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Micronutrients Status of Bio fuel Plant (Moringa) Irrigated By Diluted Seawater As Affected By Silicate And Salicylic Acid

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Abstract:*A pot experiment was conducted in the greenhouse of the National Research Centre to evaluate the effect of salt stress and foliar amendments on mineral status of moringa plants. The treatments of salinity were irrigated by diluted seawater with 2000 and 4000 ppm salts and tap water (285 ppm) as a control. The treatments of silicate treatments were 300 ppm SiO_2 as potassium silicate and 300 ppm salicylic acid + 300 ppm SiO_2 more than distilled water as a control. Significant responses were detected in Zn, Mn and Cu ppm as a result of salt stress but Fe ppm without significant responds to this treatment. The depression effect in nutrients of plants received Si+SA exceeded those induced by Si alone. Generally, the all calculated ratios (Mn with N, P, K and Na) lowered by the high salinity level and the reverse were true by the lesser level of salinity. The ratios of macronutrients and micronutrients as affected by salinity, foliar application as well as the interactive effect between them were included.*

Keywords: **Moringa (*Morirga oleifera* Lam)–Salinity-Salicylic acid –Silicate - Micronutrients.**

I. Introduction

Moringa oleifera (*M. oleifera*) Lam. is one of the most widely known and utilized species, belonging to the family Moringaceae. A native of the sub-Himalayan regions of northwest India, it is also indigenous to many countries in Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands and South America (Anwar and Bhanger, 2003). It is suggested as a viable supplement of dietary minerals. The pods and leaves of Moringa contains high amount of Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe (Aslam et al., 2005). Biodiesel is an alternative to petroleum-based conventional diesel fuel and is defined as the mono-alkyl esters of vegetable oils and animal fats. *M. oleifera* oil appears to be an acceptable feedstock for biodiesel (Rashid et al., 2008 and Mofijur et al., 2014).

Soil salinity in agriculture soils refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. These concentrations of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water by the roots. Salinity can also affect plant growth because the high concentration of salts in the soil solution interferes with balanced absorption of essential nutritional ions by plants (Tester and Devenport, 2003). The water and salt balance, just like in oceans and seas determine the formation of salty soils, where more salt comes in than goes out. Here, the incoming water from the land brings salts that remain because there is no outlet and the evaporation water does not contain salts (Jouyban, 2012).Moreover, high salinity effect on plant growth through osmotic effect, a toxicity of salt ions, and the changes in physical and chemical properties of soil (Keren, 2000). Many new cultivated soils suffer from the lack of fresh water and the use of nontraditional water such as saline well water, drainage water and diluted seawater is necessary. Use of diluted sea water caused many adverse effects on physiological (Hamdy, et al. 2005, Hussein, et al. 2010, Elhindi, 2013, and Feleafel and Mirdad, 2014).

Silicon is considered as one of the important beneficial nutrient for plant growth (Liang, et al., 2006). Most soils contain significant quantities of silica, but continuous cropping, particularly with crops that accumulate significant quantities of silica, can reduce plant-available levels of Si to the point that supplemental Si fertilization is required (Ma et al., 2001). Plants growing under natural conditions do not appear to suffer from Si deficiencies. However, Si-containing fertilizers are routinely applied to several crops including rice (Pereira et al., 2004) to increase crop yield and quality. Abou-Baker et al. (2012) reported that highest basic branch, bods number, biological and stover yield of bean plant were obtained by potassium silicate application and confirmed the pathway which called to considering silicon one of the essential elements to plant growth. Silicon addition may improve nutritional balance under saline soil conditions, thereby a better growth performance and consequence yield production was obtained (Abou- Baker et al., 2011).

Salicylic acid (SA) is considered as a hormone like substance, which play an important role in regulation of physiological process such as growth, photosynthesis, nitrate metabolism, ethylene production, heat production, oxidative defense and flowering (Hayat, et al. 2007 and Joseph, et al. 2010), and also provide protection against biotic and abiotic stresses such as salinity (Kaya, et al. 2002 and Hussein, et al. 2007). It has been shown as an important signal molecule for modulating plant responses to environmental stress (Breusegem, et al. 2001). The ameliorative effects of SA have been well documented in inducing salt tolerance in many crops (Gunes, et al. 2005 and Stevens, et al. 2006 Hussein and abou-Baker 2014). Salicylic acid considered as an active antioxidant in ameliorates the adverse effect of salt salinity stress (Seneratna, et al. 2000, El-Tayeb 2005, Hussein and abou-Baker 2014 and Li et al. 2014). Several authors reported the effects of salicylic acid on metabolic processes of different plants (Gunes, et al. 2007, Hussein et al., 2007, 2012& 2013 and Anosheh et al., 2012), and its effect on mineral status

(Khan, et al. (2010) , Wang, et al. (2011) , Hussein, et al. (2012). The response of macronutrients content to saliciylic acid was shown by El-Tayeb (2005) , Khan et al. (2010) , Hussein et al. (2012) and Hussein and abou-Baker 2014. Furthermore, the response of micro nutrients concentration or uptake was still up scarce, However, Yildirim et al. (2006) for cucumber, found out that exogenous SA applications inhibited Na accumulation, but stimulated N, P, K, Mg, Fe, Mn and Cu uptake.

Therefore, the objective of this study is to evaluate the response of micronutrients of moringa plants grown irrigated by diluted seawater to silica and salicylic application.

II. Material and Methodology

A pot experiment was conducted in the greenhouse of the National Research Centre to evaluate the effect of salt stress and foliar amendments on growth and mineral status of moringa plants. The treatments were as follows:

Salinity treatments:

1. Irrigation by tap water, 285 ppm (S0).
2. Irrigation by diluted seawater 2000ppm (S1).
3. Irrigation by diluted seawater 4000ppm (S2).

Foliar treatments:

1. Spraying with distilled water (Control).
2. Spraying with 300 ppm SiO_2 (Si) as potassium silicate.
3. Spraying with 300 ppm SiO_2 + 300ppm salicylic acid (Si+SA).

Moringa (*Morirga oleifera* Lam.) seeds were sown in April 1st 2012. Soil sample was taken from Kerdasa region, Giza Governorate, air-dried, crushed, sieved to pass through 2mm sieve and preserved for analysis. Some physical and chemical characteristics of the investigated soil were given in Table (1).

Table (1) Some physical and chemical characteristics of the investigated soil

Characteristics	Values
Chemical properties	
pH (1 : 2.5 soil : water ratio)	7.15
EC (Soil paste extraction) dSm^{-1}	1.30
Soluble cations (me/100g):	
Calcium	2.38
Magnesium	1.27
Potassium	0.23
Sodium	1.82
Soluble anions (me/100g):	
Carbonate	-
Bicarbonate	0.91
Chloride	1.90
Sulphate	1.89
Physical properties (%):	
Organic matter	1.30
Calcium carbonate	2.53
Sand	21.5
Silt	30.2
Clay	48.3
Textural class	Clayey

Every pot received 8kg soil, 1.80g of ammonium sulphate (20.6%N), 1.5g calcium super phosphate (15.5% P_2O_5) and 0.5g potassium sulfate (48.5% K_2O). Plants were transplanted to these pots at 21 day from sowing, thinned twice after one and two weeks from transplanting and left one plant/pot in 8 replicates for each treatment. The irrigation with saline water was started at 45 days from sowing. Some chemical properties of used seawater were shown in Table (2).

Table (2) Some chemical properties of used seawater

Characteristics	Values
pH	8.4
EC (dSm^{-1})	50
Total salinity (g/L)	36
Cations (g/L):	
Calcium	0.42
Magnesium	1.31
Potassium	0.42
Sodium	11.02
Anions (g/L):	
Bicarbonate	0.12
Chloride	19.88
Sulphate	2.74

Foliar application treatments were applied at two successive times 60 and 90 days after sowing. At 180 day from sowing, plants leaves were picked cleaned, dried in electric oven at 70°C, and ground in stainless steel mill. Dry powder was digested and analyzed Fe, Zn, Mn and Cu as described by Cottenee et al. (1982). Analysis of variance (ANOVA) and least significant difference (LSD) at 0.05 probability level was computed using the CoStat program and applied as described by Gomez and Gomez (1984).

III. Results and discussion

Micronutrient concentrations

Significant responses were detected in Zn, Mn and Cu ppm as a result of salt stress but Fe ppm without significant responds to this treatment. Mn and Cu ppm increased as the concentration of salt increased up to the highest level used. However, Zn ppm slightly decreased with the 1st level of salt stress but slightly increased by the highest level used (Table 3). The highest reduction was observed by Si+SA application, this may be due to dilution effect in high plant growth under these treatments as reported by Hussein and Abou-Baker (2014).

Micronutrients concentrations except Cu were significantly responded which negatively affected. The depression effect in nutrients of plants received Si+SA exceeded those induced by Si alone. Cu percentage did not show any clear effect (Table 3).

The interaction of salicylic acid or silicate and salinity on micronutrients concentration were illustrated in (Table 3).The percentage of decrement in micronutrients with salicylic acid and silicate were depressed as the percentage of salts in irrigation water increased. The highest effects of both antioxidants were shown in the concentrations of Mn% under different salt stress or fresh water. Mishra and Choudhuri (1999) found that antioxidant application increased N, P and K in rice. Na/K ratio increased as salt concentration increased but Ca: (Na+K) showed the opposite response. Salicylic acid application of 200 ppm showed the highest improve in growth criteria, uptake of the macronutrients and Ca:(K+Na) compare to the control plants. Salicylic acid application lowered the

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adverse effect of salt stress and can use for amelioration of salt stress in cotton plant (Hussein et al., 2012). SA reduced the Na uptake of plants and/or increased the uptake of N, P, K, Ca, Mg and the other minerals as compared to control treatment under salt stress as described by El-Tayeb (2005) for barley, Gunes et al,. (2005) and Gunes et al.,(2007) for maize, (Szepsi et al., 2005) for tomato and Yildirim et al., (2006) for cucumber, who found out that exogenous SA applications inhibited Na accumulation, but stimulated N, P, K, Mg, Fe, Mn and Cu uptake. Alteration of mineral uptake from SA applications may be one mechanism for the alleviation of salt stress.

Wu et al., (1998) pointed out that molar percentage of strerols and phospholipids decreased with increasing salinity. Electrolyte leakage enables cell membrane injury to be assessed when plants are subjected to salinity stress. Maintaining integrity of the cellular membranes under salt stress is considered an integral part of the salinity tolerance mechanism (Stevens, et al., 2006). SA treatments lowered the electrolyte leakage in salt stressed tomato plants (Stevens, et al., 2006).

Table (3): Micronutrient concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.

Foliar treatments	Salinity treatments	Fe ppm	Zn ppm	Mn ppm	Cu ppm
	C	122.9	47	20.4	3.8
S_0	Si	116.6	48	20.7	7.1
	Si + SA	60.4	44	11.3	7.3
Mean		100.0	46	17.5	6.1
	C	111.1	47	37.4	9.8
S_1	Si	97.1	43	24.8	6.7
	Si + SA	89.7	37	13.1	6.7
Mean		99.3	42	25.1	7.8
	C	97.3	58	34.8	9.4
S_2	Si	91.5	53	28.7	8.6
	Si + SA	94.2	48	16.2	8.2
Mean		94.4	53	26.6	8.7
	C	110.5	51	30.9	7.7
Mean	Si	101.8	48	24.7	7.5
	Si + SA	81.4	43	13.5	7.4
General mean		97.9	47	23	7.5
		S=ns	S=**5	S=***3	S=***1
$LSD_{0.05}$		F=**14	F=*5	F=***3	F=ns
		F*S=*25	F*S=ns	F*S=*6	F*S=***1

S0, S1 and S2 are irrigation by tap water (285 ppm), 2000 and 4000 ppm salts, respectively. C=control, Si=silicate and SA=salicylic acid

The mean levels of iron (Fe) in MO leaves from the semi-deciduous forest and the Guinea savanna zones recorded close mean values of 26.83 mg/100 g and 25.043 mg/100 g, respectively (Asante, et al., 2014). Iron, Mn, Zn and Cu concentrations were around 97, 47, 23 and 7.5 ppm, respectively, irrespective of salinity and foliar treatments. In this concern, Azra Yasmeen, (2011) reported that Fe, Mn, Zn and Cu concentrations in moringa leaves extract were 544, 49.7, 38 and 3.5, respectively. In this concern, Aslam et al. (2005) suggested the contents of different minerals in leaves and pods of M. oleifera to significantly differ from region to region in Pakistan. Anjorin et al. (2010) confirmed that there were variations in macro and trace minerals in M. oleifera leaves, pods and seeds from different locations.

Iron ratios with macronutrient concentrations

Except Ca/Fe and Mg/Fe ratios all other ratios responded significantly. N/Fe, P/Fe and Na/Fe were increased by salicylic acid and salicylic acid + silicate but the increases with the combined treatment at from single treatment of salicylic acid while, K/Fe or Mg/Fe ratios decreased clearly by Silicate and tended to increased by salicylic acid with silicate (Fig 1a&b).

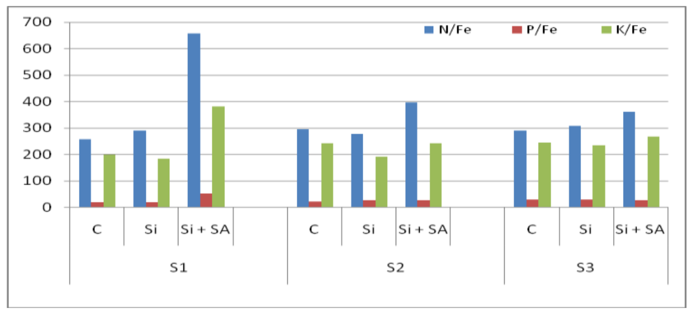

Fig.1a: Iron ratios with N, P and K concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.

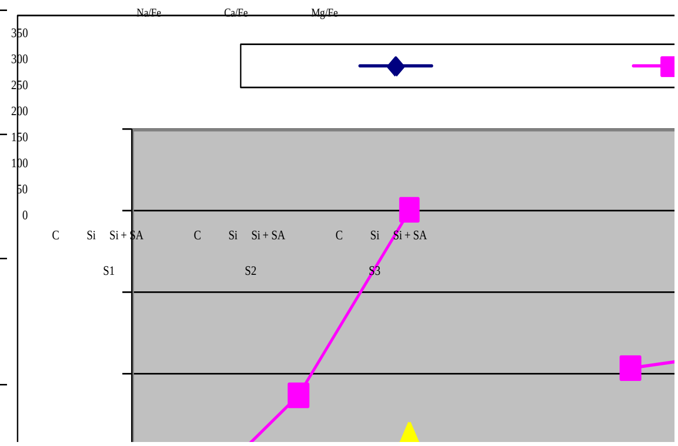

Fig.1b: Iron ratios with Na, Ca and Mg concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.
S0, S1 and S2 are irrigation by tap water (285 ppm), 2000 and 4000 ppm salts, respectively. C=control, Si=silicate and SA=salicylic acid

The N/Fe ratio lowered approximately similarly by both salinity levels, however, K/Fe, Ca/Fe and Mg/Fe ratios decreased but the depression by the first salinity level was more than that caused by its high level. Meanwhile, Na/Fe ratio did not affected by salinity level.

From general mean data, it can be observed that N concentration in moringa leaves increased by 348 fold, P and Na increased around 29 and 44 fold, K and Ca increased around 243 and 208 fold, finally Mg increased by 109 fold of Fe concentration.

Iron did not inhibit the uptake of Zn and Mn. The application of nitrogen increased the Fe content of maize (Losak et al., 2011).

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Manganese ratios with macronutrient concentrations

Generally, the calculated ratios (Mn with N, P, K and Na) lowered by the high salinity level and the reverse were true by the lesser level of salinity (Fig.2a&b).

Micronutrient deficiency is widespread in many countries due to the calcareous nature of soils, high pH, low organic matter, salt stress, continuous drought, high bicarbonate content in irrigation water, and imbalanced application of fertilizers (Alakouti, 2008).

Nitrogen nutrition increased the contents of Mn, and Micronutrient correlations in the grain were discovered between Zn and Mn contents and between Fe and Mn contents of maize (Losak et al., 2011).

A positive relation was detected between salicylic acid and the ratios of Mn and macronutrients except for Mg/Mn the differences seemed to be without effect.

Irrespective of salinity or foliar treatments, N, P, K, Na, Ca and Mg were increased by 695, 57, 489, 87, 416 and 216 fold over Mn, respectively.

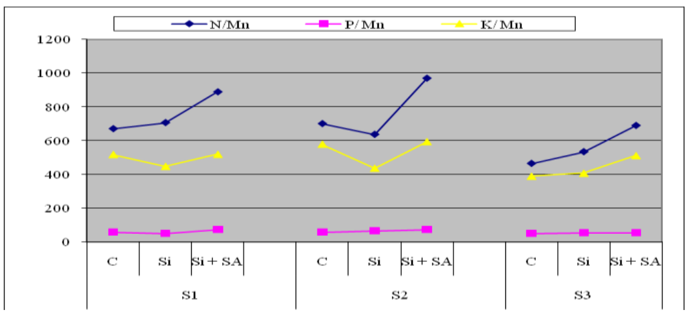

Fig.2a: Manganese ratios with N, P and K concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.

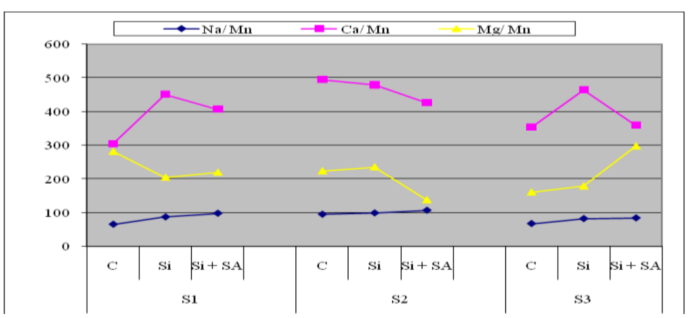

Fig.2b: Manganese ratios with Na, Ca and Mg concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.
S0, S1 and S2 are irrigation by tap water (285 ppm), 2000 and 4000 ppm salts, respectively. C=control, Si=silicate and SA=salicylic acid

Zinc ratios with macronutrient concentrations

Data illustrated in Fig 3a&b indicated that there was a continuous depression as the salt concentration increased in the water of irrigation. Moreover, the highest percentage of decrement could be shown with N/Zn ratio and lowest with Na/Zn ratio.

Furthermore, In spite of the increases of the estimated ratios, Zn ratios pronounced increases were achieved by Si+SA combination.

Foliar application led to decrease micronutrient concentrations, so it caused that increasing the gap between macro and micronutrients. Subsequently, N, P, K, Na, Ca and Mg were increased by 1675, 136, 1145, 204, 938, 504 fold over Zn, respectively.

The effect of salinity on nutrient concentration of different plants recorded by Mass et al., (1972) where reported that concentrations of Fe and Zn increased in the roots and tops of each species with increasing ambient levels of NaCl. Manganese concentrations increased in tomato and soybean tops but decreased in squash tops. Concentrations of Mn in the roots of tomato and squash were reduced at all salt levels, but they increased in all but the 100 meq/liter treatment in soybean. The changes in contents as a function of salinity were in the order of twofold or less and all were significant at the 5% level or better. Although the vegetative growth of the plant tops was depressed 45% or more by the highest saline treatment, the concentrations of these micronutrients in the tissue remained within the physiological limits necessary for normal plant growth. The influence of NaCl on the contents of Ca, Mg, K, Na, and Cl in the plant tops was comparable to that found in other investigations of salinity effects on plant mineral composition. (Influence of Salinity on Fe, Mn, and Zn uptake by Plants.

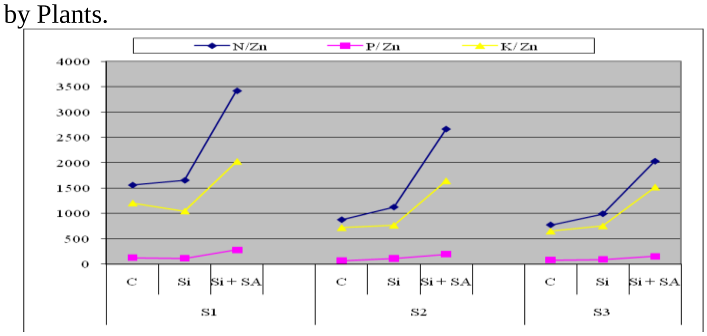

Fig 3a: Zinc ratios with N, P and K concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.

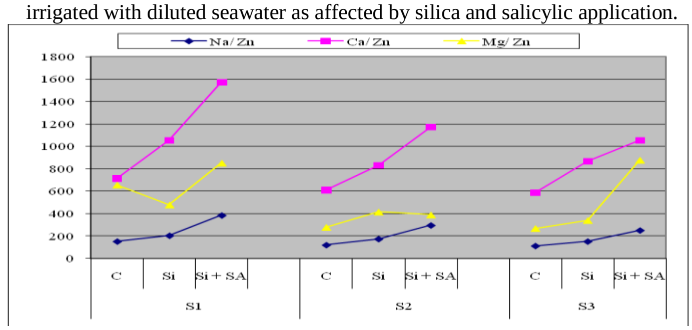

Fig 3b: Zinc ratios with Na, Ca and Mg concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.
S0, S1 and S2 are irrigation by tap water (285 ppm), 2000 and 4000 ppm salts, respectively. C=control, Si=silicate and SA=salicylic acid

Cupper ratios with macronutrient concentrations

Copper (Cu) is a heavy metal that is an essential microelement for plant metabolism. It participates in important biological reactions as an enzymatic cofactor and as an electron carrier in oxidative phosphorylation and photosynthesis (Solomon and Lowery, 1993)

Concerning the effect of salicylic acid and ratios of Cu with the other macronutrients in moringa plants, N/Cu as well as Mg/Cu decreased with both antioxidant treatments. The reverse was true for P/Cu while Ca/Cu and Na/Cu showed increases and gives higher values with silicate treatments (Fig. 4a&b).

SA has been shown as an important signal molecule for modulating plant responses to environmental stress (Breusegem et al., 2001). The ameliorative effects of SA have been well documented in inducing salt tolerance in many crops (Gunes et al., 2005 and Stevens et al., 2006)

Continuous decreases were obtained on the ratios of Cu concentration and macronutrients concentrations parallel to the increases of salts in irrigation solution. Nitrogen fertilization did not reduce the content of micronutrients in the plant or grain of maize. It is evident that the continuous single use of N fertilization so far has not resulted in a micronutrient deficiency of the plants limiting the nutrient density of the grain or reducing its quality (Losak et al., 2011).

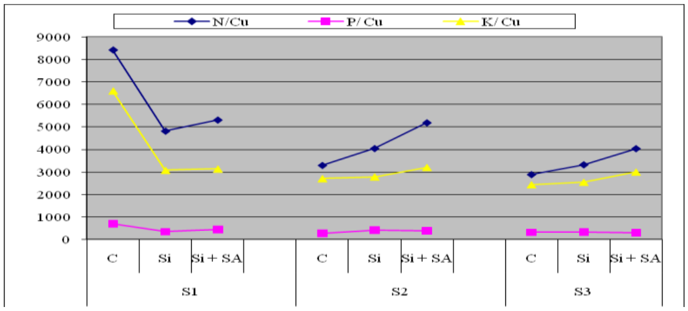

Fig.4a: Cupper ratios with N, P and K concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.

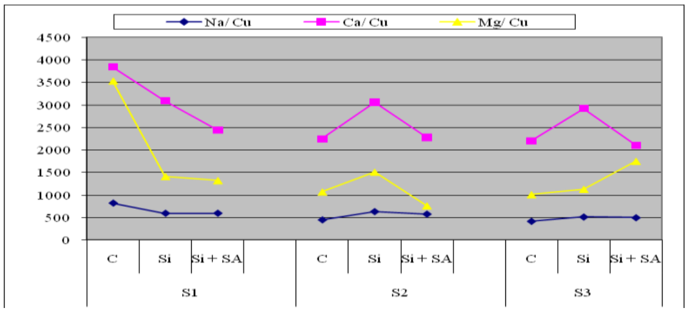

Fig.4b: Cupper ratios with Na, Ca and Mg concentrations in moringa leaves irrigated with diluted seawater as affected by silica and salicylic application.
S0, S1 and S2 are irrigation by tap water (285 ppm), 2000 and 4000 ppm salts, respectively. C=control, Si=silicate and SA=salicylic acid

IV. Conclusion

Irrespective of the harmful effect of seawater on plant growth, irrigation by diluted seawater is necessary. It can be alleviated salinity stress by silica and salicylic acid application. Micronutrient concentrations and it's ratios with the macronutrients responded to the silicate and salicylic acid. Treatment the biofuel plants by antioxidants may be used for mitigate the harmful effect of seawater salinity. The need for more researches describe the suitable dilution of seawater without harmful effect on plant growth is become urgently.

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