and scavenging in chloroplasts. In: Baker NR (ed) Photosynthesis and Environment, 16. Kluwer Academic Publishers, Dordrecht, pp 123–150
7. Bijak M (2017) Silybin, a major bioactive component of Milk thistle (*Silybum marianum* L. Gaertn.) chemistry, bioavailability and metabolism. Molecules, 22(11). Doi.org/10.3390/molecules22111942
8. Boyer JS (1982) Plant productivity and environment potential for increasing crop plant productivity, genotypic selection. Science 218:443–448
9. Cacho M, Moran M, Corchete P, Fernandez-Tarrago J (1999) Influence of medium composition on the accumulation of flavonolignans in cultured cells of *Silybum marianum* (L.) Gaertn. Plant Sci 144:63–

68

10. Cao F, Wang R, Cheng W, Zeng F, Ahmed IM, Hu X, Zhang G, Wu F (2014) Genotypic and environmental variation in cadmium, chromium, lead and copper in rice and approaches for reducing the accumulation. Sci Total Environ 496:275–281
11. Carrier DJ, Crowe T, Sokhansanj S, Wahab J, Barl B (2002) Milk thistle, *Silybum marianum* L. Gaertn. Flower head development and associated marker compound profile. J Herbs Spices Med Plants 10:65–74
12. Chiavari G, Galletti GC, Marotti M, Piccaglia R (1991) Silymarin content of different *Silybum marianum* L. Gaertn. Cultivars *Herba Hungar* 1–2:23–27
13. Chiavari G, Galletti GC, Marotti M, Piccaglia R (1991) Silymarin content of different *Silybum marianum* L. Gaertn. Cultivars *Herba Hungar* 1–2:23–27
14. Dixit N, Baboota S, Kohli K, Ahmad S, Ali J (2007) Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches. Indian Journal of Pharmacology 39:172–179. Doi.org/10.4103/0253-7613.36534
15. Drazic G, Mihailovic N (2005) Modification of cadmium toxicity in soybean seedlings by salicylic acid. Plant Sci 168:511
16. Fang Y, Sun X, Yang W, Ma N, Xin Z, Fu J, Liu X, Liu M, Mariga AM, Zhu X, Hu Q (2014) Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China. Food Chem 147:147–151. Doi.org/10.1016/j.foodchem.2013.09.116
17. Federico A, Dallio M, Loguercio C (2017) Silymarin/Silybin and chronic liver disease: A marriage of many years. *Molecules*, 22(2), pii: E191
18. Flora KMD, Hah MMD, Rosen HMD, Benner KMD (1998) Milk thistle (*Silybum marianum)* for the therapy of liver disease. Amj Gastroenterol 93:139–143
19. Galland P (1986) Croissance et strategie de Production de deuxespeces pines: *Carex firma* L. and *Drays octopetala* L. Bull. *Soc Neuchateloise Sci Nat* 109:101–112
20. Ghahreman A, Iranshahr M, Attar F (1999) Introducing two new and rare species of the genus cousinia cass, Sect. Cynaroideae (Asteraceae). Iran Journ Bot 8(1):15–22
21. Ghani A, Wahid A (2007) Varietal differences for cadmium induced seedling mortality and foliar-toxicity symptoms in mungbean (Vigna radiate). International Journal of Agriculture Biology 9:555–558
22. Ghavami N, Ramin AA (2008) Grain yield and active substances of Milk thistle as affected by soil salinity. Commun Soil Sci Plant Anal 39:2608–2618
23. Gill S, Khan N, Anjum N, Tuteja N (2011) Amelioration of cadmium stress in crop plants by nutrients management: morphological, physiological and biochemical aspects. Plant stress 5(1):1–23
24. Hemalatha G, Renugadevi J, Evera T (2017) Studies on seed priming with hydrogen peroxide for mitigating salt stress in rice. Int J Curr Microbiol App Sci 6:691–695

25. Imran M, Hussain S, El-Esawi MA, Rana MS, Saleem MH, Riaz M, Ashraf U, Potcho MP, Duan M, Rajput & I.A et al (2020) Molybdenum supply alleviates the cadmium toxicity in fragrant Rice by modulating oxidative stress and antioxidant Gene expression. *Biomology*, 10, 1582
26. Kapahi M, Sachdeva S (2019) Bioremediation options for heavy metal pollution. Journal of Health Pollution, 9(24). Doi.org/10.5696/2156-9614-9.24.191203
27. Kavasnicka F, Biba B, Sevcik R, Voldrich M, Kratka J (2003) analysis of the active components of silymarin. J Chromatogr 990:239–245
28. Khan NA, Ahmad I, Singh S, Nazar R (2006) Variation in growth, photosynthesis and yield of five wheat cultivars exposed to cadmium stress. World Journal of Agriculture Science 2:223–226
29. Khan NA, Samiullah., Singh S, Nazar R (2007) Activities of antioxidative enzymes, Sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J Agron Crop Sci 193:435–444
30. Kim YY, Yang YY, Lee Y (2002) Pb and Cd uptake in rice roots. Physiol Plant 116:368
31. Kowalenko CG, Lowe LE (1973) Determination of nitrates in soil extracts. SoilSci Soc Amer Proc 37:660
32. Kozlowski J, Holynska M (1985) Effect of fertilization in a field experiment on the crop of *Silybum marianum* Gaertn. Fruits as well as on the content and yield of silymarin. Herba Pol 1/2:51–59
33. Kurtyka R, Malkowski E, Kita A, Karcz W (2008) Effect of calcium and cadmium on growth and accumulation of cadmium, calcium, potassium and sodium in maize seedlings. Polish Journal of Environmental Studies 17(1):51–56
34. Liu C, Guo J, Cui Y, Lu T, Zhang X, Shi G (2011) Effects of cadmium and salicylic acid on growth, spectral reflectance and photosynthesis of castor bean seedlings. Plant Soil 344(1):131–141. Doi.org/10.1007/s11104-011-0733-y
35. Liu CP, Shen ZG, Li XD (2007) Accumulation and detoxification of cadmium in Brassica pekinensis and B. chinensis. Biol Plant 51(1):116–120. Doi.org/10.1007/s10535-007-0023-y
36. Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: An overview. Archieves of Biochemistry Biophysics 444:139–158
37. Marin V, Gazzin S, Gambaro SE, Dal BM, Calligaris S, Anese M, Rosso N (2017) Effects of oral administration of silymarin in a juvenile murine model of non-alcoholic steatohepatitis. Nutrients 9(9):1006. Doi.org/10.3390/nu9091006
38. Metwally A, Finkemeier I, Georgi M, Dietz KJ (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol 132:272–281
39. Mohy El-Din SM, Abdel-Kareem MS (2020) Effects of Copper and Cadmium on the Protein Profile and DNA Pattern of Marine Microalgae Chorella salina and Nannochloropsis salina. Environmental processes 7(1):189–205. Doi.org/10.1007/s40710-019-00419-1
40. Morazzoni P, Bombardelli E (1995) Silybum marianum (Carduus marianus). Fitoterapia 66:3–42

41. Ng CC, Boyce AN, Abas MR, Mahmood NZ, Han F (2020) Evaluation of Vetiver Grass Uptake Efficiency in Single and Mixed Heavy Metal Contaminated Soil. Environmental Processes 7(1):207–226. Doi.org/10.1007/s407100-019-00418-2
42. Omar AA, Hadad GM, Badr JM (2012) First detailed quantification of silymarin components in the leaves of *Silybum marianum* cultivated in Egypt during different growth stages. Acta Chromatogr 24:463–474
43. Rahman N, Khan NA, Azmi SNHJ (2004) Kinetic spectrophotometric method for the determination of silymarin in pharmaceutical formulations using potassium permanganate as oxidant. Die Pharmazie-An Int J Pharma Sci 59:112–116
44. Ramasamy K, Agarwal R (2008) Multitargeted therapy of cancer by silymarin mini-review. JCancer Lett 269:352–362
45. Recatala L, Sanchez K, Arbelo C, Sacristan D (2010) Testing the validity of a Cd soil quality standard in representative Mediterranean agricultural soils under an accumulator crop. Sci Total Environ 409(1):9–18. Doi.org/10.1016/j.scitotenv.2010.09.021
46. Ross SM (2008) Milk thistle (*Silybum marianum*): An ancient botanical medicine for modern times. Holist Nurs Pract 22(5):299–300. Doi.org/10.1097/01.HNP.0000334924.6d
47. Sairam RK, Srivastava GC (2000) Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. Biol Plant 43:381–386
48. Saleh IA, Vinatoru M, Mason TJ, Nahla S, Abdel-Azim S, Shams KA, Aboutabl E, Hammouda FM (2017) Extraction of silymarin from milk thistle (*Silybum marianum*) seeds a comparison of conventional and microwave-assisted extraction methods. J Micro Power Electron Energy 51:124–133
49. Sanchez-Sampedro AA, Fernandez-Tarrago J, Corchete P (2008) Some common signal transduction events are not necessary for the elicitor-induced accumulation of Silymarin in cell cultures of *Silybum marianum*. J Plant Physiol 165:1466–1473
50. Seregin IV, Ivanov VB (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol 48:523
51. Shi GR, Cai QS (2008) Photosynthetic and anatomic responses of peanut leaves to cadmium stress. Photosynthetica 46:627–630
52. Skorzynska-Polit E, Tukendorf A, Selstam E, Baszynski T (1998) Calcium modifies Cd effect on runner bean plants. Environ Exp Bot 40:275
53. Soto C, Pe´rez J, Garcı´a V, Urı´a E, Vadillo M, Raya L (2010) Effect of silymarin on kidneys of rats suffering from alloxan induced diabetes mellitus. Phytomedicine 17:1090–1094
54. Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2019) Phytohormone priming: Regulator for heavy metal stress in plants. J Plant Growth Regul 38:739–752
55. Taie HAA, El-Yazal MAS, Ahmed SMA, Rady MM (2019) Polyamines modulate growth, antioxidant activity, and genomic DNA in heavy metal-stressed wheat plant. Environ Sci Pollut Res 26:22338–22350

56. Uchida A, Jagendorf AT, Hibino T, Takabe T (2002) Effect of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci 163:515–523
57. Vargas-Mendoza N, Madrigal-Santillan E, Morales-Gonzalez A, Esquivel-Soto J, Esquivel-Chirino C, Garcia-Luna Y, Gonzalez-Rubio M, Morales-Gonzalez JA (2014) Hepatoprotective effect of silymarin. World J Hepatol 6(3):144–149. Doi.org/10.4254/wjh.6.i3.144
58. Wagner H, Horhammer L, Munster R (1968) The chemistry of silymarin (Silybin), the active principle of the fruits of *Silybum marianum* (L.) Gaertn. (*Carduus marianus*) (L.) *Arzneimittelforshung*, 18:688–696
59. Wahid A, Ghani A (2008) Varietal differences in mungbean (*Vigna radiata*) for growth, yield, toxicity symptoms and cadmium accumulation. Ann Appl Biol 152:59–69
60. White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. Ann Bot 105(7):1073–1080. Doi.org/10.1093/aob/mcq085
61. Wojcik P (2004) Uptake of mineral nutrients from foliar fertilization. Journal of Fruit Ornamental Plant Research 12:201–2018
62. Xu C, Gao HY, Zhang LT (2013) Effects of cadmium on growth, photosynthetic rate and chlorophyll content in leaves of soybean seedlings. Biol Plantarum 57:587–590
63. Yoshida S, Forno DA, Cock JH, Gomez KA (1976) Laboratory Manual for Physiological Studies of Rice. *International Rice Research Institute (IRRI)*, Los Banos, The Philippines
64. Zaid A, Mohammad F, Fariduddin Q (2020) Plant growth regulators improve growth, photosynthesis, mineral nutrient and antioxidant system under cadmium stress in menthol mint (Mentha arvensis L.). Physiology Molecular Biology of Plants 26(1):25–39. Doi.org/10.1007/s12298-019-00715-y
65. Zlatica M, Svetlana B, Vojin D, Aleksandar I, Lazar C, Gordana D, Larisa M (2018) Effect of priming on soybean seed germination parameters. Acta Agric Serb 45:15–26

Figures

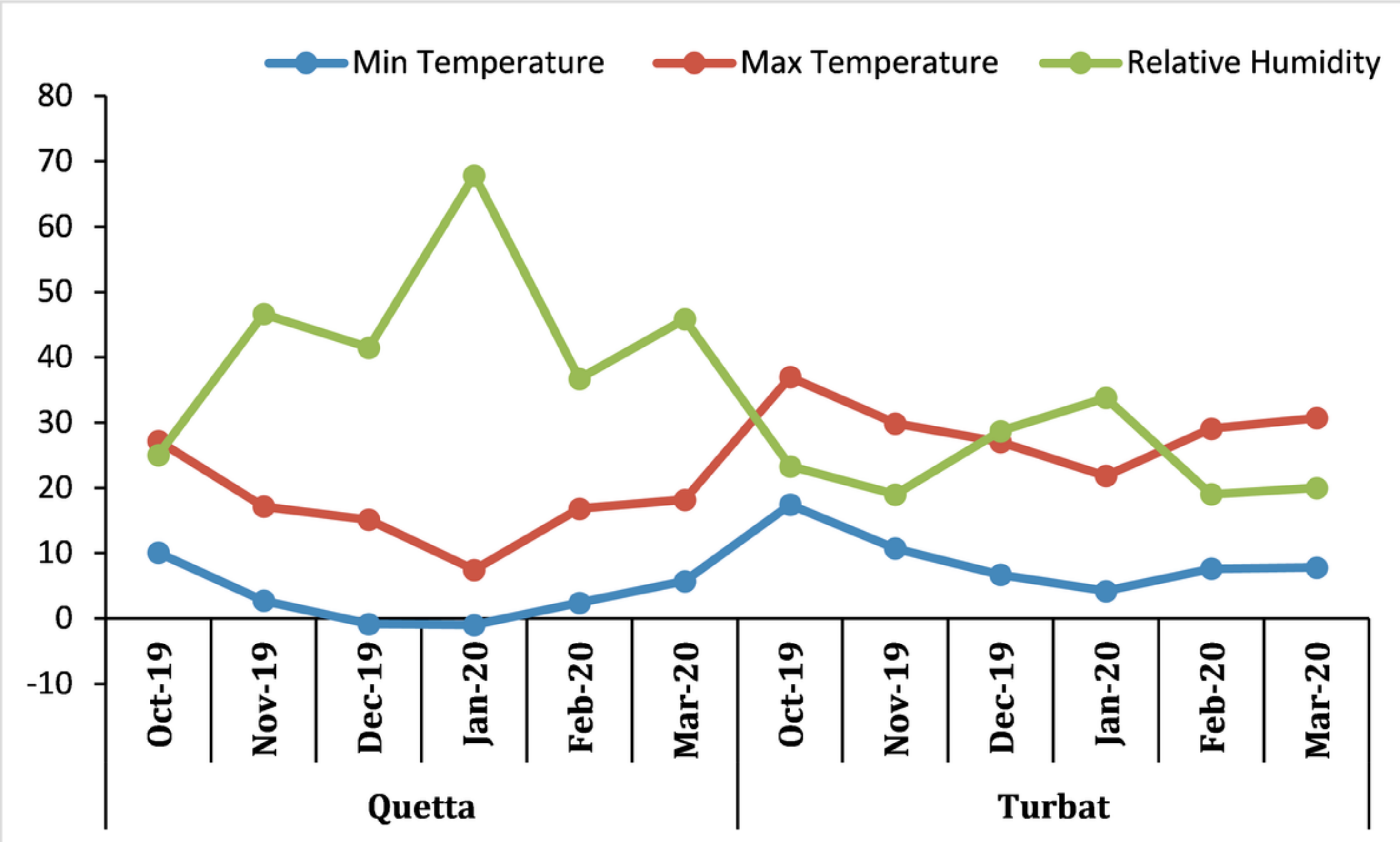

Figure 1

Average monthly maximum, minimum temperature and relative humidity during the experiment period October 2019-March 2020

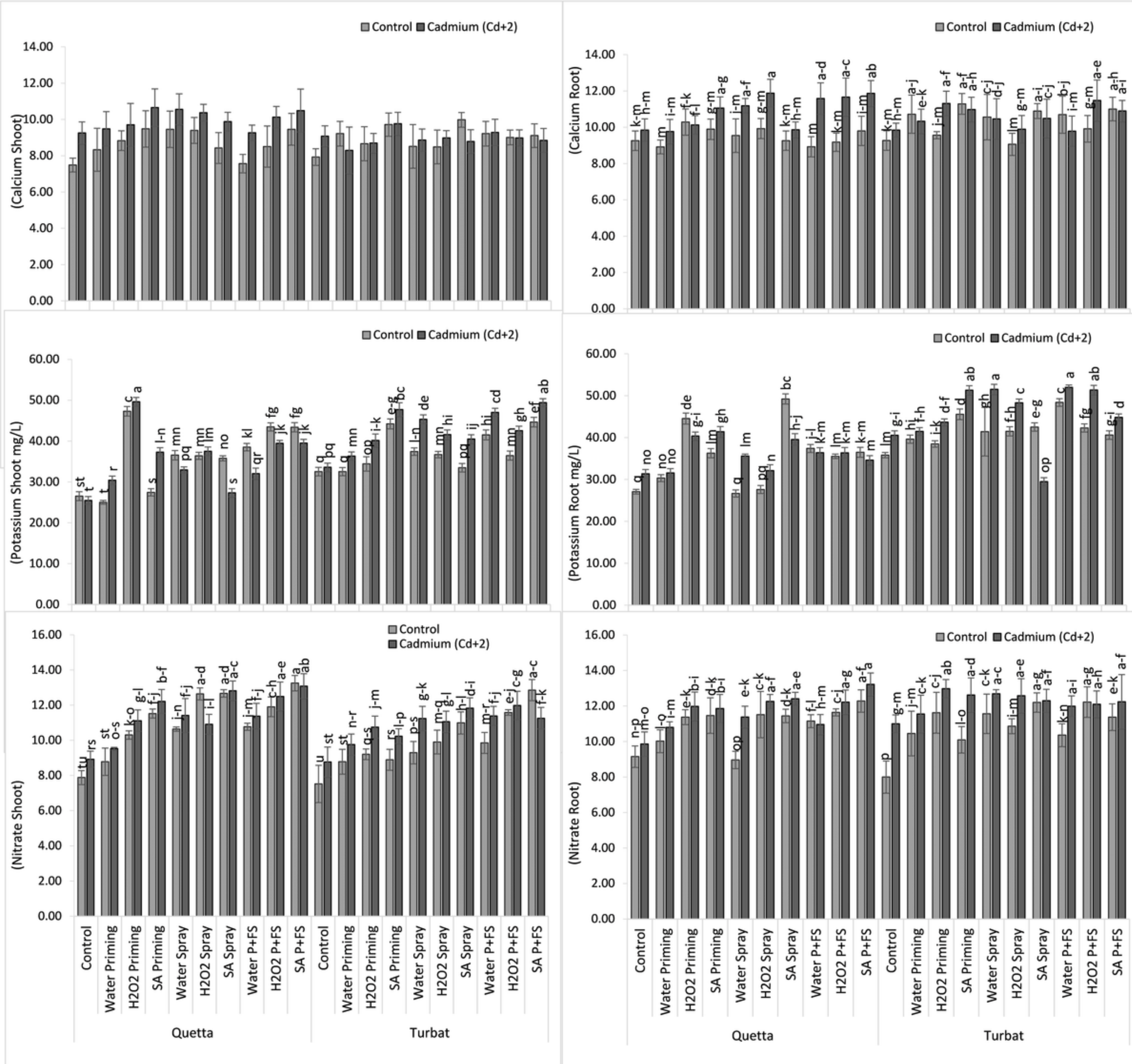

Figure 2

Changes in Calcium, Potassium and Nitrate content of Milk Thistle as affected by SA & H2O2 treatment grown at Quetta and Turbat under Cadmium stress

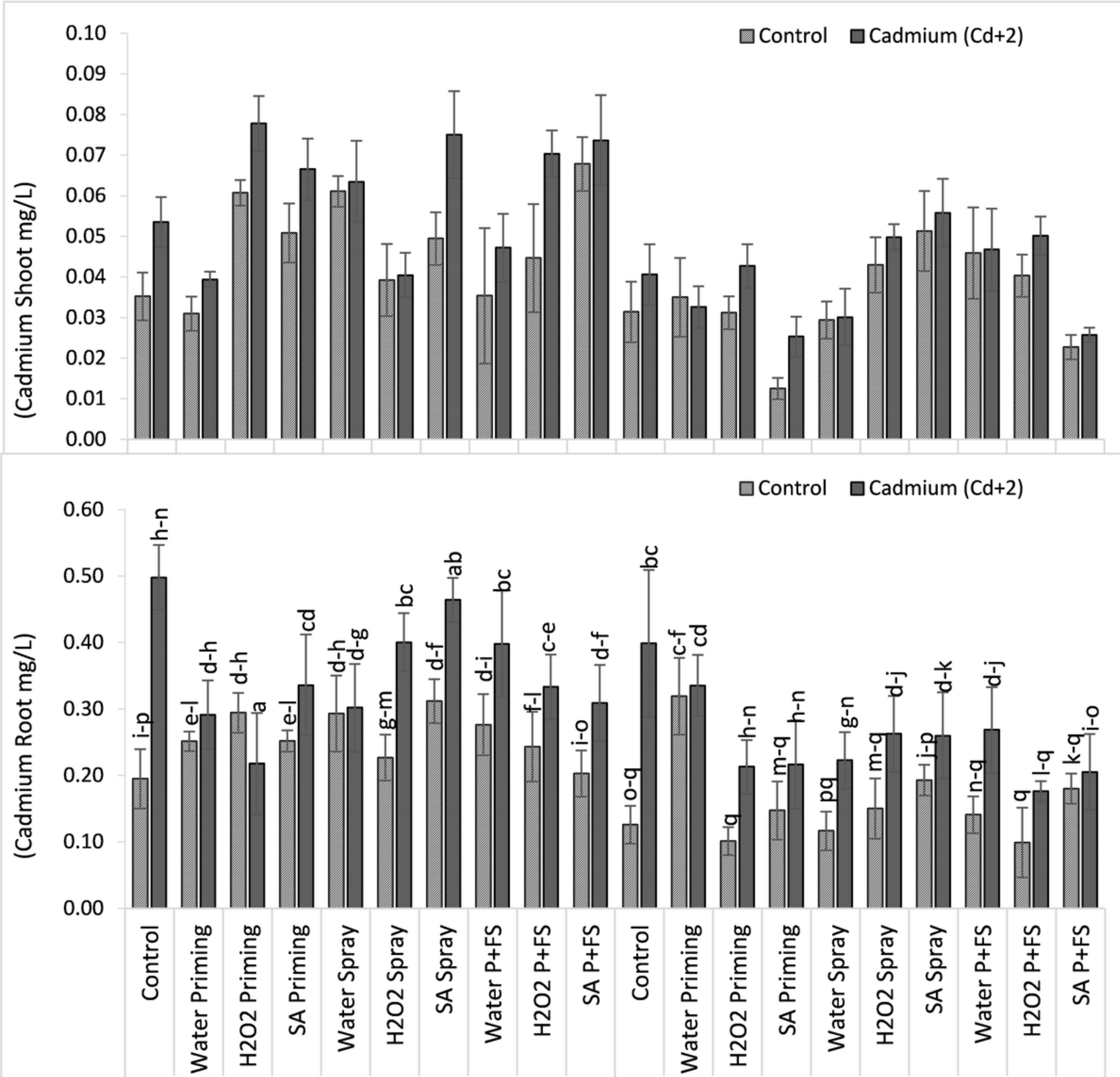

Figure 3

Changes in Cadmium content of Milk Thistle as affected by SA & H2O2 treatment grown at Quetta and Turbat under Cadmium stress

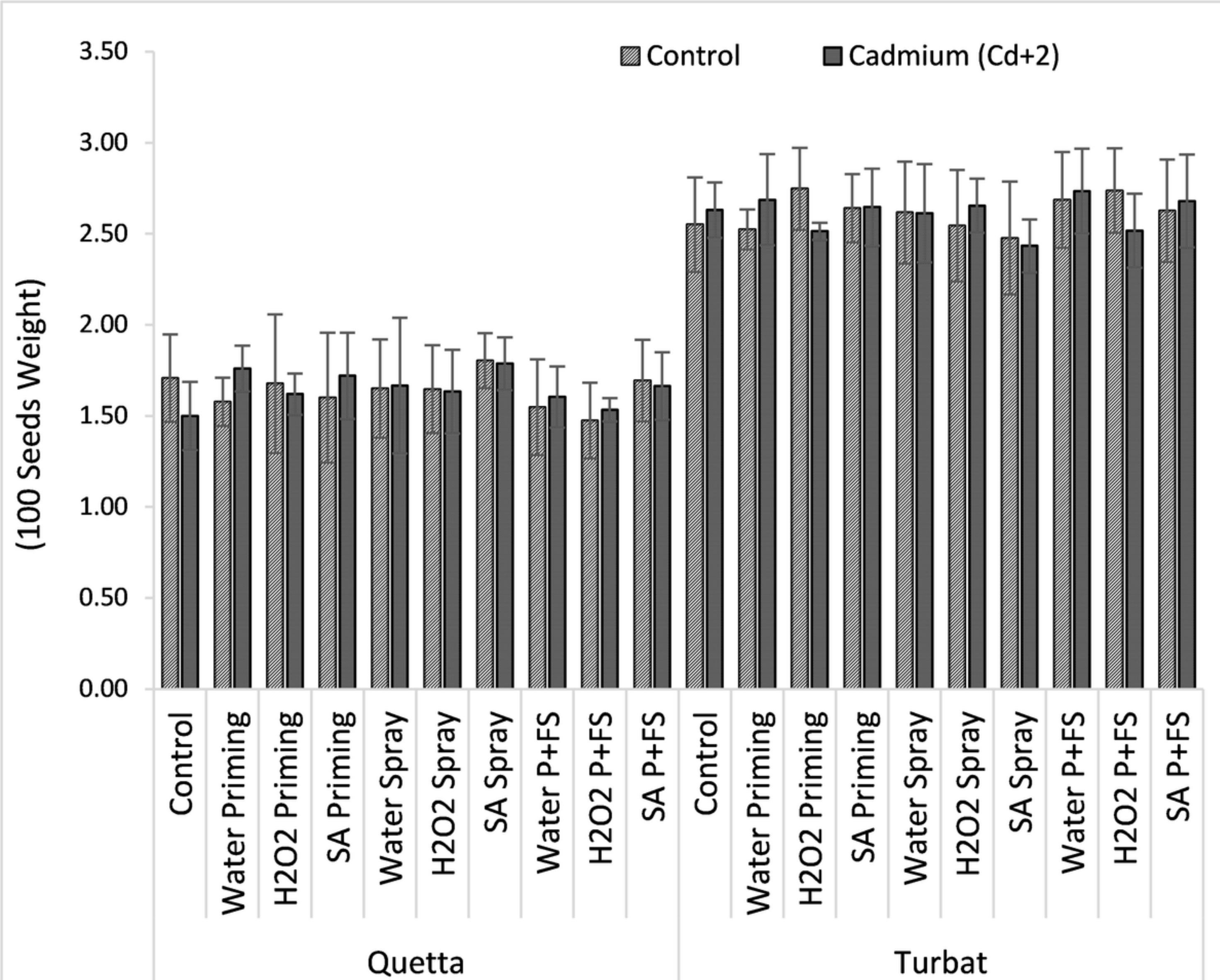

Figure 4

Changes in 100 seed weight/ plant of Milk Thistle as affected by SA & H2O2 treatment grown at Quetta and Turbat under Cadmium stress

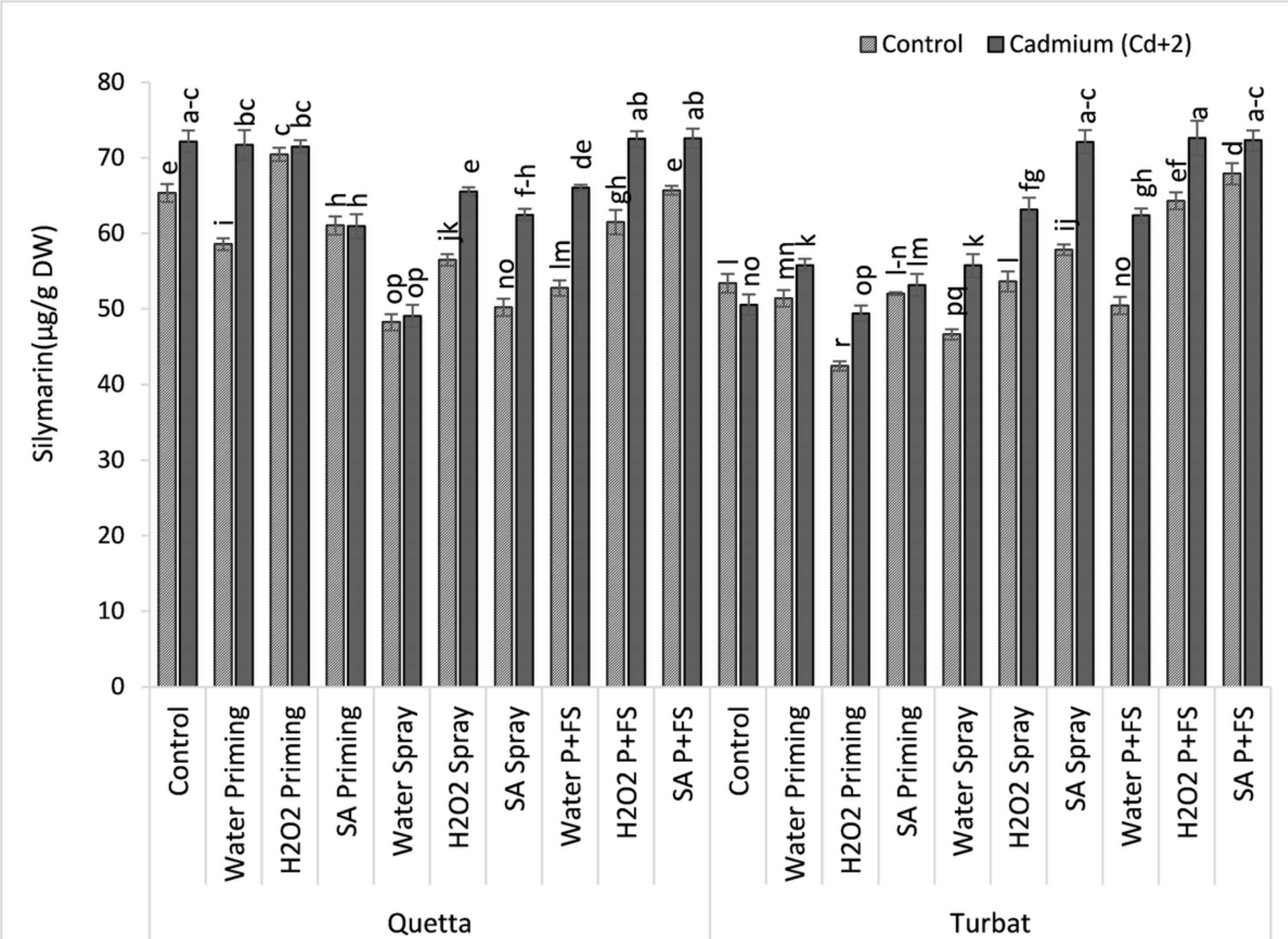

Figure 5

Changes in Silymarin content of Milk Thistle as affected by SA & H2O2 treatment grown at Quetta and Turbat under Cadmium stress