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Alleviation of Soil Salinity by Some Bio - Chemichal Amendments Salicylic Acid and Biofertilizers with tomato (*Lycopersicon esculentum* L.)

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Abstract

In a greenhouse pot experiment, growth parameters of tomato (*Lycopersicon esculentum*.) cultivated in sandy soil were studied under salinity stress by Nacl i.e. (0, 1000, 2000, and 3000 ppm). Two applications were added to alleviate salinity stress, silicon and biofertilizers either individually or mixed. The diazotrophic bacteria, namely free-living *Azotobacter* associative *Azospirillum* were inoculated to the tomato seedlings before planting. Results showed that , all vegetative growth parameters i.e. plant height , shoot and root fresh and dry weights were decreased with increasing salinity ,especially at 3000ppm. Application of silicon alleviate salinity stress on tomato plant as well as chlorophyll content. Prolien content increased in leaves with increasing salinity . Biofertilizers mixed with silicon alleviate salinity stress.

Keywords: Azotobacter, Azospirillum, N_2 Fixation, Salinity stress, Silicon

Introduction

Salinity is a common environmental stress particularly in dry climates that significantly restricts plant growth and production. In many cases, salinity has adverse effects on general plant growth, development and quality **Zörb *et al.* (2019)** that can significantly increase the production costs of agricultural crops **Souri and Hatamian (2019)**. Salinity may naturally exist particularly in arid and semi-arid regions such as Egypt. According to Alleviation of Salinity Effects on Tomato Plants by Application of different approaches including genetic and biotechnology methods or physiochemical treatment strategies have been applied to increase plant tolerance to salinity **(Manaa *et al*., 2014; Jayakannan *et al*., 2015; Methenni *et al*., 2018).** Salicylic acid is a water-soluble secondary metabolite and phenolic compound that is produced in many organisms including plants. It is a plant growth regulator with various roles in plant metabolism **Singh and Gautam (2013).** Salicylic acid has important regulatory functions in plant growth, particularly under adverse stressful conditions (**Kim *et al.*, 2018; Nie *et al.*, 2018).** Salicylic acid has roles in flower induction, general growth and development, various enzyme biosynthesis, stomata movements, membrane protections, and cell respiration **(Dong *et al*., 2011); Torun 2019)**. One of the most prominent roles of salicylic acid is in stress tolerance of plants, where it can act as a signaling molecule that induces resistance. Salinity stress is a common consequence of insufficient water supply and/or using poor quality water. Plant growth promoting (PGP) rhizobacteria are beneficial microorganisms, which enhances the nutrient bioavailability and bio assimilation, helps in detoxification of plant from toxic chemicals, protects plants from soil pathogens and can act as an alternative to inorganic and organic chemical fertilizers, pesticides and transgenic plants (**Vilchez *et al*., 2016)**. PGP rhizobacteria are capable of solubilizing inorganic P by producing organic acids and chelating agents, producing growth regulating hormones, indole-3-acetic acid (IAA) and siderophores, which are in turn beneficial for soil fertility and plant growth. **Mahmud *et al.* (2021)** mentioned that, Biofertilizers can, directly or indirectly, help in attaining food security compared to the harmful effect of chemical fertilizers. A direct mechanism of Biofertilizers refers to phyto-stimulation and nutrient mobility, while an indirect mechanism poses bio-control activity. Direct mechanisms involve phytohormone production and phosphate, potassium, zinc, etc. solubilization. While, indirect-mechanism is HCN production, siderophore production, antibiotic production, etc. The present review elucidates the diversity of microbial inoculants (biofertilizers), their impacts on agricultural production through rising soil fertility, and overall crop yield.

Tomato (*Lycopersicon esculentum.*) is a member of the family solanaceae. Tomato is the second most important vegetable crop grown in the world in outdoor fields and greenhouses after potato (*Solanum tuberosum* L.). Grape tomato (*Solanum lycopersicum* L. var. cerasiforme) is popular worldwide for its flavor, sweetness, nutritional values, and health benefits (**Simonne *et al*., 2008**). The red pigment, enriched with carotenoid lycopene, is a good antioxidant protecting cell damage and preventing cancer when cooked or processed tomatoes are consumed in daily diets in larger amount (**Gerszberg and Hnatuszko-Konka, 2017).** The aim of this study is to find an applied, environmental-friendly method to improve tomato crop grown under saline conditions.

Materials and Methods

This research work was carried out in order to study and comparatively evaluate the efficiency of silicon and biofertilizers i.e. (*Azotobacter* associative *Azospirillum*) for elevation salt stress (0,1000, 2000 and 3000 ppm Nacl on tomato seedlings under pot experiment.

Soil

Sandy soil from the farm of Environmental Studies and Research, Institute, Sadat City University, was used. Surface soil samples (0-30cm -depth) were collected from the assigned locations. The samples of surface soil were air-dried, ground, mixed well and sieved through a 2 mm - sieve. The sieved soils were subjected to initial analyses for pertinent physical and chemical properties following the standard methods described by **(Page *et al*., 1982**; **klute 1986)**. The obtained data were recorded in **Table 1.**

Table 1. Physical and chemical properties of experimental soil.

$CaCO_3$%	Organic matter,%	Particle size distr., %			Texture class
		Sand	Silt	Clay	
1.90	0.03	88.59	4.8	6.61	Sandy

PH*	EC** m mohs/cm	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	K^{+}	Na^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.63	1.82	0.36	0.32	0.13	0.56	-	0.41	0.36	0.61

*In the 1:2.5 Soil: water suspension. **In the 1:5 Soil ; water then extract.

Plant

Tomato seedlings (*Lycopersicon esculentum.*) hybrid Forbella grown in foam trays were transplanted in the mid of November of 2018 at the stage of 5th true leaf to plastic pots of 30 cm – diameter and 25 cm – depth4r that contain mixture of (peatmoss and vermiculite) with sandy soil (1:10 v:v) . Plants were hand-irrigated with fresh water and fertilized with the standard recommendations of the ministry of agriculture in Egypt for two weeks until plant establishment was assured in the growing media then salinity treatments were applied. Three levels of salinity namely 1000, 2000 and 3000 ppm were established by dissolving Rashidi salt in fresh water until reaching the targeted concentrations. Plants were divided into groups then irrigated with the targeted saline level. Irrigation was carried out daily and in each irrigation cycle, enough drain was allowed to ensure adequate leaching and until reaching the targeted level of salinity in the drain.

Diazotrophs

Inocula of *Azotobacter sp.* (free – living) and *Azospirillum sp*. (associative), supplied from the Dept. of Agriculture Microbial. of the Agriculture Research Center, were used. Such N_2-fixing bacterial agents were applied to the tomato seedlings, at the time of planting, as co-inoculants.

Layout

The study was undertaken in a greenhouse pot experiment, using plastic pots of 30 cm – diameter and 25 cm – depth .Each pot was filled with 5 kg of mixed soil with peatmoss and vermiculite. The pots, allocated for each soil, were divided into main groups, sub- groups and sub- sub groups, representing the sampling periods, levels of the salinity, silicon and bacterial inoculation, respectively. The experimental treatments were randomly arranged in a block design, and performed in four replicates for each .Controls without any treatment.

Plant analysis

A sample weighing 0.2 g of the dried fine materials of tomato shoots were digested with a mixture of 10 ml concentrated H_2SO_4 and $HClO_4$ (at a ratio 3:1), on a sand hot plate (at approximately 270 ^oC), until the digest become clear. The digest was then diluted to 100 ml with distilled water. The contents of Na, K & Ca (%)were determined following the methods stated by **(Cottenie *et al*., 1982).**

Leaf chlorophyll content were determined as SPAD value using SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan).

Determination of proline and protein content

The method of **Bates *et al.* (1973)** was used for the identification of proline content in the newly formed leaves.

Results and Discussion

Plant height, Fresh and Dry Matter Yields of the Plants

Data presented in **Table 2** indicate that, the plant height, fresh and dry matter yields of tomato plants exhibited wide variations among the treatments undertaken. Addition of salinity led to reduction of vegetative value for plant growth when the lowest value of plant height was found at 3000 ppm Nacl when it gave 21.18 Cm, 119.45 g, 24.89 g, 23.87 g and 13.25 g for plant height, shoot fresh and dry weight . and root fresh and dry weights, respectively .

Table 2. Effect of salinity, silicon and biofertilizers on tomato plant growth.

salinity	**Treat.**	**plant height(Cm)**	**Shoot fresh wt.(g)**	**Shoot dry wt.(g**	**Root fresh wt.(g)**	**Root dry wt.(g)**
0Nacl	Control	26.56	137.75	29.45	36.72	17.50
	Si	26.53	148.54	30.74	37.75	19.92
	Bio	29.54	176.25	34.04	50.65	21.87
	Si x Bio	32.98	181.75	36.97	51.73	24.81
1000 ppm Nacl	Control	27.35	180.76	31.86	37.75	21.97
	Si	28.14	206.00	37.13	49.75	27.25
	Bio	30.26	214.25	34.35	53.97	29.56
	Si x Bio	33.16	224.00	38.94	54.86	31.72
2000 ppm Nacl	Control	25.17	146.25	31.88	33.87	18.55
	Si	27.11	157.75	33.93	42.11	19.86
	Bio	26.35	170.75	32.30	34.41	18.65
	Si x Bio	28.66	225.00	33.47	41.95	22.57
3000 ppm Nacl	Control	21.18	119.45	24.89	23.87	13.25
	Si	24.36	134.52	28.44	29.33	15.59
	Bio	22.45	129.25	26.95	24.35	13.93
	Si x Bio	25.16	155.75	32.89	32.75	18.57

Results show the reduction of all vegetative parameters with increasing salinity compared to tap water irrigation (control) without any NaCL **(Table 2)**. Application of silicon or plant growth rhizobacteria either single or mixed improved the plant growth parameters of tomato plant compared to control. At 2000 and 3000 ppm Nacl the application of silicon was more effective to alleviate the Nacl stress compared to biofertilizers and control . These results are in agreement with the reports of **Win *et al*. (2018)** that found that ,Salinity stress strongly affected growth, leaf water contents, and photosynthetic performance of tomato seedlings, and inoculation with *Pseudomonas* spp. Strain ameliorated the salinity stress. Likewise, **Chakma *et al*. (2021)** found that , the application of Si in the form of monosilicic acid (MSA [H_4SiO_4]) increased tomato growth under drought stress.

Leaf chlorophyll and proline contents

Chlorophyll content is one of the main factors that reflect the photosynthetic rate. Some authors suggested that variation in pigment content can provide valuable insight into the physiological performance of leaves and indicates their photosynthetic capacity as well as the presence of stress or diseases. Data presented in **Table 3** declared the effect salinity on chlorophyll of tomato and application of silicon and biofertilizers alone or mixed. Results show that, high salinity reduced SPAD value especially at 3000 ppm. Exactly the contrary, proline content increased with increasing salinity and silicon had more effected for increased proline content in leaves.

Conclusion

This study confirmed that raised soil salinity (Nacl) decreased tomato plant growth and increased proline content in leaves. Also, the present study indicated the importance of applying the selected silicon and rhizobacteria as biofertilizers to enhance, not only the plant growth, but also alleviate salinity stress for tomato pants.

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Table 3. Effect of salinity, silicon and biofertilizers on chlorophyll and proline contents of tomato.

salinity	Treat.	Chlorophyll content	Leaf Proline (µ g/g FW)
0 Nacl	Control	48.57	35.11
	Si	45.12	38.45
	Bio	48.62	36.56
	Si x Bio	50.57	39.48
	Mean	48.22	37.4
1000 ppm Nacl	Control	42.37	41.65
	Si	46.57	48.78
	Bio	52.27	45.25
	Si x Bio	52.67	52.95
	Mean	48.47	47.16
2000 ppm Nacl	Control	46.74	54.48
	Si	48.11	61.65
	Bio	48.02	58.24
	Si x Bio	52.27	65.86
	Mean	48.78	60.03
3000 ppm Nacl	Control	37.42	69.12
	Si	44.65	74.36
	Bio	44.46	70.45
	Si x Bio	47.52	76.86
	Mean	43.51	72.69

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