EFFECTS OF AMORPHOUS SILICA ON GROWTH AND NUTRIENTS ACCUMULATION IN LETTUCE (*Lactuca sativa*)

by

Rafea Sultana Rea

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Integrated Agricultural Sciences
August 2020

Committee Members:

Ken Mix, Chair

Gary Beall

Nihal Dharmasiri

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

DEDICATION

I would like to dedicate this dissertation to my family members for all the guidance, encouragement and support throughout my life.

ACKNOWLEDGEMENTS

First, I would like to take this opportunity to express my sincere gratitude to my advisor Dr. Ken Mix for offering his guidance and the opportunity to work with him over the last two years. Dr. Mix not only helped me in academic matters, but also in adjusting to the completely new culture as a newcomer from the other side of the world. Without his supervision, it would not be possible to complete my work and this thesis.

I would also like to thank the other members of my thesis committee, Dr. Gary Beall and Dr. Nihal Dharmasiri for their time and valuable suggestions. I am especially grateful to Dr. Pratheesh Omana Sudhakaran, who helped me regarding data analysis. I wish to thank Dr. Madan M Dey for his guidance and encouragement.

I am thankful to my fellow classmate Prokash Deb, Cody Brown and Ryan Manthei for their support and valuable advice during the experimental work in greenhouse and lab. Prokash Deb not only supported during the experimental work in lab but also helped me for data analysis.

My gratitude and appreciation are not enough for my husband Dr. Mehedhi Hasan, whose support and encouragement has helped me greatly through these last two years.

I would like to acknowledge Graduate College, Texas State University for Thesis Research Support Fellowship and Department of Agriculture, TXST for financial support.

Finally, I wish to acknowledge, all the members (this can be a very big list of names) of *Bangladesh Student Association* community who made San Marcos my second home also made the graduate life eventful.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

Silicon (Si) is the second most abundant element in the Earth's crust. Plants uptake Si from the soil which impacts their growth and nutrients accumulation. It is known to increase plant resistance to abiotic and biotic stresses like drought, salinity, and heavy metal, diseases, and pest infestation. Eco-Sil^{TM} is an amorphous silica produced from rice hull, which has not been studied as a fertilizer. In this study, the effect of Eco-Sil^{TM} fertilization on the growth and nutrients accumulation of lettuce plant was analyzed. The study was conducted in the greenhouse using two Si fertilizers: 1) Eco-Sil^{TM} and 2) diatomaceous earth following three doses: 0.38, 0.75 and 1.25 g kg^{-1} soil. Eco-Sil^{TM} was applied following soil and foliar application method whereas only soil application of diatomaceous earth was followed. Both forms of Si fertilizer increased plant growth. Eco-Sil^{TM} increased plant height and weight up to 10% and 20% respectively compared with control, which was statistically similar with diatomaceous earth for certain doses and the effect was significantly ($p<0.05$) different for two different methods of application and three different doses. However, further increase of Eco-Sil^{TM} dose caused decrease in plant height and weight. There was a statistically significant effect of nutrient accumulation in leafy part of lettuce plant, but the effect for some nutrients were not adequate to improve plant growth. Lettuce accumulated higher concentration of N, Ca, Mg, Zn, B and Mn due to soil application of Eco-Sil^{TM} whereas, P, S, Fe and Cu accumulation decreased. Almost no effect was observed in case of K accumulation. Nutrients accumulation was least for foliar application of Eco-Sil^{TM}. Collectively, these

results indicate the positive effects of Si fertilizers as well as Eco-Sil^{TM} on lettuce plant growth and nutrients accumulation.

1. INTRODUCTION

Most of the major crops accumulate a significant amount of Si though it is considered non-essential for plant growth and development (Guntzer et al. 2012). While, Si does not directly contribute to plant metabolism (Tubana et al. 2016; Ma and Takahashi 2002), there is evidence that Si improves several growth aspects; crop productivity (Sandhya et al. 2018; Artyszak 2018; Amin et al. 2016; Janislampi 2012), nutrient accumulation in plants (Cuong et al. 2017; Neu et al. 2017; Mali and Aery 2009;) and drought tolerance of plants (Santi et al. 2018; Ahmad et al. 2016; Zhu and Gong 2013;). Since these benefits for crop production have been recognized, the global use of Si as a soil amendment is increasing.

The Earth's pedosphere of Si is estimated to be 28.2% by weight (Tubana et al. 2016). Si, along with oxygen and metals forms silicon dioxide (Si_2O) and water-soluble silicates. Si minerals go through various physical and chemical weathering and release Si in solution under suitable pH condition. The source of silica and silicates in soil and clays are from the weathered Si minerals like quartz and feldspar present in the pedosphere (Shakoor et al. 2014; Green and Piperno 1991). Si is present in soil mainly in three different phases such as solid, liquid, and adsorbed. Solid phases can be either amorphous or crystalline. Amorphous silica contributes significantly more to dissolve Si in soil solution because of its higher solubility than the crystalline form (Tubana et al. 2016). However, the plant does not uptake any Si as amorphous silica; rather it is taken up by plants in the form of monosilicic acid (H_4SiO_4) (Keeping 2017).

The addition of Si as a soil amendment in the crop field is a recent phenomenon, and few reports have been published on the impact of Si on plants. Recent publications report Si effects on growth include, yield (Khan et al. 2017; Kaya et al. 2007; Korndörfer and Lepsch 2001) and disease resistance (Rodrigues and Datnoff 2015; Ning et al. 2014; Ashtiani et al. 2012). The research on plant silicon relationship began after 1935. Until 1970, research on this field was minimal. However, between 1970-2000, the overall number in publications started to increase gradually (Figure 1.1). After 2000, a dramatic increase of publication indicates, as this field is getting more research attention (Coskun et al. 2018).

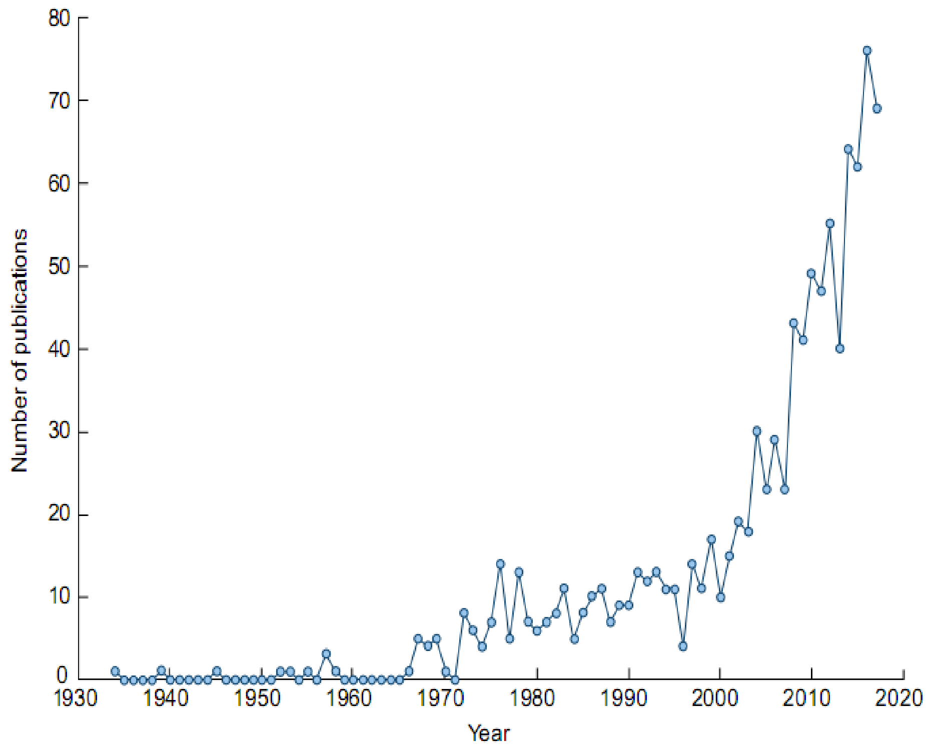


Figure 1.1: Number of Si related publications in the plant sciences from 1934 to 2017 (Coskun et al. 2018)

It is noteworthy, that among the ten major global crops, seven crops (rice, wheat, sugarcane, sugar beet, soybean, tomato, barley) are Si accumulators and the biomass of these plants consist of more than 1% Si in dry matter (Hodson et al. 2005). Production of these crops takes away large amounts of Si from the soil. For instance, rice take away about 500 kg Si ha^{-1} when harvested (Keeping 2017, Makabe et al. 2009). After several years of continuous cropping and harvesting, plant available Si for plant use declines in the soil. This fact necessitates the application of Si from external sources. In different parts of the world such as, India, Vietnam, China, researchers have used rice (Agostinho et al. 2017), wheat (Ahmad et al. 2016), potato (Pilon et al. 2013), sugarcane (Keeping 2017) for experiments on Si applications related to crop production.

It has been observed that Si has beneficial effects, including increasing crop yield, limiting abiotic stresses from salinity, increasing drought tolerance, reducing toxic effects of heavy metals etc. and also reducing biotic stresses from diseases and pests (Gong et al. 2006). Silicate application sharply decreased transpirational flow in rice about 4.2% to 0.8% (Gong et al. 2006), which essentially increases the drought tolerance. It was reported that applications of amorphous silica minimize the cadmium stress in the plants by inhibiting root to shoot transfer of cadmium along with other metals (Zaheer et al. 2018, Bocharnikova et al. 2018). When amorphous silica was applied at a rate of 1000 kg ha^{-1} it increased the availability and accumulation of mineral nutrients: P (10-40%), Ca (up to 33%), S (up to 51%), Mo (up to 54%) and Cu (10-40%) (Greger et al. 2018). Si helps make the nutrients available to plant root systems by impeding soil particles bonding with mineral nutrients.

Considering the effect of Si on plant growth, nutrient accumulation, and other factors this research was intended to find the impact of amorphous silica product named Eco-Sil™ produced from rice hull on lettuce, which is a popular representative of leafy vegetable.

2. LITERATURE REVIEW

2.1 Role of Si on Plant Growth and Yield

Application of Si based fertilizer has been reported to be effective for plant growth and yield. To determine the effect of Si fertilizer on growth, yield, and nutrient accumulation of rice plant, four different dosages of SiO_2 were applied with identical recommended dosages of N, P, K fertilizers. It was observed yield components (number of tillers, number of panicles per plant, number of grains per panicle) and yield were significantly affected by Si fertilizer doses. About 3716 kg ha^{-1} maximum grain yield was obtained when they applied SiO_2 at a rate of 329 kg ha-1, which resulted in about a 23% increase in grain yield compared to control (Cuong et al. 2017). Whereas, in a similar study with different Si fertilizer (Na_2SiO_3) dosages, up to 17.4% yield increase along with increased panicle number were recorded from a field trial in China. Increased growth and yield recorded from this experiment are given in Table 2.1.

Table 2.1: Effect of Si fertilization on rice growth and yield in China (Ma and Takahashi 2002)

Application rate (kg ha^{-1})	Number of panicles ($\times 10^4$ ha^{-1})	Number of spikelets $panicle^{-1}$	Yield (ton ha^{-1})
0	4.84	74.7	7.01
75	4.94	73.9	7.87
105	5.03	74.8	8.16
135	5.03	76.8	8.23

In India, researchers used diatomaceous earth (DE) as a source of Si and compared its use in two different moisture regimes: saturated/submerged and field capacity. It was found that biomass yield was high with almost all of the DE treatments in acidic (300 and 600 kg ha^{-1}) and alkaline (150, 300 and 600 kg ha^{-1}) soil condition. Analysis of soil and rice yield, before and after the application of DE provided evidence that applications of DE increase rice yield regardless of soil condition. The increases were 150, 300, 600 kg ha^{-1} a in alkaline, acidic, and neutral soil respectively. According to the report, DE works best in submerged condition compared to field capacity condition of the rice field (Sandhya et al. 2018). Si is also responsible for grain quality in rice. Formation of the quality hull with milky sap is high when the concentration of Si in the rice shoot is high (Savant et al. 1997).

Si fertilization has a positive impact on wheat production, as well. It increases the plant height, number of spikelets, and number of spikes per spikelet. In irrigated fields, grain yield increased by 13.4% compared to no Si application. It has been reported, the application of K_2SiO_3, at a rate of 12 kg ha^{-1}a, increased plant height, the number of effective tillers m^{-2} up to 515.33, spike length up to 12.25 cm and number of spikelets per spike on an average 16.70. A maximum grain yield of 4.38 t ha^{-1} was observed when K_2SiO_3 were applied with four irrigations (Ahmad et al. 2016). Foliar application of sodium silicate salt ($Na_2Si_3O_7$), especially at the tillering stage and anthesis stage increases the yield for various wheat cultivars (Maghsoudi et al. 2016). However, no remarkable effect was found in Idaho using amorphous volcanic tuff as a source of Si on increasing crop yield. It was claimed that in non-stress condition, Si application does not improve wheat grain yield if the proper nutrient status in soil were maintained (Walsh et al. 2018).

Notable improvements were observed when Si fertilizer was applied for maize production in water deficit condition in Pakistan. Two hybrid maize variety P-33H25 and

FH-810 were grown under 100% and 60% field capacity of water levels. In the water deficit condition, applications of Si significantly increased plant height, stem diameter and cob length (13.96 cm and 12.83 cm respectively) for both of maize variety. Si decreases evaporation loss of water by decreasing stomata opening. It also increases the number of grains per cob (235.05 and 215.35), and grain yield (46.18 g and 39.88 g) correspondingly. This increased yield was due to the increased number of cobs, grains per cob and weight of 1000 grains (Amin et al. 2016). A similar result in drought stressed condition was published on the improvement of maize plant growth and yield from Turkey and South Carolina, USA (Owino-Gerroh and Gascho 2011; Kaya et al. 2007).

Si is not only beneficial for cereal and grain crops; it also increases the production and fresh weight of vegetables. In India, studies were conducted from 2013 to 2017 on three different potato varieties. Application of additional Si (ferti-silica 50 mg L^{-1}) increased tuber yield by 15-50% (Khan et al. 2017). In a pot cultivation system, Ca and Mg silicate were used for growing potato in the absence and presence of water. According to this report, Si application enhances Si availability in soil, which increases overall tuber dry weight irrespective of water condition. Though, there was no significant improvement on an increase in the number of tubers $plant^{-1}$ (Crusciol et al. 2009).

Likewise, in soilless cultivation systems, the addition of Si increases Si content in green bean pods without any loss of biomass production (Montesano et al. 2016). Significant increase in shoot and root length of cowpea were observed in India when the water-soluble Si was applied at a concentration of 100 mg Si kg^{-1} of Si fertilizer dose. It's increased shoot yield in cowpea by 128% (Mali and Aery 2009). In a legume (soybean) crop study in Brazil, the effect of Si ($K_2Si_2O_5$ and $Na_2Si_2O_5$) and salicylic acid ($C_7H_6O_3$)

together were examined. As stated by, individual application of sodium-potassium silicate and salicylic acid does not have any significant impact on legume plant growth. However, combined foliar application of these two Si materials has an adverse effect on the yield of soybean (Barros et al. 2018).

In the same way, spraying of silicic acid increases lettuce yield quality and postharvest firmness (Olle 2017). Application of Si at a dose of 250 mg L^{-1} significantly increases the head weight and total dry matter content in lettuce. In comparison to control treatments, the yield of cucumber increased by 9.35-26.6% by weight as the number of fruits increase (Artyszak 2018). In India, several studies conducted on tomato and onion using diatomaceous earth at a rate of 500-700 kg ha^{-1} provided the highest yield for both of these crops (Ashok et al. 2017; Nazirkar et al. 2017).

2.2. Role of Si on Nutrients Availability and Accumulation

There are sixteen minerals playing essential roles in plant's cell metabolism, energy transfer, osmosis, and reproduction. Among these sixteen elements, nitrogen (N) is a major constituent of plant structure which works with a combination of H, C and P. It forms the organic compounds like protein and nucleotide in the plant. Like N, phosphorus (P) also forms some organophosphorus compound like sugar phosphate, pyrophosphate bond (ATP), phytin etc. On the other hand, potassium (K) maintain the ionic balance among the cells along with the activation of enzymes. It also provides mechanical strength against the lodging of plants in water deficit condition. Among the micronutrients, Fe and Mn work as a cofactor of enzymes and helps in N metabolism. Availability of Fe in the soil increases the amount of Fe in plant parts, which is good if the plants are used as food. Zn and Cu are two redox active micronutrients which operate to maintain of structural integrity and permeability of the plasma membrane (Hasanuzzaman et al. 2018). Si influences the availability and accumulation of these nutrients in various plants species. It also affects the presence of nutrients in root and shoot. The discussion of nutrient availability and accumulation follows in the next three sections on macronutrients, micronutrients, and silicon accumulation.

2.3 Macronutrients

In the published literature, the results of Si application reveal a mixed trend of macronutrients accumulation. Needless to say, the environmental parameters, Si dosage, and soil types of the research sites were widely variable. Therefore, one cannot argue the results are contradictory.

Some reports claim the improvement of nutrient accumulation by using Si. According to Cuong et al. (2017) application of silica has a positive impact on the availability and uptake of N, P, and K in rice plants, especially in grain crops. They reported SiO_2 application has a positive linear relationship (Figure 2.1) with uptake and availability of N, P, and K. SiO_2 application dose was ranged from 100-400 kg ha^{-1}. They fit three different linear equations for these three elements, where the slope for K uptake was highest. The increase of N, P, and K accumulation recorded up to 33%, 69%, and 36.8% respectively compared to control (Cuong et al. 2017). Similar results were also found utilizing diatomaceous earth on the rice field (Pati et al. 2016). It was concluded in another study; Si fertilization has a positive correlation with P uptake making P more available in soil (Eneji et al. 2008). Generally, P concentration increases in root areas but in potato higher concentration of P were found in leaves due to the application of Ca and Mg silicate fertilizers (Pulz et al. 2008). Plant available forms of phosphorus also increased in soil because Si binds with iron and manganese, thus preventing phosphorus opportunity to bond with those elements (Owino-Gerroh and Gascho 2011). Whereas potassium concentration in the shoot and root decreased in lettuce due to the addition of Si and increased for some other crops like maize and rice from 10-40% (Greger et al. 2018). Hence, the accumulation of potassium with the addition of Si appears plant species dependent. In addition to increased N accumulation, S and Mg accumulation also increased in total plant biomass with a high concentration in roots when Si treatments continued for a longer duration (3 weeks). Though, very high dose of Si decreases the availability of Mg (Greger et al. 2018; Reboredo et al. 2013).

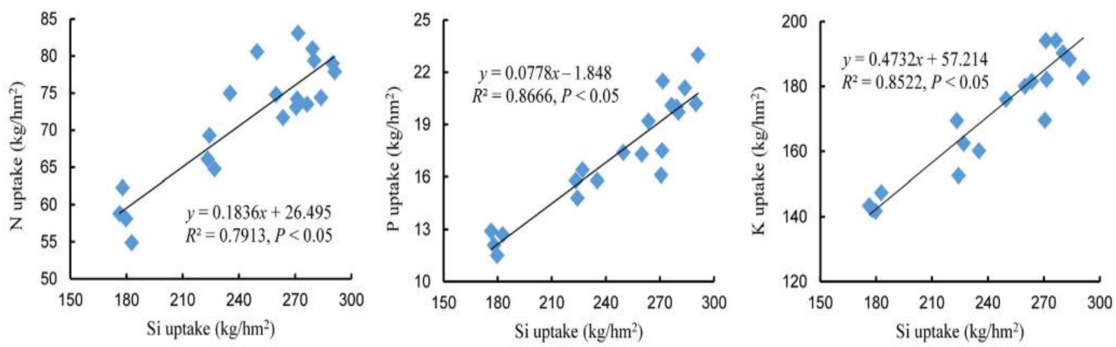


Figure 2.1: Linear regression between Si uptake and nitrogen (N), phosphorus (P), and potassium (K) uptakes in above-ground biomass of rice variety BC15 (Cuong et al. 2017)

There appears to be evidence, very high doses of Si decreased the net accumulation of N and P in plant shoots and roots, though it's thought the concentration of N decreased due to increased growth rate and decreased Mg availability caused by Si treatment (Greger et al. 2018; Reboredo et al. 2013). In contrast, another study found Si application does not have any measurable effect on increasing extractable phosphorus from soil, in fact it may increase P fixation in soil because Si increases soil pH which increases soil P ability to be adsorbed by soil particles and silicic acid is not strong enough to break that bond (Hawk et al. 2006). The pH of the soils that they used for this experiment was 6.2-7. Therefore, here the supplemental Si application slightly affects the availability of P.

2.4 Micronutrients

Very limited reports are available on Si applications associated with micronutrients accumulation; however, Si application appears to have an impact on crop accumulation of most of the micronutrients. The net accumulation of Fe and Mn has been improved by Si application as has B (Boron) accumulation in plant leaves. Fe concentration increased both in root and shoot respectively 20-40% and 10% (Greger et al. 2018). In contrast, Si treatment decreased the accumulation of Cu and Zn by 20%. In most of the cases, Si did not influence the accumulation of Cl and Mo (Mehrabanjoubani et al. 2014).

In many cases, the combined application of Si with other minerals like Zn increases the availability and accumulation of micronutrients. Zn concentration increased up to 10 µg L^{-1} in all organs of rice plants when additional Zn was applied in combination with Si fertilizers. On the other hand, in Zn deficient conditions, Si application increases the Ca concentration in rice and maize shoots and grains (Mehrabanjoubani et al. 2014; Kaya et al. 2007). In B deficient conditions, Si increases Zn, Mo, Mn, and Cu in sunflower shoots. However, it decreases Fe concentration in roots but increases Fe in fully developed leaves, thus increasing its mobility (Savić and Marjanović-Jeromela 2013).

2.5 Silicon uptake in plant

Increased Si availability in soil solution is the main reason for increased Si uptake in plants. Additional application of Si increases Si availability in the soil solution and improves root systems, which stimulates Si uptake by plants. Si uptake and accumulation are highest for Si accumulator plants, which consist of more than 1% of total biomass Si (Elsokkary 2018). In Table 2.2, a few major crops are listed with Si percentage in total biomass. Rice plants are the highest Si accumulators, followed by wheat, barley, tomato, and sugarcane. In rice cultivation systems, application of SiO_2 at a rate of 100-400 kg ha^{-1} increases Si uptake 26.8%-58.5% in total plant biomass (Cuong et al. 2017).

Table 2.2: Si percent in above ground parts of major crop plants (Elsokkary 2018; Hodson et al. 2005)

Plant Species	Si percentage in Plant Biomass
Rice (*O. sativa*)	4.17
Wheat (*Triticum aestivum*)	2.45
Barley (*Hordeum vulgare)*	1.82
Tomato (*Lycopersicon esculentum*)	1.54
Sugarcane (*Saccharum officianum*)	1.51
Soybean (*Glycine max*)	1.39
Lettuce (*Lactuca serriola*)	0.97
Corn (*Zea mays*)	0.82
Potato (*Solanum tuberosum*)	0.4

In potato, soil and foliar application of sodium metasilicate have a different effect on leaf, stem and tuber Si concentration, and accumulation. The concentration of Si was maximum in stems followed by roots for soil-applied Si compared to foliar-applied and untreated control. No significant differences were found in tubers Si concentration.

Whereas Si accumulation in the stem was maximum for foliar application of Si. Overall, soil application of Si provides maximum Si concentration and accumulation in different parts of the potato (Pilon et al. 2013). Figure 2.2 shows the comparison of Si concentration and accumulation in leaves, roots, stems and tubers of potato for soil and foliar-applied sodium metasilicate.

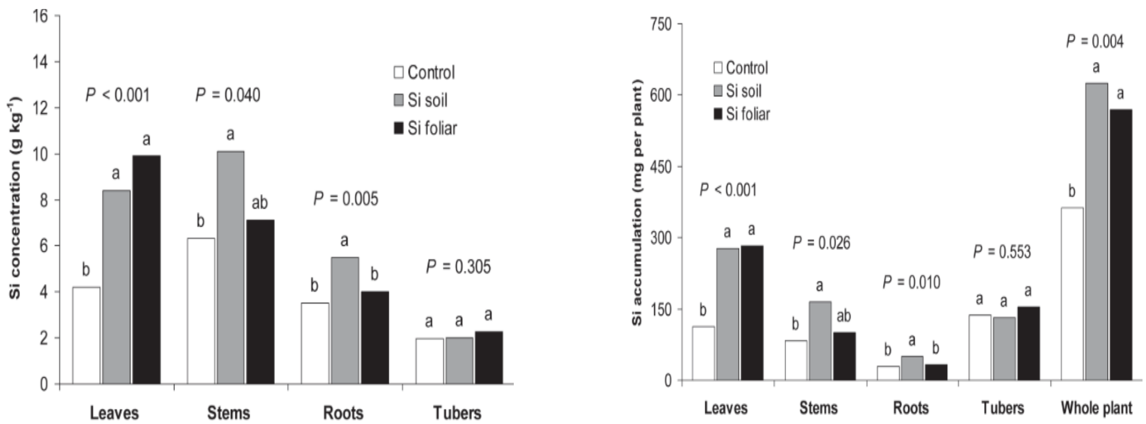


Figure 2.2: Comparison of Soil and Foliar-applied sodium metasilicate on Si concentration and accumulation in potato leaves, roots, stems, and tubers (Pilon et al. 2013).

In the same way, maximum uptake and accumulation of Si in cowpea roots and leaves were observed for 800 mg kg^{-1} soil-applied Si. Application of Si increased leaf Si concentration up to 4259.7 µg g^{-1} and root Si concentration up to 3126 µg g^{-1} (Mali and Aery 2009).

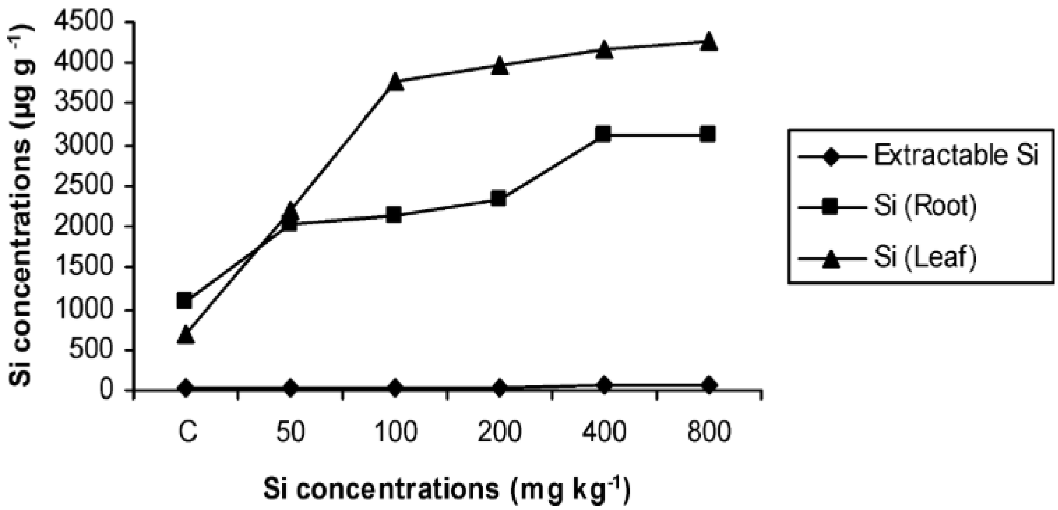


Figure 2.3: Effect of sodium metasilicate on leaf, root and extractable Si contents in cowpea (*Vigna unguiculata*) (Mali and Aery 2009).

2.6 Effect of Si on Drought Tolerance in Plants

The global prevalence of drought is increasing due to climate change, which is one of the greatest threats to crop production because one-third of the world land area is drought prone (Santi et al. 2018). Drought has several harmful impacts on plant growth, metabolic activity, photosynthesis, and nutrient uptake (Xiong et al. 2012). Lack of water due to drought stress inhibits photosynthesis, damages the cell membranes, and limits cell division. About 5% to 10% of leaf transpiration occurs through leaf cuticle, not the stomata (Taiz and Zeiger 2006). When Si deposits under the leaf cuticle, it forms a double layer which creates an extra barrier to prevent water loss (Figure 2.4). Si deposition has been observed in stomata as well, and it has been reported Si can reduce transpiration up to 30% in rice, which has a thin cuticle. It also increases structural reinforcements and changes the photosynthetic rate to increase water use efficiency (Perez et al. 2014). Furthermore, Si application was reported to increase drought tolerance by elongating roots, enabling roots to extract water from soil in drought stressed conditions (Hattori et al. 2005).

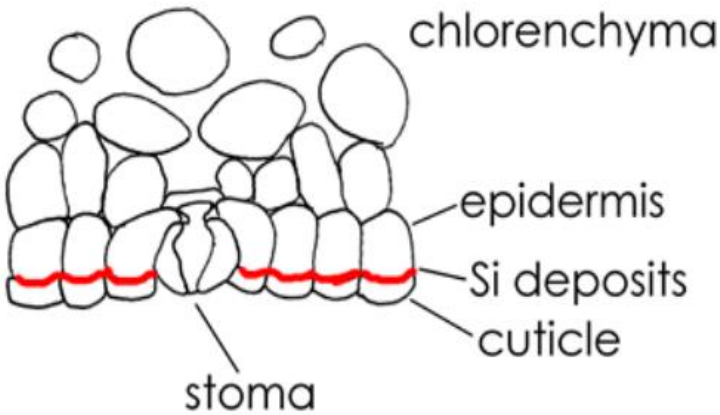


Figure 2.4: Diagram of Si deposits (red) between cuticle and epidermal cells in plant leaf (Janislampi 2012)

Drought stress is a severe constraint to rice production. Si has been verified to increase rice plant resilience to drought stress conditions. The increased drought resistance of rice with Si was seen with K_2SiO_3 applications at a concentration of 0. 0.5, 1.0, 1.5, and 2.0 mM Si. They imposed drought treatment for 15 days after 28 days of rice transplanting. The study showed that Si application decreases the leaf water potential up to -1.92 Mpa, which was -1.33 Mpa for the wet field with Si. It also increased water utilization efficiency from 0.16 g cm^{-2} to 0.39 g cm^{-2}. Biomass accumulation, nutrient accumulation of K, Ca, Mg, and root activity of rice plants were increased significantly under drought stressed condition after Si application (Chen et al. 2011). Similarly, in an Egyptian study, 70, 80, 90 100 and 120% soil saturation were maintained to identify the effect of Si on drought tolerance of rice. Reduction of water saturation from 120% to 70% without Si application decreased the plant height 32% and grain yield 27%. Whereas the application of Si 8.4 mg per 10 plants increased the plant height by 38% and grain yield by 106% compared to no Si application (Ibrahim et al. 2018). In the same way, Ca and Mg silicate applications at a rate of 200-600 kg ha^{-1} in different soil water contents, 60, 70 and 80% of field capacity, also increased the rice yield (Nolla et al. 2012).

Application of sodium silicate increased chlorophyll contents and maintained required leaf water potential by reducing leaf transpiration in drought stress condition of wheat. Sodium silicate at a rate of 6 mM increased relative water content under the drought stressed condition. Foliar application of sodium silicate at the active tillering and anthesis stage provided maximum positive influence both in drought stressed and non-stressed condition (Maghsoudi, Emam, and Ashraf 2016, Gong et al. 2008). In the same way, under reduced moisture level, potassium metasilicate (K_2SiO_3) at a rate of 12 kg ha^{-1} also increased leaf water potential by increasing K contents in wheat (Ahmad et al. 2016). Another experiment in China observed the regulatory activities of Si on water relations of wheat leaves in drought condition. They maintained drought treatments in the field by withholding irrigation and used moveable rainwater shelter. They did not find any differences in soil moisture contents under drought condition, but Si applied plants had better water potential and moisture content in leaf area (Gong and Chen 2012).

A study in Utah found that additional application of Si also increased drought tolerance in corn, under three cultivation techniques: hydroponic and subjected to salt stress, gradual drought stress in low Si soil less medium and acute drought stress in low Si soil less medium. They found inconsistent result in the case of drought tolerance but about 18% increase in the corn dry mass. According to the study, Si increased water use efficiency in corn up to 36% (Janislampi 2012). It was also found applications of Si increase corn growth and grains per cob in drought-stressed plants (Amin et al. 2016).

Recently, in a study in Pakistan, it was reported that application of plant growth promoting rhizobacterium in combination with Si in drought stress condition, increases the drought tolerance and mineral nutrients (K, Ca and Mg) accumulation in tomato. They followed two drought conditions: 45% field capacity and 35% field capacity with 50 ppm Si as sodium silicate. Maximum fruit yields were reported 92.9 g $plant^{-1}$ at 45% field capacity and 8.6 g $plant^{-1}$ at 35% field capacity (Ullah et al. 2016). On the other hand, foliar application of Si reduces flower drop and young pod drop of soybean. An experiment was conducted on soybean following 25%-70% water capacity of the soil. They used Optysil and Silvit stimulator at a concentration of 0.25%. It was observed that Optysil and Silvit stimulator decreased flower drop and increased number of pods per plants compared to control by 20% and 18% respectively (Artyszak 2018). Antioxidant parameters and photosynthetic rate of soybean were also influenced significantly by Si application (Kaushik and Saini 2019). It also has been reported that Si also increases drought tolerance in cucumber (Hattori et al. 2008), sorghum (Hattori et al. 2005) and sunflower (Gunes et al. 2008).

Table 2.3: Si used as a fertilizer for major crops in different part of the world with time of research, materials used as a source of Si and the outcomes (SA: Soil Application, FA: Foliar Application)

Crop species	Place & Time	Materials Used	Dosage	Application Method	Findings	References
Rice (*O. sativa*)	[a]India (2018) [b]Vietnam (2017) [c]Florida (1997)	[a]Diatomaceous earth, [bc]SiO2	- [a] 0, 150, 300, 600 kg ha^{-1} (DE) - [bc] 100, 200, 300,400 kg ha^{-1} (SiO_2)	[a] SA (pot) [bc] Broadcast in 3 split doses	- [ab] Significant increase in biomass and grain yield (up to 600 kg ha^{-1}) - [c] Compete with arsenic ions in root entry point. - [bc] Disease management and yield of rice	[a]Sandhya et al. 2018, [b]Cuong et al. 2017, [c]Datnoff ET AL.1997
Wheat (*Triticum aestivum*)	[a]India (2016) [b]Florida, [b]Louisiana (2017), [c]Iran (2015)	K_2SiO_3	- [a]0 and 12 kg ha^{-1} (Iran) - [b]Survey on different dose (LA)	SA	- [ac]Si application causes increased grain yield, no. of spikelet's, number of grains per spike - [b]Increased K concentration up to 28.65 mg g^{-1} shoot and 3.5 mg g^{-1} grain. - [abc]Increased drought tolerance	[a](Ahmad et al. 2016), [b](Dupree et al. 2017), [c](Maghsoudi, Emam, and Ashraf 2016)
Potato (*Solanum tuberosum*)	[c]Israel (2012) [a]Brazil (2013) [b]India (2017), [c]Poland (2018)	[bc]Silicic acid (H_4SiO_4), [a]Ferti-silica, aSilamol	- [a]250 L ha^{-1} (Silamol) - [a]50 mg dm^{-3} Si (Ferti-silica)	[c]SA [ab]FA	- [a]Delayed skin maturation - [abc]Average tuber weight increase - [b]Yield increase approximately 15%	[a](Pilon, Soratto, and Moreno 2013), [b](Khan, Goyal, and Jain 2017), [c](Artyszak 2018)
Lettuce (*Lactuca sativa*), Pea (*Pisum sativum*), Carrot (*Daucus carota*)	Sweden (2018)	K_2SiO_3	- 80 and 1000 kg Si ha^{-1} Soil - 100, 500, 1000 and 5000 µM Si in nutrient medium	SA	- Increases mineral nutrients (Ca, P, S, Mn, Zn, Cu) accumulation	(Greger, Landberg, and Vaculík 2018)
Chard (Beta vulgaris), Kale (*B. oleracea var. sabellica*)	Brazil (2019)	$NaKSiO_3$, K_2SiO_3	- 0.00; 0.84; 1.68 and 2.52 g L^{-1}	FA	- increased accumulated Si in both sources and vegetables - increased fresh matter content	(Pedreira et al. 2018)
Corn (*Zea mays*)	Turkey (2017)	Exogenous Si	0, 300, 750 kg Si ha^{-1}	SA (broadcasting)	- Formation of less soluble zinc-silicates in cytoplasm	(Keeping 2017)

3. MATERIALS AND METHODS

The experiment was conducted at the green house of Texas State University, San Marcos during November 2019 – January 2020. The aim was to study the effect of amorphous silica on yield and nutrient accumulation of lettuce for soil and foliar application method in lettuce. The objectives and hypothesis of the research experiments are presented below.

3.1 Objectives and hypothesis

The main objectives of the study were to evaluate the effect of foliar application (0.38, 0.75 and 1.25 g kg^{-1} soil) and soil application (0.38, 0.75 and 1.25 g kg^{-1} soil) of amorphous silicon on growth, yield and nutrient accumulation of lettuce. As part of this objectives, the following hypothesis was tested:

i. H_0: The weight of fresh lettuce fertilized with silicon (Eco-Sil™ and Diatomaceous earth) will not be statistically different than the weight of fresh lettuce without supplemental silicon.

   Ha: There will be a statistically significant difference in the weight of fresh lettuce fertilized with silicon compared to lettuce with supplemental silicon.

ii. H_0: Nutrients (N, P, K, B, Zn, Fe, Mn) contents of lettuce fertilized with silicon will not be statistically different than lettuce without supplemental silicon.

   Ha: There will be statistically significant difference in nutrients (N, P, K, B, Zn, Fe, Mn) contents of lettuce fertilized with silicon compared to lettuce without supplemental silicon.

iii. H_0: Growth and yield of water stressed lettuce fertilized with silicon will not be statistically different than growth and yield of water stressed lettuce without supplemental silicon.

Ha: There will be statistically significant difference in growth and yield of water stressed lettuce fertilized with silicon compared to lettuce without supplemental silicon.

iv. H_0: Soil and foliar application of silicon will have similar effects like a commercial Si fertilizer named diatomaceous earth on growth, yield and nutrient accumulation of lettuce.

Ha: Soil application of silicon will exhibit a significantly different effect on growth, yield and nutrient accumulation of lettuce comparing to foliar application.

3.2 Lettuce (*Lactuca sativa*)

Lettuce is the most important leafy vegetable crop. It belongs to the family Asteraceae, which is one of the largest plant families comprising more than 32,000 plant species (Anon. 2020). The origin of consumable lettuce is Mediterranean region (Kesseli 1991). Due to high nutritive value, the consumption of lettuce is now globally common.

The United States is the second highest lettuce producing country in the world after China. In 2019, lettuce was cultivated on 2627 thousand acres in United States. The total production of lettuce in the United States was 8131.9 million pounds in 2019 where about 51% is head lettuce, 15% leaf lettuce and 33% romaine lettuce. Total value of utilized production of lettuce is (USDA 2020) 3.49 billion dollars.

The growth and yield of lettuce greatly depends on the essential elements like N, P and K (Hasan et al. 2017; Mahlangu et al. 2016). N and K are mostly accumulated nutrition by vegetable plants in conventional farming system. All these three elements (N, P and K) play important roles in photosynthesis and disease resistance of plants, which are consequently responsible for better plant growth (Souza et al. 2017). The application of Si fertilizers can promote plant growth and better quality. It was also observed in a previous study that Si application increases the breadth of lettuce up to 17% compared to control (Olle 2017). In hydroponic condition, the concentrations of N, P, K, Ca, Mg, Na and Fe were higher than in the control when treated with silica sol (Kleiber et al. 2015). Considering the possible benefit of Si fertilization on lettuce, this study intended to evaluate the effect of varying doses of Si fertilizer (Eco-Sil^{TM}) following soil and foliar application methods on lettuce.

3.3 Soil

Black soil was used as growing media. The physical and chemical characteristics of the soil (before doing any on this) are shown in the Table 3.1

Table 3.1: Physical and chemical characteristics of the soil sample

Characteristics	Value
Soil type	Black soil
Particle size analysis	
Sand	11.8%
Silt	34.5%
Clay	53.7%
Textural class	Clay
pH	7.7
Electrical conductivity	406 umho cm^{-1}
Organic matter	20.4%
Nitrate-nitrogen (NO_3-N)	42 ppm
Phosphorus (P)	250 ppm
Potassium (K)	713 ppm
Calcium (Ca)	9500 ppm
Magnesium (Mg)	377 ppm
Sulphur (S)	58 ppm
Iron (Fe)	6.38 ppm
Zinc (Zn)	3.87 ppm
Manganese (Mn)	6.66 ppm
Copper (Cu)	2.45 ppm

3.4 Planting time and climate

The plants were seeded on October 4th, 2019 and were kept outside of the greenhouse for two weeks under full sunshine. Full sunshine was important for proper germination of the seeds to avoid etiolation. After two weeks they were relocated inside the greenhouse. Temperature was relatively high during the germination with enough sunshine hours and no rainfall compared with overall time period of the experiment. The average high and low temperature in outside and inside of the greenhouse are presented in table 3.2.

Table 3.2: Average greenhouse temperature during experiment

Outside temperature (ºF)			Inside temperature (ºF)		
Average high temperature	Average low temperature	Average temperature	Average high temperature	Average low temperature	Average temperature
86	62.5	74	77	53	65

3.5 Plant Variety and Planting Method

Lettuce (*Lactuca sativa*) were used as green vegetable plant. Plastic seed trays were used for germination of lettuce seed. Three different plastic trays were used, for Eco-Sil™ treatments, diatomaceous earth treatments and for control where no silicon was used. Almost all of the seeds were germinated within two days of seeding. After three weeks of germination, the seedlings were transferred to 2.5 L plastic pots, which contained about 2 L of soil and moved inside the greenhouse. Total of 12 plant samples were used for each type of silicon dosages. The population size was 120. The plant samples were harvested after 11 weeks on December 18th, 2019. The pictures of the plants taken at different times are presented in the following figure.

A

B

C

Figure 3.1: Different stages of lettuce plant growth. A. After 7 days of seeding, B. After 3 weeks when transplanted in larger pots, and C. At 11^{th} week before harvesting

3.6 Silica sources

The goal of this research is to investigate the impact of two types of amorphous silica on nutrient accumulation and growth of lettuce. One product was derived from rice hulls (Eco-Sil^{TM}) and the other silica source was a natural mined product, Diatomaceous earth. The two products allowed the ability to test the difference between sources and manufacturing process, as well the overall impact of Si applications on lettuce.

3.6.1. Eco-Sil^{TM}

A commercial Si product, Eco-Sil^{TM}, produced from rice hulls were used as Si source. The main features of this amorphous silica are-

a. Contains SiO_2 >99.8%

b. No heavy metal

c. Surface area 260-320 m^2 g^{-1}

d. pH 6.5-7.0

A B

Figure 3.2: Image of A. Rice Hulls, B. Eco-Sil^{TM} powder

3.6.2. Diatomaceous earth

Mined diatomaceous earth, the other source of amorphous Si that we used in our experiments has the following composition (Sandhya et al. 2018):

a. SiO_2 remove these 54.8%

b. Particle size 10-200 µm

c. Cation exchange capacity 52 cmol P^+ kg^{-1}

d. pH 9.21

e. Al_2O_3 18.3%

f. Fe_2O_3 4.9%

g. MgO 3%

h. CaO 1.6%

i. Na_2O 1.2%

j. K_2O 0.4%

3.7 Treatments

Following dosages were used for Lettuce (*Lactuca sativa*), (the doses are chosen based on reported similar experiments and also the recommended dose of amorphous silica manufacturers):

Table 3.3: Description of treatments

Treatments	Group	Si Concentration in actual g applied	Soil treatment g kg^{-1}	Foliar treatment g L^{-1}, applied 3 times	Application type
T1	Control	0 g applied	0		Control
T2	Eco-Sil™*	0.38	0.38		SA
T3	Eco-Sil™	0.74	0.75		SA
T4	Eco-Sil™	1.24	1.25		SA
T5	Eco-Sil™			1.26	FA
T6	Eco-Sil™			2.5	FA
T7	Eco-Sil™			4.16	FA
T8	DE†	0.21	0.38		SA
T9	DE	0.41	0.75		SA
T10	DE	0.68	1.25		SA

*****>99%

†54.8%

3.8 Application of fertilizers

Before seeding, 1g Eco-Sil™ powder was mixed with the soil and the Eco-Sil™ tray was filled with that soil. Similarly, diatomaceous earth was used for diatomaceous earth tray. For soil application, full dose of Eco-Sil™ were mixed with soil for all the three treatments T2, T3 and T4 before 15 days of planting. Foliar application of Eco-Sil™ (T5-T7) and diatomaceous earth (T8-T10) were sprayed manually in three installments, first one after 30 days, second one at 45 days and third one after 60 days. For fulfilling the basic requirements of essential plant nutrients, a commercial fertilizer named "Miracle Gro" were used. Miracle Gro contains 24% Total Nitrogen (N), 8% Available Phosphate (P_2O_5), 16% Potassium (K). It also contains calcium and magnesium. About 4.2 g of Miracle Gro were mixed in one gallon of water and each plant received about 26 ml of the solution.

3.9 Watering and weeding

The plants were watered twice a day at their germination stage. When they were transplanted in larger pot, they were watered once a day. At the later growth stage, sometimes they were watered once in two days depending on the climatic condition and temperature. Overflow of water was always avoided. Emerged weeds were removed in the white root stage.

3.10 Data on Plant Growth and yield components

The plant height was measured using meter stick from the soil surface level to the tip of the longest leaf. All of the plants from each treatment were measured and averaged, n=12 treatments. Total number of edible lettuce leaf for each plant and treatments were counted and averaged. After 11th week, the plants were harvested, and mass of fresh product were recorded using a digital balance.

3.11 Soil and Plant Sample Collection

About 150 g soil sample were collected from all the pots of each treatment after harvesting. Plant samples were also collected after harvesting. About 5-6 leaves were collected from each plant, which were neither so young nor over matured.

3.12 Preparation of soil samples

All soil samples were removed from the paper bags and placed in shallow aluminum container for drying. The soil samples were oven dried at 65°C ± 2°C for 24 hours. After oven drying, soil samples were pulverized and then sieved using a 2mm sieve. All soil particles greater than 2 mm were removed. The graded soil samples were stored and used for further chemical analysis.

3.13 Soil Sample Analysis

All soil samples were performed in Soil Lab, Department of Agriculture, Texas State University. All soil results expressed on a dry basis. The methods of analysis are discussed under following sections.

3.13.1 Soil pH

Soil pH was determined using a pH meter having a hydrogen selective electrode. Soil solution of 1:2 soil: water (w/v) was made using deionized water. Soil solution extracts were stirred for 5 minutes and then allowed to equilibrate for 30 minutes. After settled down, soil water was used to determine actual soil water pH. Every time before taking a new pH reading, pH meter was calibrated using a buffer solution (Schofield and Taylor 1955).

3.13.2 Electrical Conductivity (EC)

Soil electrical conductivity was determined using a conductivity probe meter. Soil solution of 1:2 soil: (w/v) water was made using deionized water following same way as pH determination. Soil solution extracts were stirred for 5 minutes and then allowed to equilibrate for 30 minutes. After settled down, soil water was used to determine actual soil electrical conductivity and expressed in uhm cm^{-1} (Page et al. 1982).

3.13.3 NO_3-N determination

Nitrate-nitrogen (NO_3-N) was determined using cadmium column reduction method and finally by spectrophotometer measurement. In cadmium column reduction method nitrite (NO_2-N) was formed from the reduction of nitrate (NO_3-N).

Before performing the soil sample analysis via Cd reduction, 1 M KCl solution was used to extract NO_3-N from the soil samples. To determine NO_3-N 1 M KCl was used with 2 mm pulverized soil that we prepared in soil sample preparation stage at a ratio of 10:1 respectively. About 2 gm of soil sample was taken in an extraction cup, agitated for 5 minutes and then filtered through Whatman 2 filter paper to make it ready for Cd reduction and spectrophotometer (Kachurina et al. 2000; Page et al. 1982).

3.13.4 P, K, Ca, Mg and S extraction

First of all, P, K, Ca, Mg and S were extracted using an extractant chemical called Mehlich III. Mehlich III is a dilute acid-fluoride EDTA with a pH of 2.5. Mehlich III consists of 0.013 N HNO_3, 0.2 N CH_3-COOH, 0.25 N NH_4NO_3, 0.015 N NH_4F and 0.001 M EDTA solution. The extracted soil solution was then used nutrient analysis via ICP (Inductively Coupled Plasma) method (Mehlich 1984; Mehlich 1978).

3.13.5 Micronutrients (Cu, Zn, Fe and Mn) determination

To determine plant available Cu, Zn, Fe and Mn in soil, the ICP (Inductively Coupled Plasma) method was again used following extraction of the micronutrients. The nutrients were extracted using DTPA extractant solution consisting of 0.005 M DTPA, 0.01M CaCl and 0.1 M triethanolamine. About 20gm of soil sample were placed in an extraction cup and agitated for 120 minutes and then filtered using a Whatman 2 filter paper. After that the extracted solution was run through ICP (Lindsay and Norvell 1978).

3.14 Plant Sample Analysis

All plant samples were performed in Soil Lab, Texas A & M University. All soil results expressed on a dry basis. The methods of analysis are discussed under following sections.

3.15 Preparation of plant samples

The plant samples were oven dried at 65^{o}C ± 2^{o}C for 24 hours before they were grounded by a grinding machine. The prepared samples were then stored into paper bags and were used for chemical analysis.

3.15.1 Total Nitrogen determination

Plant nitrogen (or protein) is determined by high temperature combustion process. About 0.1 g of oven dried grounded sample was taken in a combustion flask. A catalyst mixture of K_2SO_4, $CuSO_4.5H_2O$ and Se at a ratio of 100:10:1 (v/v/v), with 2 ml 30% H_2O_2 and 3 ml H_2SO_4 were added in the flask. It was allowed to stand for 30 minutes. After completion of digestion, the extract was used to determine total nitrogen. It was reported as dry plant basis. (Nelson and Sommers 1973).

3.15.2 Plant Minerals (Excluding N and Cl)

Plant P, K, S, Ca, Mg, Fe, Cu, Zn, Mn and B were determined by ICP analysis after digestion with nitric acid (Havlin and Soltanpour 1980).

3.16 Statistical Analysis and graphs

Data were analyzed statistically and separately to compare with each treatment using ANOVA, with contrast for each treatement interaction, at 5% significant level and JMP Pro statistical discovery software Some of the graphs were also made using JMP Pro and other graphs were made using Origin.

4. RESULT AND DISCUSSION

4.1 Introduction

Data on different yield contributing characters (plant height, number of edible leaves, plant weight) were recorded to determine Si fertilizers effect on lettuce plant growth. To identify the differences in plant physical parameters of height, weight and edible leaves with respect to treatments, ANOVA tests were performed. In this experiment, two different Si formulations were used and compared with the control (no Si). Moreover, the Si formulations were applied in three doses and two application methods. Both application type: soil and foliar, were used for Eco-Sil^{TM} whereas, only soil application was followed for diatomaceous earth. Therefore, growth data analysis requires several groupings to elucidate the outcome of the experiments in terms of application method and doses compared with control. The statistical analysis was divided into following groups in Table 3.3 and analysis for differences were performed for both plant height and weight.

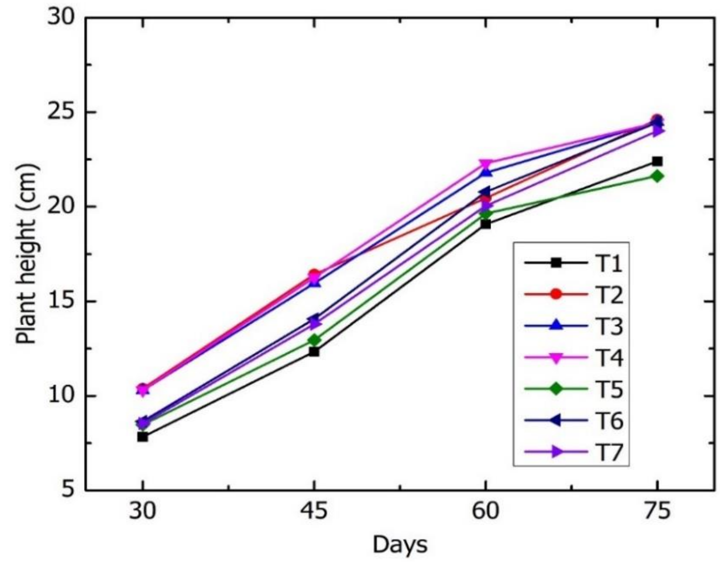
1. Type of fertilizers
2. Dose of fertilizers
3. Application methods

4.2 Plant physical data

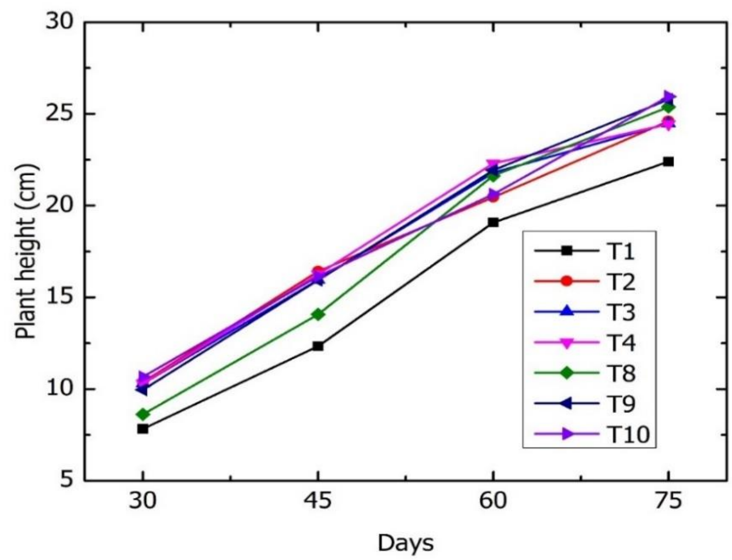
4.2.1 Plant Height

Due to application of different doses of Eco-Sil^{TM} and diatomaceous earth (DE), different average lettuce plant heights were observed which was not liner in fashion with the increase or decrease of doses at 45, 60 and 75 days after transplanting (Figure 4.1). At 45 days after transplanting (DAT), all Si applications showed greater mean height over control except T5 which was not statistically different from the control (Table 4.1). Similarly, all plants with soil applied Si had mean heights significantly greater than control or foliar applications. At 60 days DAT, the same effect appears with foliar application T5 and T7 not significantly different from the control (Table 4.2). Yet notable is, there was little difference between either soil applied Si treatments, but a few foliar applications were not significantly different from Eco-Sil^{TM} applications (T2, T3, T4). However, Eco-Sil^{TM} application T4 produced the greatest mean height at 60 days, with only T8 and T9 not being significantly different. At 75 days DAT all soil applied DE produced the greatest mean heights, with control producing the second lowest mean height (Table 4.3). The control was significantly different from all treatments except T5 foliar application which was the shortest mean height.

Eco-Sil^{TM} (T1, T2-T4, T5-T7).



A



B

Figure 4.1: Increase of plant height over the time, A. soil and foliar application of Eco-Sil^{TM} (T1, T2-T4, T5-T7) and B. soil application of Eco-Sil^{TM} and DE (T1, T2-T4, T8-T10).

Table 4.1: Effect of amorphous silica application on plant height after 45 days.

Levels of significant differences between treatments

Treatments	Average plant height (cm)	T2 (SA) Eco-Sil™ 0.38 g kg^{-1}			T3 (SA) Eco-Sil™ 0.75 g kg^{-1}			T4 (SA) Eco-Sil™ 1.25 g kg^{-1}			T5 (FA) Eco-Sil™ 0.38 g kg^{-1}			T6 (FA) Eco-Sil™ 0.75 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	12.33	29.37	<0.0001	1.28	57.02	<0.0001	1.17	67.68	<0.0001	1.16	0.99	0.329	1.49	13.67	0.0013	1.12
T2	16.42	-	-	-	2.945	0.1002	1.11	5.708	0.025	1.11	13.44	0.001	1.44	6.21	0.0211	1.06
T3	15.95	-	-	-	-	-	-	0.556	0.463	0.98	28.41	<0.0001	1.35	24.07	<0.0001	0.92
T4	16.25	-	-	-	-	-	-	-	-	-	34.72	<0.0001	1.33	33.004	<0.0001	0.91
T5	12.95	-	-	-	-	-	-	-	-	-	-	-	-	3.959	0.060	1.30
T6	14.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	13.79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T8	14.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T9	15.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	16.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.1 Continued

Levels of significant differences between treatments (cont.)

Treatments	Average plant height (cm)	T7 (FA) Eco-Sil™ 1.25 g kg^{-1}			T8 (SA) DE 0.38 g kg^{-1}			T9 (SA) DE 0.75 g kg^{-1}			T10 (SA) DE 1.25 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	12.33	6.255	0.0203	1.43	35.09	<0.0001	1.26	67.51	<0.0001	1.07	18.53	0.0003	1.39
T2	16.42	5.928	0.023	1.38	0.344	0.563	1.21	3.496	0.074	1.01	0.325	0.57	1.34
T3	15.95	16.94	0.0005	1.28	1.136	0.298	1.09	0.0005	0.981	0.87	4.53	0.045	1.24
T4	16.25	22.19	0.0001	1.27	3.015	0.097	1.08	0.759	0.392	0.87	7.407	0.012	1.23
T5	12.95	1.603	0.219	1.58	16.71	0.0006	1.44	32.35	<0.0001	1.25	8.057	0.0102	1.56
T6	14.06	0.275	0.605	1.24	10.17	0.0046	1.02	32.71	<0.0001	0.78	2.3532	0.140	1.19
T7	13.79	-	-	-	8.492	0.0083	1.37	19.38	0.0002	1.19	2.844	0.106	1.49
T8	14.07	-	-	-	-	-	-	1.359	0.256	0.98	1.173	0.291	1.33
T9	15.94	-	-	-	-	-	-	-	-	-	5.24	0.0325	1.14
T10	16.14	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.2: Effect of amorphous silica application on plant height after 60 days.

Treatments	Average plant height (cm)	Levels of significant differences between treatments														
		T2 (SA) Eco-Sil™ 0.38 g kg^{-1}			T3 (SA) Eco-Sil™ 0.75 g kg^{-1}			T4 (SA) Eco-Sil™ 1.25 g kg^{-1}			T5 (FA) Eco-Sil™ 0.38 g kg^{-1}			T6 (FA) Eco-Sil™ 0.75 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	19.08	5.66	0.026	1.42	27.6	<0.0001	1.26	49.09	<0.0001	1.12	1.05	0.315	1.31	9.08	0.006	1.35
T2	20.46	-	-	-	5.177	0.033	1.43	11.72	0.0024	1.31	1.766	0.198	1.48	0.247	0.623	1.51
T3	21.8	-	-	-	-	-	-	1.155	0.294	1.13	15.23	0.0008	1.32	3.204	0.087	1.36
T4	22.3	-	-	-	-	-	-	-	-	-	29.03	<0.0001	1.17	8.814	0.007	1.22
T5	19.64	-	-	-	-	-	-	-	-	-	-	-	-	3.579	0.073	1.41
T6	20.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	20.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T8	21.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T9	21.93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	20.62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.2 Continued

Levels of significant differences between treatments (cont.)

Treatments	Average plant height (cm)	T7 (FA) Eco-Sil™ 1.25 g kg^{-1}			T8 (SA) DE 0.38 g kg^{-1}			T9 (SA) DE 0.75 g kg^{-1}			T10 (SA) DE 1.25 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	19.08	2.369	0.138	1.55	33.51	<0.0001	1.05	41.56	<0.0001	1.08	9.85	0.005	1.21
T2	20.46	0.349	0.56	1.69	4.926	0.037	1.26	7.982	0.009	1.27	0.115	0.737	1.38
T3	21.8	7.455	0.012	1.56	0.133	0.718	1.07	0.099	0.755	1.1	4.974	0.036	1.22
T4	22.3	14.34	0.001	1.45	3.177	0.089	0.89	0.882	0.357	0.93	13.55	0.0014	1.06
T5	19.64	0.377	0.545	1.6	17.52	0.0005	1.11	23.21	<0.0001	1.14	3.566	0.073	1.59
T6	20.78	1.113	0.303	1.64	2.959	0.1008	1.16	5.469	0.029	1.18	0.044	0.834	1.31
T7	20.05	-	-	-	7.170	0.014	1.41	10.55	0.0037	1.42	0.902	0.353	1.53
T8	21.63	-	-	-	-	-	-	0.757	0.394	0.84	5.356	0.031	0.98
T9	21.93	-	-	-	-	-	-	-	-	-	8.974	0.006	1.02
T10	20.62	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.3: Effect of amorphous silica application on plant height after 75 days.

Levels of significant differences between treatments

Treatments	Average plant height (cm)	T2 (SA) Eco-Sil^{TM} 0.38 g kg^{-1}			T3 (SA) Eco-Sil^{TM} 0.75 g kg^{-1}			T4 (SA) Eco-Sil^{TM} 1.25 g kg^{-1}			T5 (FA) Eco-Sil^{TM} 0.38 g kg^{-1}			T6 (FA) Eco-Sil^{TM} 0.75 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	22.4	7.71	0.011	1.94	8.78	0.007	1.72	7.49	0.012	1.88	1.19	0.288	1.72	6.4	0.019	1.92
T2	24.61	-	-	-	0.019	0.891	2.05	0.012	0.912	2.19	11.87	0.002	2.51	0.033	0.86	2.24
T3	24.49	-	-	-	-	-	-	0.0004	0.983	2.002	13.63	0.001	1.85	0.004	0.95	2.04
T4	24.44	-	-	-	-	-	-	-	-	-	11.77	0.002	2.01	0.0062	0.94	2.18
T5	21.63	-	-	-	-	-	-	-	-	-	-	-	-	10.24	0.004	2.05
T6	24.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	24.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T8	25.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T9	25.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	25.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.3 Continued

Levels of significant differences between treatments (cont.)

Treatments	Average plant height (cm)	T7 (FA) Eco-Sil™ 1.25 g kg^{-1}			T8 (SA) DE 0.38 g kg^{-1}			T9 (SA) DE 0.75 g kg^{-1}			T10 (SA) DE 1.25 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	22.4	4.59	0.043	1.84	32.28	0.0001	1.25	30.03	0.0001	1.504	18.61	0.0003	1.21
T2	24.61	0.45	0.51	2.15	1.177	0.29	1.707	2.302	0.143	1.88	0.32	0.57	1.87
T3	24.49	0.35	0.56	1.96	2.192	0.153	1.44	3.601	0.07	1.65	0.683	0.417	1.63
T4	24.44	0.327	0.57	2.1	1.641	0.214	1.63	2.908	0.102	1.82	0.526	0.476	1.80
T5	21.63	8.428	0.0085	1.97	38.45	<0.0001	1.42	36.29	<0.0001	1.65	24.54	<0.0001	1.62
T6	24.46	0.218	0.644	2.14	1.764	0.199	1.66	2.976	0.099	1.85	0.617	0.441	1.84
T7	24.01	-	-	-	4.264	0.051	1.58	5.873	0.024	1.77	1.99	0.172	1.76
T8	25.38	-	-	-	-	-	-	0.661	0.425	1.15	0.497	0.488	0.70
T9	25.78	-	-	-	-	-	-	-	-	-	1.545	0.227	1.38
T10	25.95	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

These results indicate that Si fertilizers (Eco-sil and DE) have positive impact on the growth of lettuce and it results in higher lettuce plant height over control. Comparing with control group, the null hypothesis was rejected ($p<0.05$), except a few foliar applications (T5 and T7) for all of the three doses treatment levels of two Si fertilizers, Eco-Sil™ (soil and foliar) and DE. This observation indicates Si applications of Eco-Sil™ and DE has a positive impact on plant height. In general, the differences in lettuce plant height were not largely distinguishable at the early stages (45 DAT) of growth. However, at the time of harvesting, lettuce plant height increased for the application of Si fertilizers which support the results of Pati et al. (2016). Pati et al. (2016) conducted the experiment on rice plant and used diatomaceous earth as source of Si. Another study was conducted by Pilon et.al. (2013) on potato growth. They used a Si product named silamol, which is a combination of orthosilicic acid, disilicic acid and polyethylene glycol. According to their study, plant height increases more in case of soil application of Si fertilizers compared with foliar application. Thus, our study confirms the results of Pilon et.al. (2013).

4.2.2 Number of Edible leaves

Number of edible leaves per plant varied due to application of different treatments of Eco-Sil^{TM} and DE (Table 4.2). Edible leaves were determined by careful observation after removing damaged and yellow lower leaves. There were significant differences ($p<0.05$) among the treatments. T2 and T3, soil applied Eco-Sil^{TM}, produced the most edible leaves per plant. Aside from T10 compared to both and T9 compared to T2, all other comparisons were significantly different with T2 and T3 having the most edible leaves (Table 4.4). Overall, Si fertilizers (Eco-Sil^{TM} and DE) increased the number of leaves per plant which support the result of Pilon et. al. (2013). However, the increases with doses were different for Eco-Sil^{TM} and DE. It should be noted, the Eco-Sil Eco-Sil^{TM} applications, T2 and T3, were similar in overall Si concentration to T9 and T10, which may explain the similarity in edible leaf count.

Table 4.4: Effect of amorphous silica application on number of edible leaves plant^{-1}.

Levels of significant differences between treatments

Treatments	Number of edible leaves plant^{-1}	T2 (SA) Eco-Sil™ 0.38 g kg^{-1}			T3 (SA) Eco-Sil™ 0.75 g kg^{-1}			T4 (SA) Eco-Sil™ 1.25 g kg^{-1}			T5 (FA) Eco-Sil™ 0.38 g kg^{-1}			T6 (FA) Eco-Sil™ 0.75 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	14	8.82	0.007	1.71	18.58	0.0003	1.33	3.14	0.09	1.38	6.18	0.021	1.66	o.292	0.594	1.61
T2	16.08	-	-	-	0.148	0.703	1.58	2.62	0.119	1.62	23.30	<0.0001	1.89	10.09	0.004	1.84
T3	16.33	-	-	-	-	-	-	7.183	0.013	1.21	40.68	<0.0001	1.52	19.38	0.0002	1.46
T4	15	-	-	-	-	-	-	-	-	-	17.17	0.0005	1.57	4.616	0.935	1.52
T5	12.27	-	-	-	-	-	-	-	-	-	-	-	-	3.16	0.09	1.79
T6	13.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	12.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T8	14.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T9	14.91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	15.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.4 Continued

Levels of significant differences between treatments (cont.)

Treatments	Number of edible leaves plant^{-1}	T7 (FA) Eco-Sil™ 1.25 g kg^{-1}			T8 (SA) DE 0.38 g kg^{-1}			T9 (SA) DE 0.75 g kg^{-1}			T10 (SA) DE 1.25 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	14	4.27	0.05	1.48	0.247	0.623	1.31	1.95	0.176	1.61	4.22	0.052	1.48
T2	16.08	22.5	<0.0001	1.72	7.44	0.012	1.58	2.433	0.133	1.83	1.255	0.275	1.73
T3	16.33	43.55	<0.0001	1.33	19.06	0.0003	1.13	5.567	0.027	1.47	3.679	0.068	1.32
T4	15	15.81	0.0006	1.38	2.112	0.16	1.19	0.018	0.894	1.52	0.223	0.641	1.38
T5	12.27	0.469	0.5006	1.67	9.49	0.005	1.52	12.55	0.0019	1.78	17.56	0.0005	1.67
T6	13.63	1.728	0.202	1.61	1.042	0.319	1.46	3.113	0.092	1.73	5.586	0.028	1.62
T7	12.75	-	-	-	7.670	0.011	1.32	10.83	0.003	1.61	16.51	0.0006	1.48
T8	14.27	-	-	-	-	-	-	1.108	0.304	1.46	3.2011	0.088	1.31
T9	14.91	-	-	-	-	-	-	-	-	-	0.277	0.604	1.62
T10	15.33	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

4.2.3 Plant weight

Arguably, plant weight is a major factor in lettuce production, as much of sales are based on weight and the majority of plant weight is water. The application of different doses of Eco-Sil^{TM} and DE had positive impact on average lettuce plant weight. Both increased the average weight of plants. Figure 4.2 represents the distribution of lettuce plant weights using box plot after harvesting at 75th day for different treatments of Si fertilizers. The results are grouped for control, Eco-Sil^{TM} and DE, and color coded for soil and foliar application. At the time of harvesting, the greatest mean plant weight of 99.39 g was observed from DE (T10:1.25 g kg^{-1} SA), which was significantly greater than all other treatments, except T2, T3, and T9 and markedly heavier than the control weight of 81.53 g (Table 4.5).

Table 4.5: Effect of amorphous silica application on plant weight.

Levels of significant differences between treatments

Treatments	Average plant weight (g)	T2 (SA) Eco-Sil™ 0.38 g kg^{-1}			T3 (SA) Eco-Sil™ 0.75 g kg^{-1}			T4 (SA) Eco-Sil™ 1.25 g kg^{-1}			T5 (FA) Eco-Sil™ 0.38 g kg^{-1}			T6 (FA) Eco-Sil™ 0.75 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	81.53	21.13	<0.0001	8.66	16.31	0.0005	8.21	1.36	0.26	8.61	35.11	<0.0001	8.43	6.99	0.015	9.89
T2	97.9	-	-	-	0.597	0.447	8.60	30.82	<0.0001	8.98	101.45	<0.0001	8.82	40.46	<0.0001	10.24
T3	95.21	-	-	-	-	-	-	25.55	<0.0001	8.55	97.07	<0.0001	8.36	35.47	<0.0001	9.84
T4	77.53	-	-	-	-	-	-	-	-	-	20.91	0.0002	8.76	2.572	0.123	10.19
T5	60.8	-	-	-	-	-	-	-	-	-	-	-	-	5.303	0.032	10.1
T6	70.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T7	68.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T8	81.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T9	96.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T10	99.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Table 4.5 Continued

Levels of significant differences between treatments (cont.)

Treatments	Average plant weight (g)	T7 (FA) Eco-Sil™ 1.25 g kg^{-1}			T8 (SA) DE 0.38 g kg^{-1}			T9 (SA) DE 0.75 g kg^{-1}			T10 (SA) DE 1.25 g kg^{-1}		
		F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err	F-value	p-value	Std Err
T1*	81.53	10.08	0.004	10.48	3.92	0.06	8.29	9.27	0.005	12.1	30.91	<0.0001	8.24
T2	97.9	45.91	<0.0001	10.79	6.711	0.017	8.7	0.054	0.817	12.39	0.632	0.435	8.65
T3	95.21	40.58	<0.0001	10.43	3.794	0.064	8.22	0.096	0.759	12.09	2.679	0.116	8.18
T4	77.53	4.683	0.041	10.75	9.227	0.006	8.64	14.44	0.001	12.36	41.93	<0.0001	8.59
T5	60.8	2.63	0.119	10.69	59.11	<0.0001	8.45	48.27	<0.0001	12.38	124.58	<0.0001	8.4
T6	70.71	0.290	0.595	11.89	17.44	0.0005	9.98	21.51	0.0001	13.43	50.27	<0.0001	9.94
T7	68.04	-	-	-	21.405	0.0001	10.6	26.16	<0.0001	13.73	55.205	<0.0001	10.5
T8	81.12	-	-	-	-	-	-	2.567	0.124	12.29	12.15	0.0023	8.26
T9	96.72	-	-	-	-	-	-	-	-	-	0.627	0.437	12.2
T10	99.39	-	-	-	-	-	-	-	-	-	-	-	-

* Control = no Si application

Compared to the control group, the null hypotheses were rejected at 5% level of significance (Table 4.5) for most of all three doses (excepting T4 soil applied Eco-Sil™ of 1.25 g kg^{-1}) of two Si fertilizers, Eco-Sil™ and DE. That means, control, Eco-Sil™ and DE has significance differences among them. However, lettuce plant weight increased for the application of Si fertilizers, which support the results of Pati et al. (2016), Meena et al. (2014) and Ahmed et al. (2011). Both Pati et al. (2016) and Meena et al. (2014) conducted the experiment to find the effect of silicon on rice plant. Whereas Ahmed et al. (2011) did their study on sorghum. There are also significant differences in plant weight due to soil and foliar application methods (Table 4.5). Plant weight increased more in case of soil application of Eco-Sil™ comparing with foliar application which supports the results of Pilon et al. (2013). Like the number of leaves per plant, plant weight followed the same trend line in case of increasing doses of fertilizers. The plant weight decreased with the increasing level of Eco-Sil™ whereas, increasing level of DE increased average plant weight.

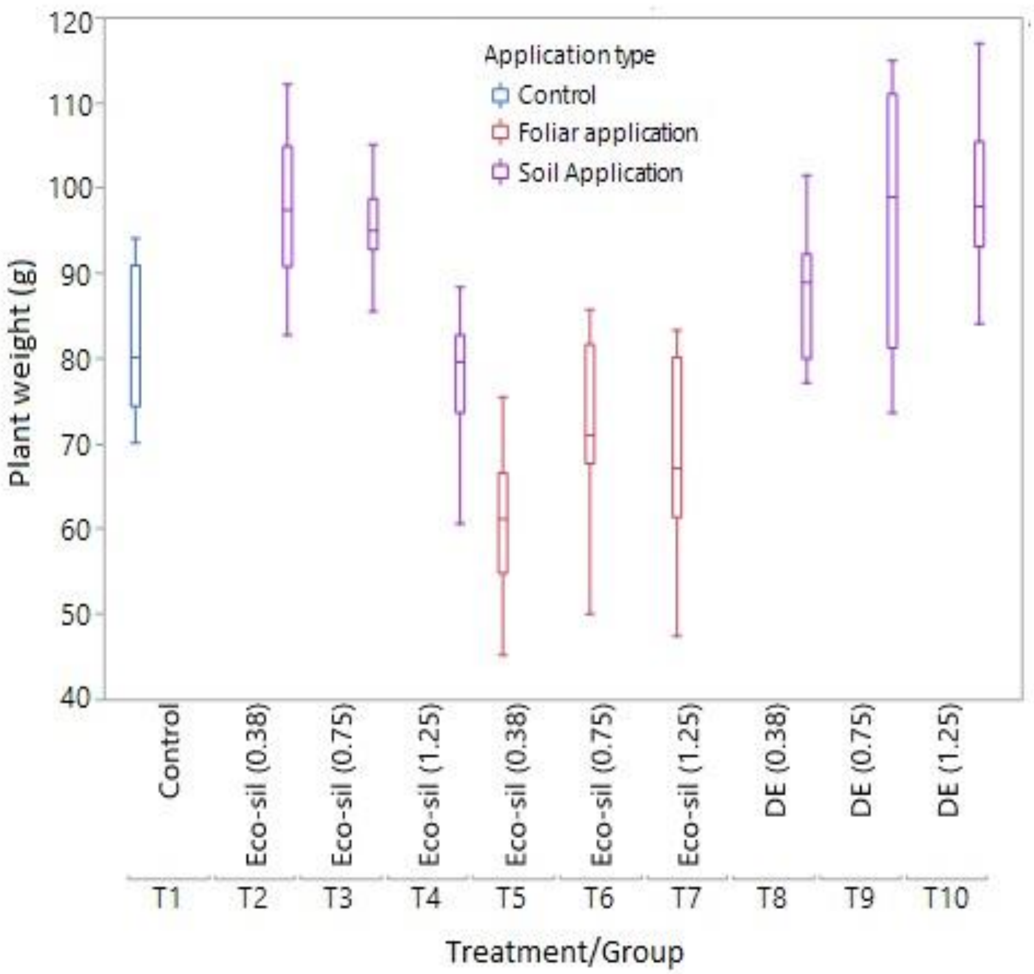


Figure 4.2: Effect of silicon fertilization on lettuce plant weight

4.3 Plant chemical data

The chemical analysis of lettuce plant tissue was conducted to determine if silica applications affected the tissue concentrations of plant essential elements. The results are grouped in four figures (4.3 and 4.4) and represent macronutrient data; figures 4.5 and 4.6 represent micronutrient data. The presentation style of all four graphs is same, with the first column showing the control data point. The second and third column shows the data for DE and Eco-Sil^{TM} respectively with dose amount. The nutrients representatives are stacked for comparison. Note that, two applications were applied for Eco-Sil^{TM} whereas, DE applied using one method only. The graphs are color coded based on application type. The sample size was too small for statistical analysis; thus, the figures are only used for inference and reference to further research opportunity.

4.3.1 Accumulation of N, P and K in lettuce leaf

As shown in fig. 4.3 top row, FA of Eco-Sil^{TM} and SA of DE have minimal impact on accumulation of N. A slow gradual increase of N accumulation observed with doses in both cases. On the other hand, Eco-Sil^{TM} SA resulted sharp increase of N accumulation up to 6.24% N for Eco-Sil^{TM} (T4:1.25 g kg^{-1} SA) (figure 4.3). Whereas the minimum N 4.84% was recorded from control (T1: No Si fertilizers). The increase in the percentage of N in plant leaves support the results of Pilon et al. (2013) and Pati et al. (2016) but contradict with the findings of Greger et al. (2018). According to Greger et al. (2018), the amount of N tented to decrease due to application of Si.

DE application increased the amount of P percentage in lettuce leaf comparing with control (0.44%). Maximum P% 0.55 was recorded from DE (T10:1.25 g kg^{-1} SA). However, the percentage of P in leaf decreased with the application of increased rates of

Eco-Sil™ (figure 4.3). This decreasing trend of P percentage also agrees with the result of Greger et al. (2018) but does not agree with Olle et al. (2017). Olle et al. (2017) found about 25% higher P content in lettuce leaf comparing with control.

In case of K, no clear pattern was observed with various doses of DE. Although it appears that both soil and foliar application causes linear increase of K percentage, the highest accumulation level is comparable with control. This implies, Si fertilization does not have any general effect on K percentage in leaf. This result conforms with literature (Greger et al. 2018; Olle 2017).

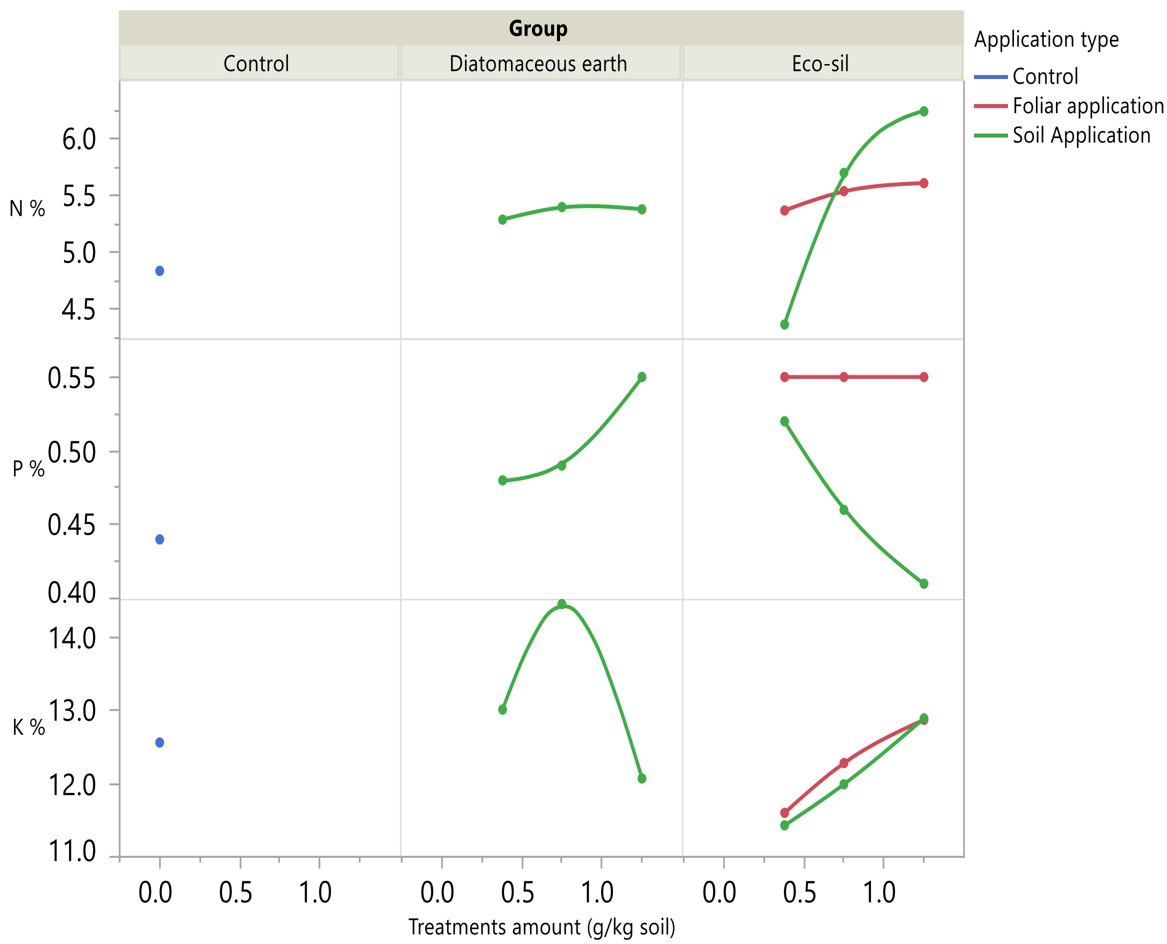


Figure 4.3: Effect of silicon fertilization on N, P, K accumulation in plant

4.3.2 Accumulation of Ca, Mg and S in lettuce leaf

Both Ca and Mg percentage in lettuce leaf increased in a linear fashion due to soil application of Eco-Sil^{TM} (figure 4.4). There is up to 2.6% increase in Ca percentage and 9.5% increase in Mg percentage for Eco-Sil^{TM} (T4: 1.25 g kg^{-1} SA) comparing with control. No remarkable changes were observed from foliar application of Eco-Sil^{TM}. DE also has no remarkable change in Ca percentage, but it increases the Mg percent up to 10%. These results also support the work of Greger et al. (2018). They conducted their experiment on maize, lettuce, wheat, carrot and pea. According to their study, Ca and Mg increased in all plant species when Si applied.

For S percentage in lettuce leaf, all of the groups of Si fertilizers: Eco-Sil^{TM} SA - and FA application, and DE FA application have similar trend (Figure 4.4). In all cases, with the application of Si fertilizers S percentage increased up to a level but then decreased with increased level of doses. With further increment of the doses, S percentage started to increase again. According to literature, Si also increases S content in plant under varying stress condition like salt stress and mineral stress (Miyake 1993; Ribera and Marı 2015).

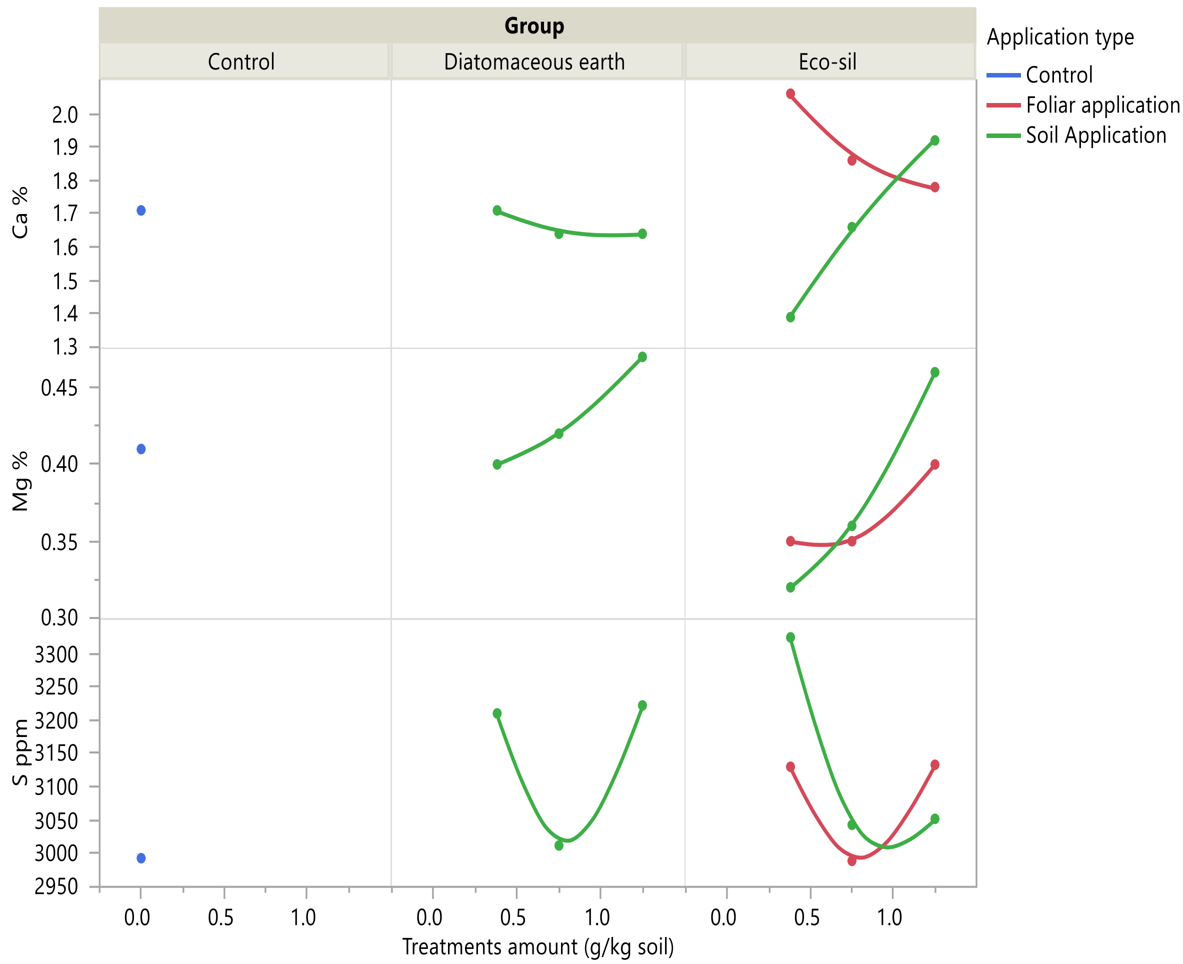


Figure 4.4: Effect of silicon fertilization on Ca, Mg and S accumulation in plant

4.3.3 Accumulation of Zn, Cu and Fe in lettuce leaf

Zn accumulation in leaf followed identical pattern of increase (Figure 4.5) with dose for both fertilizers: Eco-Sil^{TM} and DE although the level of increase is different (Eco-Sil^{TM}: 2.5% and DE: 5.5%). In contrast, the amount of Fe declined in case of soil application of Eco-Sil^{TM} and DE which conforms the results of (Islam and Saha 1969). Decreasing Cu content in plant leaves support the result of Greger et al. (2018), but according to Greger et al. (2018), Si slightly increased Fe contents in plant which is opposite of this study. Foliar application of Eco-Sil™ also decreased Fe and Cu comparing with control but almost no change with the increased doses of Eco-Sil™ (FA).

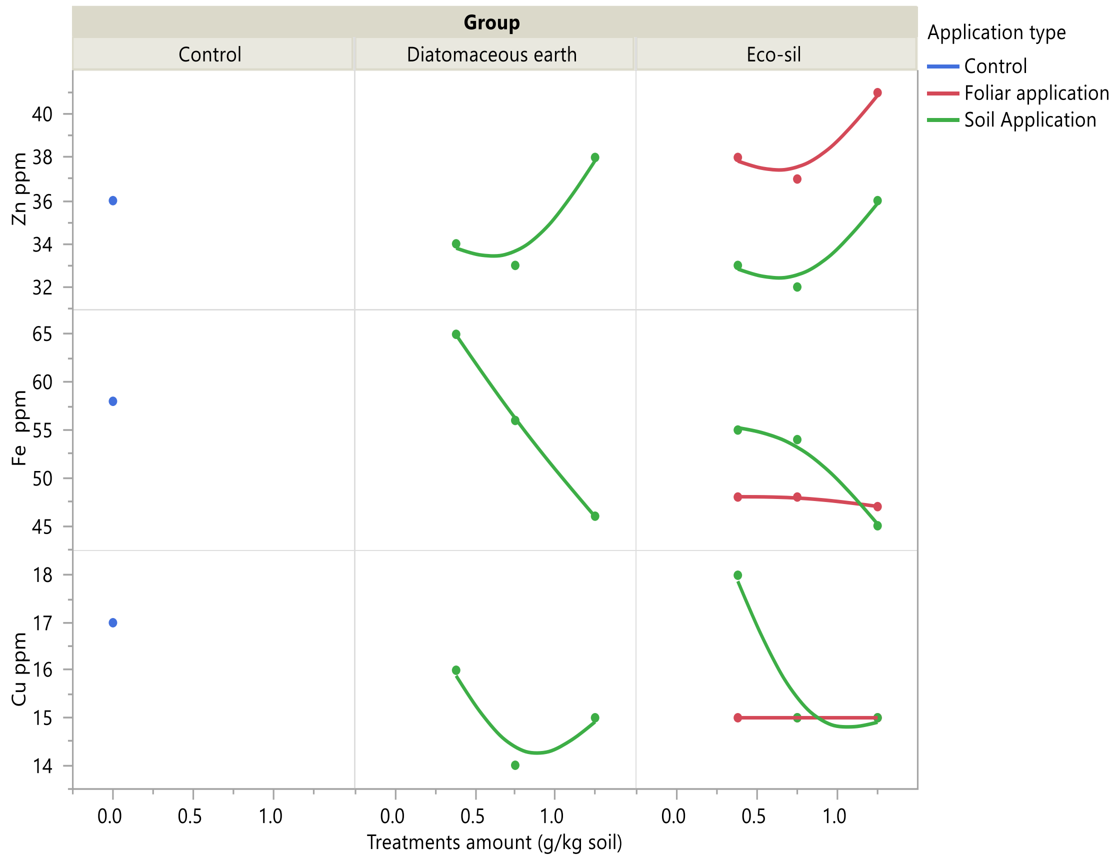


Figure 4.5: Effect of silicon fertilization on Cu, Fe and Zn uptake in plant

4.3.4 Accumulation of Mn and B in lettuce leaf

Both Mn and B increased in case of soil application of Eco-Sil™ and DE (figure 4.6) which completely support the result of Greger et al. (2018). In comparison with control, DE increased B up to 11% whereas, Eco-Sil™ increased up to 24%. The results recorded from foliar application are almost similar with control. The percent increase of Mn is higher for Eco-Sil™ SA than that of SA of DE. Mn increased up to 13% because of soil application of Eco-Sil™ and the accumulation was increasing with the increased doses of Eco-Sil™.

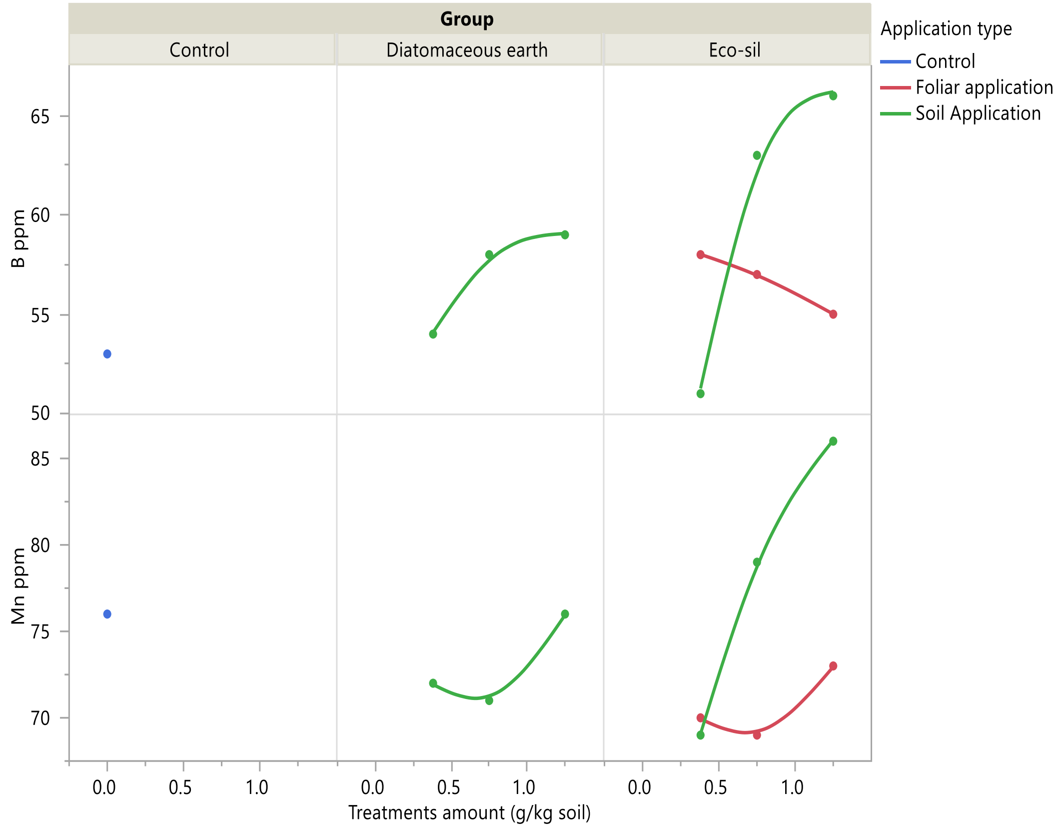


Figure 4.6: Effect of silicon fertilization on Mn and B accumulation in plant

5. CONCLUSION AND FUTURE SCOPE

5.1 Conclusion

From commercial point of view, plant height, weight and number of edible leaves per plants are the parameters of interest. The key objective of this research was to assess the effects of Si application on lettuce. The secondary goal was to find out suitable application method and dose. DE treatments are chosen for comparison since it is commercially available and popularly used in crop production. Note that, DE contains P, K, Ca, Mg etc. along with SiO_2 (Sandhya et al. 2018) whereas Eco-Sil™ contains only SiO_2. In general, soil application of Eco-Sil™ resulted in higher yield over control. In the case of some treatments, yield parameters are comparable with DE. However, foliar application of Eco-Sil™ resulted in lower yield compared with control. Low yield with foliar application can be interpreted by comparative chemical analysis of soil and foliar application of Eco-Sil™ treatments. Further, the T4 treatment, at harvest, showed consistent lower values for all measures, when compared to T2 and T3, the other Eco-Sil™ soil applications. While these lower values were not always significantly different, there may be a Si toxicity effect appearing that warrants further research for higher concentrations. As can be seen from figure 4.3-4.6, N, Ca, Mg, Fe, B and Mn accumulation significantly increased with doses for SA Eco-Sil™ compared with FA. In FA the P, K, Mg, Cu and Mn remain unchanged with increased level of doses. The majority of nutrient accumulation increased in case of SA of Eco-Sil™ which resulted higher growth (Sinclair 1992). As per statistical data presented in table 4.2, among the three doses of Eco-Sil™ SA, T2 and T3 are statistically similar and gave much higher production relative to T4. At the same time, Eco-Sil™ T2 and T3 are also similar with

DE T9 and T10. In summary, certain doses of Eco-Sil^{TM} SA have similar performance like DE and capable of increasing leafy green vegetable growth, yield and nutrient accumulation.

5.2 Future scope

There exists considerable research work supporting the role of Si for reduction of biotic and abiotic stress, and nutrient availability. However, only a few references are available on the role of silicon in plant growth and yield. This work is the first research attempt to evaluate Eco-Sil^{TM} as a Si fertilizer. As a result, the work done here opens the window of further potential research. Research should focus on understanding the effect of Si fertilizers on plant growth and yield, as well as Eco-Sil^{TM}'s high SiO_2 influence when compared to lower SiO_2 products like DE. Further research on Eco-Sil^{TM} should be considered for monocot crops like rice, wheat and maize as these are known to be very good conductor of Si (Elsokkary 2018). There are also scopes to work on the growth and accumulation of nutrients in other leafy green vegetables. Current research does not include interactions of Eco-Sil^{TM} and biotic-abiotic stress of plants. Future researchers need to address the lack of knowledge on effect of Eco-Sil^{TM} application to plants for reduction of biotic and abiotic stress. Along with, research needs to be less reliant on laboratory and greenhouse research and consider the effect of Eco-Sil^{TM} under field conditions.

REFERENCES

Agostinho, F., B. Tubana, M. Martins, and L. Datnoff. 2017. Effect of Different Silicon Sources on Yield and Silicon Uptake of Rice Grown under Varying Phosphorus Rates. *Plants* 6, no. 3: 35.

Ahmad, M., M.H. El-Saeid, M.A. Akram, H.R. Ahmad, H. Haroon, and A. Hussain. 2016. Silicon Fertilization – A Tool to Boost up Drought Tolerance in Wheat (Triticum Aestivum L.) Crop for Better Yield. *Journal of Plant Nutrition* 39, no. 9: 1283–1291.

Ahmed, M., U. Qadeer, and M.A. Aslam. 2011. Silicon Application and Drought Tolerance Mechanism of Sorghum. *African Journal of Agricultural Research* 6, no. 3: 594–607.

Amin, M., R. Ahmad, A. Ali, I. Hussain, R. Mahmood, M. Aslam, and D.J. Lee. 2016. Influence of Silicon Fertilization on Maize Performance Under Limited Water Supply. *Silicon* 10, no. 2: 177–183.

Anon. 2020. The Plant List. http://www.theplantlist.org/1.1/browse/A/Compositae/.

Artyszak, A. 2018. Effect of Silicon Fertilization on Crop Yield Quantity and Quality—A Literature Review in Europe. *Plants* 7, no. 3: 54.

Ashok, L.B., G.S.K. Swamy, and N.B. Prakash. 2017. Diatomaceous Earth as Source of Silicon in Tomato Crop. In *7th International Conference on Silicon in Agriculture*, 128. Bengaluru, India.

Ashtiani, F.A., J. Kadir, A. Nasehi, S.R.H. Rahaghi, and H. Sajili. 2012. Effect of Silicon on Rice Blast Disease. *Pertanika Journal of Tropical Agricultural Science* 35, no. SUPPL.: 1–12.

Barros, T.C., R. De Mello Prado, C. Garcia Roque, G. Ribeiro Barzotto, and C. Roberto Wassolowski. 2018. Silicon and Salicylic Acid Promote Different Responses in Legume Plants. *Journal of Plant Nutrition* 41, no. 16: 2116–2125.

Bocharnikova, E.A., D.M. Khomiakov, P. Zhang, V. V. Matichenkov, Y. Liu, and E.P. Pakhnenko. 2018. Effect of Amorphous Silicon Dioxide on Cadmium Behavior in the Soil–Rice Plant System. *Moscow University Soil Science Bulletin* 73, no. 1: 34–38.

Chen, W., X. Yao, K. Cai, and J. Chen. 2011. Silicon Alleviates Drought Stress of Rice Plants by Improving Plant Water Status , Photosynthesis and Mineral Nutrient Absorption. *Biological Trace Research* 142, no. 1: 67–76.

Coskun, D., R. Deshmukh, H. Sonah, J.G. Menzies, O. Reynolds, J.F. Ma, H.J. Kronzucker, and R.R. Bélanger. 2018. The Controversies of Silicon's Role in Plant Biology. *New Phytologist* 221, no. 1: 67–85.

Crusciol, C.A.C., A.L. Pulz, L.B. Lemos, R.P. Soratto, and G.P.P. Lima. 2009. Eff Ects of Silicon and Drought Stress on Tuber Yield and Leaf Biochemical Characteristics in Potato. *Crop Science* 49: 949–954.

Cuong, T.X., H. Ullah, A. Datta, and T.C. Hanh. 2017. Effects of Silicon-Based Fertilizer on Growth, Yield and Nutrient Uptake of Rice in Tropical Zone of Vietnam. *Rice Science* 24, no. 5: 283–290.

Datnoff, L.E., C.W. Deren, and G.H. Snyder. 1997. Silicon Fertilization for Disease Management of Rice in Florida. *Crop Protection* 16, no. 6: 525–531.

Dupree, P., T. Babu, B. White, B. Tubana, and L. Datnoff. 2017. Survey of the Plant-Available Silicon Status of Agricultural Soils in Louisiana. *Journal of Pla nt Nutrition* 41, no. 3: 273–287.

Elsokkary, I.H. 2018. Silicon as a Beneficial Element and as an Essential Plant Nutrient: An Outlook (Review). *Alexandria Science Exchange Journal* 39, no. July-September: 534–550.

Eneji, A.E., S. Inanaga, S. Muranaka, J. Li, T. Hattori, P. An, W. Tsuji, et al. 2008. Growth and Nutrient Use in Four Grasses Under Drought Stress as Mediated by Silicon Fertilizers. *Journal of Plant Nutrition* 31: 355–365.

Gong, H., and K. Chen. 2012. The Regulatory Role of Silicon on Water Relations , Photosynthetic Gas Exchange , and Carboxylation Activities of Wheat Leaves in Field Drought Conditions. *Acta Physiologiae Plantarum* 34: 1589–1594.

Gong, H.J., K.M. Chen, Z.G. Zhao, G.C. Chen, and W.J. Zhou. 2008. Effects of Silicon on Defense of Wheat against Oxidative Stress under Drought at Different Developmental Stages. *Biologia Plantarum* 52, no. 3: 592–596.

Gong, H.J., D.P. Randall, and T.J. Flowers. 2006. Silicon Deposition in the Root Reduces Sodium Uptake in Rice (Oryza Sativa L.) Seedlings by Reducing Bypass Flow. *Plant, Cell and Environment* 29, no. 10: 1970–1979.

Green, S.W., and D.R. Piperno. 1991. Phytolith Analysis: An Archaeological and Geological Perspective. *American Journal of Archaeology* 95, no. 4: 741.

Greger, M., T. Landberg, and M. Vaculík. 2018. Silicon Influences Soil Availability and Accumulation of Mineral Nutrients in Various Plant Species. *Plants* 7, no. 2: 41.

Gunes, A., D.J. Pilbeam, A. Inal, and S. Coban. 2008. Influence of Silicon on Sunflower Cultivars under Drought Stress, I: Growth, Antioxidant Mechanisms, and Lipid Peroxidation. *Communications in Soil Science and Plant Analysis* 39, no. 13–14: 1885–1903.

Guntzer, F., C. Keller, and J.D. Meunier. 2012. Benefits of Plant Silicon for Crops: A Review. *Agronomy for Sustainable Development* 32, no. 1: 201–213.

Hasan, M.R., A.K.M.M. Tahsin, M.N. Islam, M.A. Ali, and J. Uddain. 2017. Growth and Yield of Lettuce (Lactuca Sativa L .) Influenced As Nitrogen Fertilizer and Plant Spacing 10, no. 6: 62–71.

Hasanuzzaman, M., M. Fujita, H. Oku, K. Nahar, and B. Hawrylak-Nowak. 2018. *Plant Nutrients and Abiotic Stress Tolerance*.

Hattori, T., S. Inanaga, H. Araki, P. An, S. Morita, M. Luxová, and A. Lux. 2005. Application of Silicon Enhanced Drought Tolerance in Sorghum Bicolor. *Physiologia Plantarum* 123, no. 4: 459–466.

Hattori, T., K. Sonobe, S. Inanaga, P. An, and S. Morita. 2008. Effects of Silicon on Photosynthesis of Young Cucumber Seedlings under Osmotic Stress. *Journal of Plant Nutrition* 31, no. 6: 1046–1058.

Havlin, J.L., and P.N. Soltanpour. 1980. A Nitric Acid Plant Tissue Digest Method for Use with Inductively Coupled Plasma Spectrometry. *Communications in Soil Science and Plant Analysis* 11, no. 10: 969–980.

Hawk, C., J. Hyland, R. Rupert, M. Colonvega, and S. Hall. 2006. A Comparison of Soil and Foliar-Applied Silicon on Nutrient Availability and Plant Growth and Soil-Applied Silicon on Phosphorus Availability. *Chiropractic & Osteopathy* 14: 3.

Hodson, M.J., P.J. White, A. Mead, and M.R. Broadley. 2005. Phylogenetic Variation in the Silicon Composition of Plants. *Annals of Botany* 96: 1027–1046.

Ibrahim, M.A., A.M. Merwad, and E.A. Elnaka. 2018. Rice (Oryza Sativa L .) Tolerance to Drought Can Be Improved by Silicon Rice (Oryza Sativa L .) Tolerance to Drought Can Be Improved by Silicon Application. *Communications in Soil Science and Plant Analysis*: 1532–2416.

Islam, A., and R.C. Saha. 1969. Effects of Silicon on the Chemical Composition of Rice Plants. *Plant and Soil* 30: 446–458.

Ismail, H., and B. Mirza. 2015. Evaluation of Analgesic , Anti-Inflammatory , Anti-Depressant and Anti-Coagulant Properties of Lactuca Sativa (CV . Grand Rapids) Plant Tissues and Cell Suspension in Rats. *BMC Complementary and Alternative Medicine* 15, no. 199: 1–7.

Janislampi, K.W. 2012. Effect of Silicon on Plant Growth and Droucht Stress Tolerance. *Zhurnal Eksperimental'noi i Teoreticheskoi Fiziki*: 100.

Kachurina, O.M., H. Zhang, W.R. Raun, and E.G. Krenzer. 2000. Simultaneous Determination of Soil Aluminum , Ammonium - and Nitrate - Nitrogen Using 1 M Potassium Chloride Extraction. *Communications in Soil Science and Plant Analysis* 31: 893–903.

Kaushik, P., and D.K. Saini. 2019. Silicon as a Vegetable Crops Modulator—A Review. *Plants* 8, no. 6: 148.

Kaya, C., L. Tuna, and D. Higgs. 2007. Effect of Silicon on Plant Growth and Mineral Nutrition of Maize Grown under Water-Stress Conditions. *Journal of Plant Nutrition* 29, no. 8: 1469–1480.

Keeping, M.G. 2017. Uptake of Silicon by Sugarcane from Applied Sources May Not Reflect Plant-Available Soil Silicon and Total Silicon Content of Sources. *Frontiers in Plant Science* 8, no. May: 1–14.

Kesseli, R. 1991. Variation at RFLP Loci in Lactuca Spp . and Origin of Cultivated Lettuce (L . Sativa). *Genome* 34.

Khan, M.A., V. Goyal, and N. Jain. 2017. Impact of Ortho Silicic Acid Formulation on Yield and Disease Incidence of Potatoes. In *In Proceedings of the 7th International Conference on Silicon in Agriculture*, 137. Bengaluru, India.

Korndörfer, G.H., and I. Lepsch. 2001. Effect of Silicon on Plant Growth and Crop Yield. *Studies in Plant Science* 8, no. C: 133–147.

Llndsay, W.L., and W.A. Norvell. 1978. Development of a DTPA Soil Test for Zinc , Iron , Manganese , and Copper. *Soil Science Society of America* 42: 421–428.

Ma, J.F., and E. Takahashi. 2002. *Soil, Fertilizer, and Plant Silicon Research in Japan*. Amsterdam: Elsevier.

Maghsoudi, K., Y. Emam, and M. Ashraf. 2016. Foliar Application of Silicon at Different Growth Stages Alters Growth and Yield of Selected Wheat. *Journal of Plant Nutrition* 39, no. 8: 1194–1203.

Mahlangu, R.I.S., M.M. Maboko, D. Sivakumar, P. Soundy, and J. Jifon. 2016. Lettuce (Lactuca Sativa L .) Growth , Yield and Quality Response to Nitrogen Fertilization in a Non-Circulating Hydroponic System 4167, no. June: 0–28.

Makabe, S., K. Kakuda, Y. Sasaki, T. Ando, H. Fujii, and H. Ando. 2009. Relationship between Mineral Composition or Soil Texture and Available Silicon in Alluvial Paddy Soils on the Shounai Plain , Japan. *Soil Science and Plant Nutrition* 55: 300–308.

Mali, M., and N.C. Aery. 2009. Effect of Silicon on Growth, Biochemical Constituents, and Mineral Nutrition of Cowpea. *Communications in Soil Science and Plant Analysis* 40: 1041–1052.

Meena, V.D., M.L. Dotaniya, V. Coumar, S. Rajendiran, Ajay, S. Kundu, and A. Subba Rao. 2014. A Case for Silicon Fertilization to Improve Crop Yields in Tropical Soils. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences* 84, no. 3: 505–518.

Mehlich, A. 1978. New Extractant for Soil Test Evaluation of Phosphorus , Potassium , Magnesium , and Zinc. *Communications in Soil Science and Plant Analysis* 9, no. 6: 477–492.

———. 1984. Mehlich 3 Soil Test Extractant : A Modification of Mehlich 2 Extractant. *Communications in Soil Science and Plant Analysis* 15, no. 12: 1409–1416.

Mehrabanjoubani, P., A. Abdolzadeh, H.R. Sadeghipour, and M. Aghdasi. 2014. Impacts of Silicon Nutrition on Growth and Nutrient Status of Rice Plants Grown under Varying Zinc Regimes. *Theoretical and Experimental Plant Physiology* 27, no. 1: 19–29.

Miyake, Y. 1993. *Silicon in Soil and Plants*. *Sci. Rep. Faculty of Okayama Univ.*

Montesano, F.F., M. D'Imperio, A. Parente, A. Cardinali, M. Renna, and F. Serio. 2016. Green Bean Biofortification for Si through Soilless Cultivation: Plant Response and Si Bioaccessibility in Pods. *Scientific Reports* 6, no. July: 1–9.

Nazirkar, R.B., B. Narale, and A.G. Durgude. 2017. Effect of Sources and Levels of Silicon on Soil Properties, Uptake, Yield and Quality of Kharif Onion. In *7th International Conference on Silicon in Agriculture*, 141. Bengaluru, India.

Nelson, D.W., and L.E. Sommers. 1973. Determination of Total Nitrogen in Plant Material 1. *Agronomy* 65: 109–112.

Neu, S., J. Schaller, and E.G. Dudel. 2017. Silicon Availability Modifies Nutrient Use Efficiency and Content, C:N:P Stoichiometry, and Productivity of Winter Wheat (Triticum Aestivum L.). *Scientific Reports* 7, no. September 2016: 3–10.

Ning, D., A. Song, F. Fan, Z. Li, and Y. Liang. 2014. Effects of Slag-Based Silicon Fertilizer on Rice Growth and Brown-Spot Resistance. *PLoS ONE* 9, no. 7: 1–9.

Nolla, A., G.H. Korndorfer, R.J. de Faria, and R.B. Silva. 2012. Effect of Silicon on Drought Tolerance of Upland Rice. *Journal of Food, Agriculture and Environment* 10, no. 1: 269–272.

Olle, M. 2017. The Effect of Silicon on the Organically Grown Iceberg Lettuce Growth and Quality. *Journal of Agricultural Science* 28, no. 2: 82–86.

Owino-Gerroh, C., and G.J. Gascho. 2011. Effect of Silicon on Low PH Soil Phosphorus Sorption and on Uptake and Growth of Maize Effect of Silicon on Low PH Soil Phosphorus. *Communications in Soil Science and Plant Analysis* 35, no. June 2013: 2369–2378.

Page, A.L., D.E. Baker, J.R. Ellis, D.R. Keeney, R.H. Miller, and J.D. Rhoades. 1982. Methods of Soil Analysis : Part 2. In *AGRONOMY A Series of Monographs*, 167–178. Second. Madison, WI, USA: American American Society of Agronomy and Soil Science Society of America.

Pati, S., B. Pal, S. Badole, and G.C. Hazra. 2016. Effect of Silicon Fertilization on Growth , Yield , and Nutrient Uptake of Rice. *Communications in Soil Science and Plant Analysis* 3624, no. February: 1532–2416.

Pedreira, N.G., J.P. de Souza Junior, A.C.A. dos Santos, R.A. Flores, A.S. Aragão, R. de Mello Prado, E.M. Arruda, and C.F. da Costa. 2018. Nutrition and Production of Helianthus Annuus in a Function of Application of Leaf Silicon . *Journal of Plant Nutrition* 42, no. 2: 137–144.

Perez, C.E.A., F.Á. Rodrigues, W.R. Moreira, and F.M. Damatta. 2014. Leaf Gas Exchange and Chlorophyll a Fluorescence in Wheat Plants Supplied with Silicon and Infected with Pyricularia Oryzae. *Biochemistry and Cell Biology* 104, no. 2: 143–149.

Pilon, C., R.P. Soratto, and L.A. Moreno. 2013. Effects of Soil and Foliar Application of Soluble Silicon on Mineral Nutrition, Gas Exchange, and Growth of Potato Plants. *Crop Science* 53, no. 4: 1605–1614.

Pulz, A.L., C.A.C. Crusciol, L.B. Lemos, and R.P. Soratto. 2008. Silicate and Limestone Effects on Potato Nutrition, Yield and Quality under Drought Stress. *Revista Brasileira de Ciência Do Solo* 32: 1651–1659.

Reboredo, F., F.C. Lidon, F. Pessoa, M.P. Duarte, and M.J. Silva. 2013. The Uptake of Macronutrients by an Active Silicon Accumulator Plant Growing in Two Different Substrata. *Emirates Journal of Food and Agriculture* 25, no. 12: 986–993.

Ribera, A., and L.G. Marı. 2015. Silicon in Vascular Plants : Uptake , Transport and Its Influence on Mineral Stress under Acidic Conditions. *Planta* 242, no. 1: 23–37.

Rodrigues, F.A., and L.E. Datnoff. 2015. *Silicon and Plant Diseases*. *Silicon and Plant Diseases*.

Sandhya, K., N.B. Prakash, J.D. Meunier, K. Sandhya, N.B. Prakash, and J.D. Meunier. 2018. Diatomaceous Earth as Source of Silicon on the Growth and Yield of Rice in Contrasted Soils of Southern India. *Journal of Soil Science and Plant Nutrition* 18, no. 2 : 344-360.

Santi, L.P., Nurhaimi-Haris, and D. Mulyanto. 2018. Effect of Bio-Silicon on Drought Tolerance in Plants. In *Earth and Environmental Science*.

Savant, N.K., L.E. Datnoff, and G.H. Snyder. 2008. Depletion of Plant-Available Silicon in Soils: A Possible Cause of Declining Rice Yields. *Communications in Soil Science and Plant Analysis* 28, no. 13–14: 1245–1252.

Savant, N.K., G.H. Snyder, and L.E. Datnoff. 1997. Silicon Management and Sustainable Rice Production. *Advances in Agronomy* 58: 151–199.

Savić, J., and A. Marjanović-Jeromela. 2013. Effect of Silicon on Sunflower Growth and Nutrient Accumulation under Low Boron Supply. *Helia* 36, no. 58: 61–68.

Schofield, R.K., and A.W. Taylor. 1955. The Measurement of Soil PH 1. *Soil Science Society of America* 19, no. 2: 164–167.

Shakoor, S.A., M.A. Bhat, and S.H. Mir. 2014. Phytoliths in Plants : A Review. *Journal of Botanical Sciences* 3, no. 1: 10–24.

Sinclair, T.R. 1992. Mineral Nutrition and Plant Growth Response to Climate Change. *Journal of Experimental Botany* 43, no. 253: 1141–1146.

Souza, R.S. De, R. Rezende, T.L. Hachmann, C.S. Lozano, A. Felipe, B. Alves, P. Sérgio, and L. De Freitas. 2017. Lettuce Production in a Greenhouse under Fertigation with Nitrogen and Potassium Silicate. *Acta Scientiarum* 39, no. 2: 211–216.

Taiz, L., and E. Zeiger. 2006. *Plant Physiology*. 4th ed. Sunderland, MA: Sinauer Associates.

Tubana, B.S., T. Babu, and L.E. Datnoff. 2016. A Review of Silicon in Soils and Plants and Its Role in Us Agriculture: History and Future Perspectives. *Soil Science* 181, no. 9–10: 393–411.

Ullah, U., M. Ashraf, S.M. Shahzad, A.R. Siddiqui, M.A. Piracha, and M. Suleman. 2016. Growth Behavior of Tomato (Solanum Lycopersicum L .) under Drought Stress in the Presence of Silicon and Plant Growth Promoting Rhizobacteria Characterization Bacterial Strain. *Soil Environment* 35, no. 1: 65–75.

USDA. 2020. *Vegetables 2019 Summary*.

Walsh, O.S., S. Shafian, J.R. Mcclintick-chess, K.M. Belmont, and S.M. Blanscet. 2018. Potential of Silicon Amendment for Improved Wheat Production. *Plants* 7: 26.

Xiong, J., L. Zhang, G. Fu, Y. Yang, C. Zhu, and L. Tao. 2012. Drought-Induced Proline Accumulation Is Uninvolved with Increased Nitric Oxide, Which Alleviates Drought Stress by Decreasing Transpiration in Rice. *Journal of Plant Research* 125, no. 1: 155–164.

Zaheer, M.M., N.A. Yasin, S.R. Ahmad, W.U. Khan, A. Ahmad, A. Ali, and S.U. Rehman. 2018. Amelioration of Cadmium Stress in Gladiolus (Gladiolus Grandiflora L.) by Application of Potassium and Silicon. *Journal of Plant Nutrition* 41, no. 4: 461–476.

Zhu, Y., and H. Gong. 2013. Beneficial Effects of Silicon on Salt and Drought Tolerance in Plants. *Agronomy for Sustainable Development* 34, no. 2: 455–472.