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Alleviation of Natural Salinity Stress on Seedlings and Growth of Cowpea Plants Using Some different Protective Treatments

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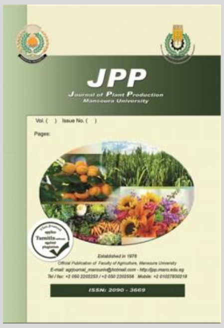

ABSTRACT

The current research was conducted to alleviate natural salinity stress on seed germination by seed priming and within turn on vegetative development using some different treatments. In laboratory, seeds were soaked in chitosan (200ppm), silicon (200ppm), and yeast extract (50ml/l) compared to control treatment under different salinity stress (0, 5 and 7 dS/m) for 3½ hours. Previous seeds were planted under the same levels of natural saline stress in two fields of El-Serw Agricultural Research Center in Damietta governorate, during 2018-2019 seasons. Two types of treatments were used, sulfur (0.4 ton/fed.) and sulfuric acid (10 L/ fed.) as soil amendments and the same previous soaking compounds used to seed priming as foliar application treatments. Results reveal that elevated the salinity stress had a negative feedback on cowpea seedlings and growth traits. Meanwhile, each of chosen protective treatments had significantly improvement of seed priming and vegetative growth traits. The major improved interaction for the formerly characteristics was the combined addition of sulphur in soil followed with sulfuric acid (10 L/ fed.) with spraying chitosan or with yeast extract under natural salinity stress. Therefore, we recommend adding sulfur to the soil (0.4 ton/fed.) before cultivation and seed priming of cowpea (Kaha 1) as well as spraying plants by chitosan (200ppm) or spraying with yeast extract (50ml/l) after 20 days after sowing, three times, ten days intervals, to increase plant tolerance to natural soil salinity for obtain the best vegetable growth under the same conditions.

Keywords: salinity stress, seed priming, soil amendment, sulfur, sulphuric acid, foliar application, chitosan, silicon, yeast extract.

INTRODUCTION

Cowpea, (*Vigna unguiculata* L.), is an important grain legume that is widely cultivated in various agro-ecological regions (Agbicodo, 2009). Due to its importance as its seeds and leaves reduce malnutrition and the cultivation of pulse crop can improve soil fertility (Anyango *et al.*, 2011 and Lal, 2017). Regarding to its nutritional value, it is a valuable source of a dietary protein and mineral source, complementing cereals, tuberous vegetables, and root vegetables in diets in addition to a fodder crop for livestock, as a green vegetable, and for dry beans (Phillips *et al.*, 2003; Manzeke *et al.*, 2017; Boukar *et al.*, 2019). Soil salinity is one of the most brutal environmental factors that limit the productivity of cowpea which almost reduces aspects of plant development (Win and Oo, 2015). Numerous studies to alleviate the dangerous impact of natural soil salinity stress on plant growth have been carried out, but protective treatments by soil amendments and foliar growth stimulants have the most promising trend in this field.

Seed priming is the recent and practical, cost-effective and low-risk alternative to improve seed germination and seedling emergence by inducing the metabolic activity of pre-germination under adverse conditions (Jiménez-Arias *et al.*, 2015 and Migahid *et al.*, 2019). Seed priming with foliar application and growth-stimulants is one of the agronomic approaches recently that reduces the negative feedback of abiotic stress, improves plant tolerance and increases the yield and quality of many crops. Chitosan, yeast and silicon have been reviewed as important and successful plant stimulants. Chitosan is widely used in agriculture either in seed germination or foliar spraying as it is environmentally friendly (Zargar *et al.*, 2015) and induces plants to be more resistant against unfavorable conditions, acts as germination accelerator and a growth stimulator and improves the quality and productivity of yield in many crop species (Lizárraga-Paulín *et al.*, 2011 and Helaly *et al.*, 2018). Meanwhile, the promising biostimulant; yeast, considers natural and safe plant growth promoting for various crops due to its natural nutrient elements. These elements have a reflection on enhancing vegetative, productivity and crop quality (Ibraheim, 2014 and Mohamed *et al.*, 2018). The importance of silicon has arisen from inducing plants to be more resistant, ameliorate biotic and abiotic stresses and increase the rate of germination and growth (Khan *et al.*, 2015 and Guerriero *et al.*, 2016).

On the other hand, soil amendments have a long history in improving physical and chemical properties under soil salinity stress. Among of them, sulphur and sulfuric acid are widely applied especially in Egyptian soils due to role in reduction the pH value of soil by oxidizing sulphur to sulphate, improving the availability of microelements, the chemical properties of alkaline soils and increasing yields and related characteristics (Kineber *et al.*, 2004). Moreover,

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mineral sulfur is recognized as one of major important mineral for growth and development of higher plants (Nazar *et al.*, 2011). While, sulfuric acid is characterized by its low cost and most abundant around world (Zia *et al.*, 2007). Alleviation of natural salinity stress as a main target on cowpea traits is processed into two directions in the current study; seed priming with the same foliar applications in lab. and using both protective applications; soil amendments and foliar applications. Additionally, determination of more efficient application by interactions optimizes on vegetative plant growth of cowpea cv. Kaha

MATERIALS AND METHODS

1. Study area characteristics

An experimental field study was conducted during the two successive 2018 & 2019 at a farm following to El-Serw Agricultural Research Center (EARC), Damietta governorate, Egypt. The locality was at North East of Nile Delta and characterized with a heavy clay salty affected soil because this region was cut out of El-Manzala lake in 1940s. The main source of irrigation is fresh water from Nile throughout El-Shocacanal plus the agricultural drained water from both El-Serw and El-Harana drained canals. Two different levels of natural soil salinity were chosen at 31°14′N and 31°48′E, as shown in Fig. (1) and the coordinates were recorded using a hand-held Global Positioning System (Garmin, GPS III plus). Meanwhile, climatic data revealed the mean temperature which ranged between 28.29 to 31.84ºC corresponding also with the minimum (19.70 to 22.68ºC) and maximum degrees of temperature (39.24 to 42.85ºC) and relative humidity (37.75 to 46.55%) with moderate to hot season. Whenever, the minimal mean of climatic factors was recorded during May and the maximum value was during July. All climatic data were according to in meteorological station in Egypt.

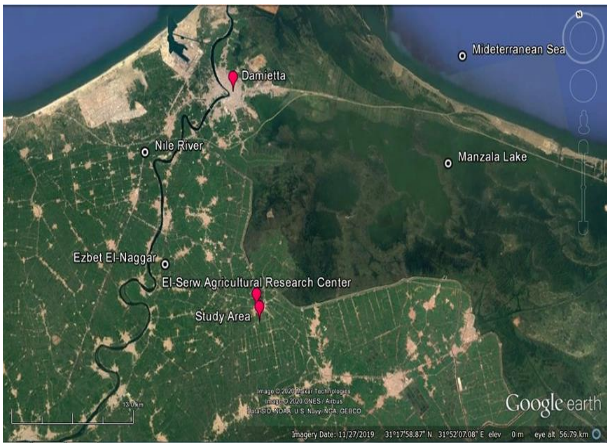

Fig .1. Map clarified main locality of the study area at the agricultural farm, El-Serw Agricultural Research Center (EARC), Damietta governorate, Egypt.

2. Soil sampling and analysis

Soil samples were separately collected from different study sites, which represented profile at a depth of 0-50 cm. For chemical analysis of air-dry soil samples, the estimation of total phosphorous and nitrogen were carried out. Total phosphorus was determined by digestion and followed by direct stannous chloride method (APha, 1998). Meanwhile, semi-micro modification of Kjeldahl method (Pirie and Springer Verlage, 1955) was conducted for evaluation the total soluble nitrogen. Moreover, Walkely and Black rapid titration method was used for determination the oxidizable organic carbon at the soil samples (Piper, 1947). Organic carbon is indication of organic matter and is calculated from the following to Jackson (1958): Soil water extract with ratio 1:5 was prepared for estimating the value of soil reaction (pH value) and the electrical conductivity (EC) as the salinity index (Jackson, 1958; Pansu and Gautheyrou, 2007). As defined, the relative amount of sodium ion that present in soil surface is Exchangeable Sodium Percentage (ESP). It is calculated by the following equation:

$$ESP = (\text{Exchangeable sodium in soil} / \text{cation exchange capacity}) \times 100$$

Cation exchange capacity or sum of exchangeable cations (Ca^{++}, Mg^{++}, Na^{+}, K^{+}) can be used in the before equation (Levy and Shainberg, 2005).

According to Allen *et al.* (1974), the extractable cations: sodium, potassium by using Flame Photometer and calcium and magnesium by atomic absorption spectrometer were measured in the soil extract. On the other hand, different soluble ions were determined as the following: Chloride content was determinate by APha (1998). Meanwhile, sulphates were occurred according the calculated equation and methodology of Piper (1947).

3. Experimental design, seed sowing and cultivation

The layout of the current experiment was planned as split-split plot design in a completely randomized blocks design with three replicates for each treatment. The main effect plot was both different levels of natural soil salinity, which different both types of protective treatments were randomly distributed as sub plots for soil amendments and sub-sub plots for second type of protective treatments; foliar applications. Net treatments from this experiment included twenty four treatments which were the interaction between two levels of natural soil salinity (main plot), three sub plot of soil amendments (control without any treatments, sulfuric acid and sulphur, and finally four sub-sub plots of foliar applications (control (tap water), potassium silicate, yeast extract, and chitosan as the following :

A. Main plots: Natural soil salinity level (dS/m)

1.Medium salinity level (Area 1, EC 5.0 dS/m)
2.High salinity level (Area 2, EC 7.0 dS/m)

B. Sub-plots, soil amendments

1.Control (without any treatments)
2.Sulfuric acid (10L./fed.)
3.Sulphur (0.4 tan/ fed.)

C. Sub-sub plots, foliar applications

1. Without (Tap water)
2. Silicon (200ppm)
3. Yeast extract (50ml/l)
4. Chitosan (200ppm)

The practical study began with an experiment of seed priming of cowpea (Vigna unguiculata L., cv. Kaha 1) under split plot design in a completely randomized blocks design with three replicates for each treatment, by their soaking for 3 hours and half with four different solutions and dried to a certain moisture level before sowing (Singh et al. 2014a, b). First one consists of tap water, meanwhile, other soaking solutions from each of foliar or spray substances either chitosan at 200 ppm, or silicon at 200 ppm, or yeast extract at 50ml/l. under3 levels stress conditions (0, 5, 7.0 dS/m).

Seed-priming of cowpea was cultivated during the first week of May, sowing of seeds was manually on one side of the ridge (4 meters length and 0.70 meters width), at a spacing of 15 cm between hills within the same row, each hill contain about 3-4 seeds and thinned to one plant, The sub-sub experimental plot contained six ridges making an area of 16.8 m^2.

Regarding to foliar applications, the first spray of foliar application at different treatments began after twenty days from seeds sowing, then spray three times, ten days intervals. Meanwhile, soil amendments were add for their sub plots and sub-sub plots as the following: sulphuric acid at 10 L./fed. This was added with first irrigation by slow dropping at the inner of treated subplots and sub-subplots with sulphuric acid. The addition of sulphur at 0.4 tan/fed. This was amended with soil during land preparation directly before sowing to subplots and sub-subplots of sulphur (0.4 ton/ fed.). The first irrigation was after one week after sowing then regularly irrigated every 10 -15 days until full flowering stage of plants.

4. Data collection

The obtained data were recorded at fifty-five days after seed sowing for three random plants. Data divided per each treatment at each salinity level for two successive seasons as following:

Seed priming indices were categorized into: firstly, germination indices by promptness index was calculated by number of germinated seeds while germination percentage (Hartman *et al.*, 2002), germination rate by Abdul-Baki and Anderson (1973) were calculated and seedling Vigor Index as represented by the ability of seedling under salinity stress and water absorption. Meanwhile, vegetative indices were expressed by length and weight of radicle and plumule.

Growth vegetative characteristics were expressed by different parameters as length of plant, number of branches/plant, number of leaves/plant, leaf area as calculate by equation of Murray (1960); the product of the value of leave length* leave width* 0.86, weighting the fresh plant for expressing fresh weight /plant and dry weight/plant.

5. Data analysis

All recorded data were processed by SPSS (Statistical Package for Social Sciences, Inc.) version 20.0 for Windows 7. The main statistical analyses were one way ANOVA with its Post-hoc analysis Duncan's Multiple Range Test at 5% Level for detecting a statistically significant variance between the different treatments at P<0.05 (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

1. Main characteristics of soil

Physical and chemical properties of soil samples were represented in (Table 1) for the study area during both cultivation seasons. Heavy clay soil is the main soil type due to the increased percentage of clay and silt with the decreased percentage of coarse and fine sand at study area. High saline soil in the study area was attributed to increasing in the mean of electric conductivity (7.05dSm^{-1}) and corresponds to an increase in the concentration of ions and cations corresponding with elevated content of ESP (10.94) in soil of area 2 than area 1. Alkaline pH of soil was detected in both study areas. Moreover, the maximum content of organic matter, dissolved nitrogen, phosphorus and potassium was at lower level of natural soil salinity (area 1).

2. Seed priming

Effect of different levels of natural soil salinity

Indices of seed priming were expressed by germinative and vegetative parameters. As shown in Table (2), obtained data in cowpea seeds clarified significantly variation between different levels of salinity concentrations.

Table 1. Clarifying the physical and chemical characteristics of soil samples collected from both study area (El-Serw Agricultural Research Center), Damietta Governorate, Egypt during the two successive 2018& 2019

Area	Type class			pH			Electric conductivity (dSm^{-1})			Exchangeable Sodium Percentage		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
Area 1	Heavy clay soil			8.33	8.11	8.22	5.1	4.8	4.95	9.22	8.78	9.0
Area 2				8.54	8.22	8.38	7.2	6.9	7.05	11.77	10.11	10.94
Percentage of soil particle size												
	C. Sand (%)			F. Sand (%)			Silt (%)			Clay (%)		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
Area 1	1.55	1.73	1.64	9.78	9.22	9.50	22.23	23.05	22.64	67.11	65.01	66.06
Area 2	1.72	1.68	1.7	10.81	12.12	11.47	22.05	20.88	21.47	66.18	66.33	66.26
Soluble Ions Concentration (%)												
Area	Chloride (Cl^-)			Bicarbonate (HCO_3^-)			Carbonate (CO_3^{--})			Sulphate (SO_4^{--})		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
Area 1	34.2	32.1	33.15	1.55	1.4	1.48	----	----	----	13.9	15.8	14.85
Area 2	43.2	43.5	43.35	1.88	1.82	1.85	----	----	----	27.3	26.2	26.75
Soluble Cations (mg/100g dry soil)												
Area	Sodium (Na^+)			Potassium (K^+)			Calcium (Ca^{++})			Magnesium (Mg^{++})		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
Area 1	36.2	31.6	33.9	0.14	1.13	0.64	7.33	7.1	7.22	7.2	7.18	7.19
Area 2	45.4	43.9	44.65	0.22	0.22	0.22	12.9	13.3	13.1	12.23	12.65	12.44
Nutrients												
Area	Organic matter (%)			Available nitrogen (ppm)			Available phosphorus (ppm)			Available potassium (ppm)		
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean
Area 1	9.4	9.7	9.55	32	37	34.5	8.11	9.55	8.83	458	471	464.5
Area 2	9.2	9.3	9.25	30	34	32.0	7.46	8.26	7.86	449	462	455.5

Table 2. The effect of different salinity concentration on seed priming characteristics during the current study

Salinity concentration (dSm^{-1})	Germination development indices			Vegetative indices							
	Promptness index	Germination %	Germination rate	Seedling vigor index	Radicle length (cm)	Plumule length (g)	Radicle fresh W. (g)	Plumule fresh W (g).	Radicle dry W.	Plumule dry W.
0	8.50^{a}	91.00^{a}	82.67^{a}	935.33^{a}	4.30^{a}	6.10^{a}	0.53^{a}	0.86^{a}	0.04^{a}	0.20^{a}
4.7	5.52^{b}	75.25b	71.58^{b}	702.23^{b}	3.73^{b}	5.58^{b}	0.48^{b}	0.78^{b}	0.033^{b}	0.18^{b}
7.0	4.13^{c}	66.83^{c}	67.17^{c}	555.57^{c}	2.82^{c}	5.38^{c}	0.43^{c}	0.72^{c}	0.029^{c}	0.14^{c}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

Regarding to germinative indices, promptness index (PI) which was expressed by the number of germinated seeds recorded the highest mean of germinated seeds at the lowest level of salinity at zero concentration of salinity as control and sharply reduced at high saline level (dSm^{-1}). That was similarity corresponded with increasing of germination percentage and rate towards lower concentration of salinity as clearly detected in Table (3); zero dSm^{-1} > 4.7dSm^{-1} > 7.0 dSm^{-1}. Meanwhile, seedling vigor index (SVI) which represented the ability of seedling under salinity stress and water absorption, significant decreased respectively with increasing the level of salinity as the following; SVI, 0> 4.7dsm^{-1}> 7dsm^{-1}.

Indices of vegetative development were expressed throughout the length and weights of radicle and plumule. These indices were significantly increased towards decreasing the salinity level; 0 > 4.7dSm^{1}> 7dSm^{-1}. The longer and heavier radicle and plumule was at the lower salinity level at zero dSm^{-1} in opposite to the higher level of salinity at 7dSm^{-1}. The current result is in harmony with Farooq *et al.* (2020) which confirmed that seed priming hastened the seedling emergence and increasing of salt-stress increased the reduction percentage in seedling indices. In this trend, Khan and Rizvi (1994) and Munns (2002) clarified the negative feedback of salinity stress which may cause alteration of enzymes and hormones contained in the seeds, the toxicity of salt constituents or lower osmotic potential of germination media lead to imbalance in water uptake.

Table 3. The effect of different foliar applications on vegetative indices of seed priming during current study

B. Foliar applications	Vegetative indices					
	Radicle length	Plumule length	Radicle fresh W. (g)	Plumule fresh W. (g)	Radicle dry W. (g)	Plumule dry W. (g)
Without	2.83^{d}	4.90^{d}	0.41^{d}	0.63^{d}	0.028^{d}	0.12^{d}
Pot. Silicate (1.1 g/L)	3.25^{c}	5.45^{c}	0.44^{c}	0.76^{c}	0.029^{c}	0.17^{c}
Yeast extract (20 g/ L)	3.40^{b}	5.60^{b}	0.47^{b}	0.80^{b}	0.030^{b}	0.175^{b}
Chitosan (0.2 g/ L)	3.62^{a}	5.95^{a}	0.50^{a}	0.83^{a}	0.035^{a}	0.182^{a}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

Effect of applied foliar applications

As shown in Fig. (2), significant effect of foliar application was indicated on the parameters of seed priming and more improved in the main characters of seed priming in comparing to control. Such data revealed that the most foliar application in alleviating salinity stress during germination was chitosan, followed by yeast extract then potassium silicate during this study.

The current result is accordant with the conclusion of Mahdavi *et al.* (2015) which confirmed the significant effect of seed priming with chitosan which increased germination percentage and rate, vigor index, shoot length, root length and dry weights of shoots under salinity stress. Which chitosan optimizes the character of seeds to be more resistant to stress and increase the availability of amino compounds (Chibu and Shibayama, 2001), in addition of increase the total protein, total carbohydrates, N, P and K in seeds (El-Tanahy *et al.*, 2012).

Also, soaking seeds with extract of dry bread yeast led to has improve their ability to germinate and seedling growth because of the benefits that related to stimulating and accelerating the metabolites that occur in the seed during the germination process; as well as dry bread yeast extract contains many compounds which contribute directly and indirectly to the growth and development of the embryo and this is consistent with what mentioned by (Barnett *et al.*, 1990). These results are harmony with results of Mustafa *et al.* (2020) and Abraheem *et al.* (2016) were on other crops.

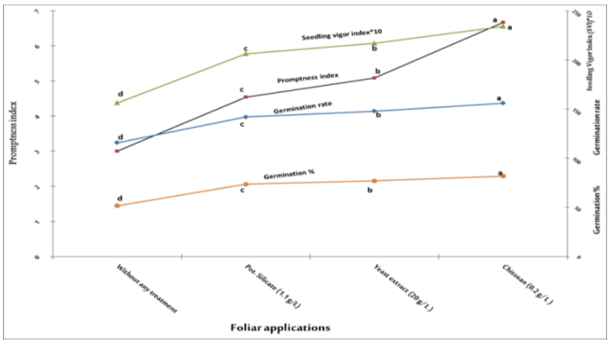

Fig .2. Effect of foliar applications on the main germinative indices of seed priming under salinity stress compared by control.

Effect of interaction

As shown in Table (4), the combined interaction effect between different concentrations of salinity and different applied foliar applications had significantly effect on different indices expressing germinative and vegetative development for treated seeds under salinity stress in comparing to untreated ones at control. Additionally, more improved for seeds priming indices was revealed in comparing with normal seeds.

Table 4. The effect of different foliar applications on the seed priming characteristics during the current study

Sal. conc.		Germination development indices				Vegetative indices					
		Promptness index	Germination %	Germination rate	Seedling vigor index	Radicle length (cm)	Plumule length (cm)	Radicle fresh W. (g)	Plumule fresh W. (g)	Radicle dry W. (g)	Plumule dry W. (g)
Zero	Without	8.50^{b}	91.00^{b}	$82.6\ 7^{c}$	935.33^{d}	4.30^{d}	6.10^{c}	0.53^{c}	0.86^{bc}	0.043^{c}	0.20^{d}
	silicon (200ppm)	9.00^{ab}	92.67^{ab}	83.67^{bc}	1001.72^{c}	4.47^{c}	6.34^{b}	0.57^{b}	0.86^{bc}	0.06^{bc}	0.27^{c}
	Yeast extract (50ml/l)	9.17^{a}	93.67^{a}	85.67^{ab}	1058.4^{b}	4.70^{b}	6.60^{a}	0.59^{b}	0.89^{ab}	0.08^{b}	0.38^{b}
	Chitosan (200ppm)	9.23^{a}	95.00^{a}	86.67^{a}	1104.9^{a}	4.90^{a}	6.73^{a}	0.63^{a}	0.95^{a}	0.13^{a}	0.48^{a}
$4.7dSm^{-1}$	Without	3.50^{f}	58.00^{h}	68.00^{f}	475.40^{k}	3.20^{g}	5.00^{g}	0.43^{f}	0.67^{f}	$.028^{e}$	0.13^{ef}
	silicon (200ppm)	5.25^{d}	77.00^{e}	69.67^{f}	716.43^{g}	3.70^{f}	5.60^{e}	0.46^{de}	0.80^{cd}	$.033^{d}$	0.19^{d}
	Yeast extract (50ml/l)	5.83^{d}	80.00^{d}	73.00^{de}	758.07^{f}	3.90^{e}	5.70^{de}	0.49^{d}	0.82^{bcd}	$.037^{cd}$	0.19^{d}
	Chitosan (200ppm)	7.50^{c}	86.00^{c}	75.67^{d}	859.0^{e}	4.13^{d}	6.00^{c}	0.52^{c}	0.85^{bc}	$.040^{c}$	0.20^{d}
$7dSm^{-1}$	Without	2.50^{g}	45.33^{i}	60.33^{g}	332.00^{l}	2.47^{i}	4.80^{g}	0.38^{g}	0.59^{g}	0.026^{f}	0.10^{f}
	silicon (200ppm)	3.83^{ef}	70.33^{g}	67.00^{f}	567.00^{j}	2.80^{h}	5.30^{f}	0.42^{f}	0.72^{ef}	0.03^{d}	0.15^{def}
	Yeast extract (50ml/l)	4.33^{e}	74.00^{f}	68.67^{f}	621.60^{i}	2.90^{h}	5.50^{ef}	0.44^{ef}	0.77^{de}	$.034^{d}$	0.16^{def}
	Chitosan (200ppm)	5.83^{d}	77.67^{de}	72.67^{e}	701.67^{h}	3.10^{g}	5.90^{cd}	0.47^{d}	0.80^{cd}	$.035^{cd}$	0.17^{de}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

The more combined interaction effect for improvement of seed priming (Table 4) was related between seeds without any salinity stress (zero salinity concentration) and applied with chitosan or yeast extract at same salinity level. Meanwhile, un treated seeds at higher concentration of salinity at 7dSm-1 was the minimal one in germinative, vegetative developments and less stress tolerance.

3. Vegetative characteristics

Effect of different levels of natural soil salinity

The represented data in Table (5) clarified a significant effect of different levels in natural soil salinity on the main vegetative growth characteristics of cowpea plant statistically. Moreover, a clearly decrease in average means of vegetative traits was significantly corresponded with the increase of soil salinity levels during both growing seasons.

In this trend, the current result is agreement with the conclusion of many researches on different crops such as cowpea (Netondo *et al.* (2004); Win and Oo, 2015; Lima *et al.*, 2017; Desoky *et al.*, 2020). Meanwhile, Wilson *et al.* (2006) as well as Manaf and Zayed (2015) pinpointed to negative feedback of salinity on leaves function that disrupts the normal metabolism throughout lipids peroxidation, protein denaturation and nucleic acids, disrupts cell division, cell elongation and other metabolic processes. That all reduced overall vegetative characteristics of plants under soil salinity.

Effect of applied soil amendments

A significant improvement in vegetative characteristics under salt stress (Table 6) was recorded towards the application of soil amendments i.e., sulfuric acid and sulfur in comparing to un-amended control over both grown seasons.

Table 5. Effect of natural soil salinity level on vegetative growth characteristics during 2018 and 2019 seasons

Soil salinity	Plant length (cm)		No. of branches/plant		No. of leaves/plant		Leaf area (cm^2)	
	2018	2019	2018	2019	2018	2019	2018	2019
Area 1 (EC 5.0)	41.72^{a}	41.72^{a}	5.61^{a}	6.64^{a}	41.25^{a}	40.92^{a}	82.00^{a}	82.86^{a}
Area 2 (EC7.0)	33.58^{b}	33.58^{b}	4.67^{b}	5.53^{b}	30.94^{b}	33.00^{b}	67.64^{b}	71.50^{b}

	Fresh weight (g)		Dry weight (g)	
	2018	2019	2018	2019
Area 1 (EC 5.0)	55.44^{a}	57.56^{a}	9.19^{a}	9.28^{a}
Area 2 (EC7.0)	45.39^{b}	47.31^{b}	8.39^{b}	8.37^{b}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

Table 6. Effect of soil amendments on vegetative growth characteristics under natural soil salinity conditions during 2018 and 2019 seasons.

Soil amendments	plant length (cm)		No. of branches/plant		No. of leaves/plant		Leaf area (cm^2)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control (0)	34.23^{c}	36.18^{c}	4.58^{c}	5.88^{c}	30.79^{c}	32.42^{c}	65.12^{c}	68.75^{c}
Sulfuric acid (10 L/ fed.)	38.47^{b}	40.81^{b}	5.96^{b}	6.50^{b}	37.83^{b}	38.29^{b}	77.83^{b}	79.88^{b}
Sulphur (0.4 ton/ fed.)	40.25^{a}	42.63^{a}	6.38^{a}	6.88^{a}	39.67^{a}	40.17^{a}	81.50^{a}	82.91^{a}

	Fresh weight (g)		Dry weight (g)	
	2018	2019	2018	2019
Control (0)	45.29^{c}	47.46^{c}	8.42^{b}	8.47^{c}
Sulfuric acid (10 L/ fed.)	51.58^{b}	53.50^{b}	8.88^{ab}	8.90^{b}
Sulphur (0.4 ton/ fed.)	54.38^{a}	56.33^{a}	9.07^{a}	9.10^{a}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

The most efficient one was using the sulfur, followed by sulfuric acid than the untreated control with extremely significance increasing in those characters. That is clear during both growing seasons. The current result is coincided with the conclusion of increasing growth characteristics in various legumes under salt stress by sulphur, among of them are pea by Osman and Rady (2012), faba bean by Abdelhamid *et al.* (2013) and cowpea (Stamford *et al.*, 2015). Others clarified the bi-importance of sulphur in soil and plants which reduce pH value and the electric conductivity (EC) and increases availability of many minerals and the solubility of sulphate, manganese, zinc, iron, phosphorus, and copper and suppress the uptake of undesired toxic elements in saline soil. That all improve the absorption of minerals and water uptake from soil and plant growth in special, root, shoot and leaves in addition to ameliorate the negative stress of salinity (El-Eweddy *et al.*, 2005; Nazar *etal.*, 2011; El-Kholy *et al.*, 2013; Aghajanzadeh *et al.*, 2019)

Effect of foliar applications

Data in Table (7) showed a significant effect of different foliar applications with potassium silicate, yeast extract, and finally chitosan on the vegetative growth characteristics in comparing with untreated plants. Over two growing seasons, it's obvious the more vegetative growth characteristics were significantly shown in plants sprayed with chitosan or with yeast extract followed with potassium silicate while, the lowest values of growth parameters were recorded in untreated plants.

Oppositely, the least plants were in untreated plants at control subplot. In this trend, numerous researches were accordant with the efficiency of chitosan in increasing of vegetative characteristics at different legumes as in harmony with the current results. Among of them are on bean (Sheikha, 2011 and Amiri *et al.*, 2015), and similarity Farouk and Ramadan (2012) and Shabana *et al.* (2019) were on cowpea. All these study have concluded the efficiency of chitosan for improving the vegetative growth of plant.

The significant effect of chitosan on the improvement of vegetative characteristics is attributed to its success as efficient, natural and successful biostimulant that clarified this improvement of vegetative characteristics as summarize in the following: it induces vital biological processes of plants for physiological and biochemical changes which ultimately leads to changes in the molecular level and the expression of the gene, therefore it serves as a successful growth promoters. Consequently, it has a vital role in improvement of leaves growth which enhances the capacity of antioxidant enzyme activities and key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improves the transportation of nitrogen in the functional leaves and thus the greater availability of amino compounds released from it which enhances plant growth and development (Chibu and Shibayama, 2001 and Mondal *et al.*, 2012). While, for the efficiency of yeast extract in increasing of vegetative characteristics growth, Nassar *et al.* (2016); Al-Amery and Mohammed (2017); Mohamed *et al.* (2018); Abdel Latef *et al.* (2019) and Abdelaal *et al.* (2020) confirmed that.

Table 7. Effect of foliar applications on vegetative growth characteristics under natural soil salinity conditions during 2018 and 2019 seasons

Foliar applications	plant length (cm)		No. of branches/plant		No. of leaves/plant		Leaf area (cm^2)	
	2018	2019	2018	2019	2018	2019	2018	2019
Without (Tap water)	32.77 ^{d}	34.69 ^{d}	4.33 ^{d}	5.06 ^{d}	27.00 ^{d}	29.28 ^{d}	59.83^{d}	62.17^{d}
silicon (200ppm)	37.12 ^{c}	39.62 ^{c}	5.67 ^{c}	6.27 ^{bc}	35.61 ^{c}	37.94 ^{c}	76.00^{c}	78.55^{c}
Yeast extract (50ml/l)	39.35 ^{ab}	41.79 ^{ab}	6.22 ^{ab}	7.72 ^{ab}	39.39 ^{ab}	39.33 ^{ab}	80.28^{ab}	82.56^{ab}
Chitosan (200ppm)	41.38 ^{a}	43.38 ^{a}	7.33 ^{a}	7.67 ^{a}	42.39 ^{a}	41.28 ^{a}	83.17^{a}	85.44^{a}

	Fresh weight (g)		Dry weight (g)	
	2018	2019	2018	2019
Without (Tap water)	41.61 ^{d}	43.83 ^{d}	8.12 ^{d}	8.14 ^{d}
silicon (200ppm)	50.17 ^{c}	52.50 ^{c}	8.80 ^{c}	8.82 ^{c}
Yeast extract (50ml/l)	52.78^{ab}	55.06 ^{ab}	9.04 ^{ab}	9.09 ^{ab}
Chitosan (200ppm)	57.11 ^{a}	58.33 ^{a}	9.19 ^{a}	9.25 ^{a}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

Effect of interaction

The effect of triple interaction among different levels of natural soil salinity, both different treatments; soil amendments and foliar applications was displayed on vegetative growth characteristics of plant (Table 8 a and b) during both growing seasons. There was a significant effect between the protective treatments towards the different levels of salinity and between treated plants and untreated plants either in lower level of salinity (Area 1) and high level of salinity (Area 2).

The major improved interaction for the formerly characteristics was the combined addition of sulphur or sulfuric acid in soil with spraying chitosan or by spraying with yeast extract as seed priming and foliar application at lower level of salinity (Area 1), over all study seasons which was significantly recommended in alleviating natural salinity stress and improvement of cowpea growth.

Table 8a. Effect of triple interaction among natural soil salinity level X soil amendments X foliar applications on some vegetative growth characteristics under natural soil salinity conditions during 2018 and 2019 seasons

A- Soil salinity	B. Soil amend.	C. Foliar applications	Plant length (cm)		No. of branches/plant		No. of leaves/plant	
			2018	2019	2018	2019	2018	2019
Area 1 (EC 5.0)	Control	Without	30.67 ^{im}	31.17 ^{l}	3.67 ^{ij}	4.67 ^{ij}	28.00 ^{i}	28.67 ^{m}
		silicon (200ppm)	39.33 ^{fg}	41.33 ^{ef}	5.00 ^{de}	6.00 ^{cd}	34.67 ^{e}	38.00 ^{h}
		Yeast extract (50ml/l)	40.67 ^{ef}	42.67 ^{de}	5.33 ^{cd}	6.33 ^{bc}	38.67 ^{d}	38.67 ^{gh}
		Chitosan (200ppm)	42.33 ^{cd}	44.00 ^{cd}	6.67 ^{b}	7.00 ^{b}	43.00 ^{c}	40.67 ^{f}
	Sulfuric acid	Without	38.33 ^{gh}	40.33 ^{f}	5.00 ^{ef}	6.00 ^{de}	31.33 ^{fg}	34.00 ^{j}
		silicon (200ppm)	41.67 ^{de}	43.33 ^{d}	6.33 ^{b}	7.00 ^{b}	45.33 ^{b}	43.67 ^{e}
		Yeast extract (50ml/l)	43.67 ^{c}	45.67 ^{bc}	6.67 ^{ab}	7.67 ^{ab}	47.67 ^{a}	44.67 ^{de}
		Chitosan (200ppm)	46.33 ^{b}	47.33 ^{ab}	7.33 ^{ab}	8.00 ^{a}	49.33 ^{a}	47.33 ^{bc}
	Sulphur	Without	40.00 ^{f}	42.67 ^{de}	5.33 ^{ef}	6.33 ^{de}	33.67 ^{e}	35.00 ^{ij}
		silicon (200ppm)	43.33 ^{cd}	45.67 ^{bc}	6.67 ^{b}	7.33 ^{b}	45.67 ^{b}	45.33 ^{cd}
		Yeast extract (50ml/l)	45.67 ^{ab}	47.33 ^{ab}	7.00 ^{ab}	8.00 ^{a}	48.00 ^{a}	46.33 ^{bc}
		Chitosan (200ppm)	48.67 ^{a}	49.00 ^{a}	8.00 ^{a}	8.33 ^{a}	49.67 ^{a}	48.67 ^{a}
Area 2 (EC7.0)	Control	Without	26.70 ^{n}	27.10 ^{m}	3.33 ^{j}	3.67 ^{k}	21.33 ^{k}	23.33 ^{o}
		silicon (200ppm)	30.27 ^{m}	33.27 ^{jk}	4.33 ^{ij}	4.67 ^{h}	24.00 ^{ij}	28.00 ^{mn}
		Yeast extract (50ml/l)	31.30 ^{kl}	34.30 ^{ji}	4.67 ^{hij}	5.33 ^{g}	26.00 ^{i}	30.33 ^{l}
		Chitosan (200ppm)	32.61 ^{jk}	35.61 ^{hi}	5.67 ^{ghi}	6.33 ^{fg}	30.67 ^{g}	31.67 ^{k}
	Sulfuric acid	Without	29.43 ^{m}	32.43 ^{kl}	4.00 ^{j}	4.33 ^{hi}	23.00 ^{jk}	26.67 ^{n}
		silicon (200ppm)	33.13 ^{j}	36.13 ^{h}	5.00 ^{ghi}	5.67 ^{g}	30.67 ^{g}	35.00 ^{ij}
		Yeast extract (50ml/l)	37.61 ^{h}	40.61 ^{f}	5.67 ^{g}	6.00 ^{ef}	37.67 ^{d}	36.33 ^{j}
		Chitosan (200ppm)	37.61 ^{h}	40.61 ^{f}	6.00 ^{g}	7.00 ^{ef}	37.67 ^{d}	38.67 ^{gh}
	Sulphur	Without	31.47 ^{kl}	34.47 ^{hij}	4.67 ^{ij}	5.00 ^{ef}	24.67 ^{e}	28.00 ^{mn}
		silicon (200ppm)	34.99 ^{i}	37.99 ^{g}	5.33 ^{gh}	6.33 ^{fg}	33.33 ^{e}	37.67 ^{h}
		Yeast extract (50ml/l)	37.19 ^{h}	40.19 ^{f}	6.00 ^{g}	7.00 ^{ef}	38.33 ^{d}	39.67 ^{fg}
		Chitosan (200ppm)	40.70 ^{f}	43.70 ^{d}	6.67 ^{f}	7.67 ^{de}	44.00 ^{bc}	40.67 ^{f}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

In this trend, both interaction reviewed applications as soil amendments and foliar application isn't enough applied in field study of legumes. Only study refers to Mansour (2017) on sweet potato who evaluated the improvement of growth by interaction between soil amendment; sulphur and foliar application; chitosan, had significantly increased growth in comparing to apply of sulfur or chitosan alone or untreated plants. Meanwhile, triple interactions between salinity stresses, different reviewed soil amendments and foliar applications haven't applied that characterize the current study in this field. Those are attributable to the vital role of sulphur and spraying chitosan for improvement of vegetative traits as discussed formerly and alleviate salinity stress. The lower level of soil salinity was the higher improved in its characteristics than likes at higher level of soil salinity.

Table 8b. Effect of triple interaction among natural soil salinity level X soil amendments X foliar applications on some vegetative growth characteristics during 2018 and 2019 season

A- Soil salinity	B. Soil amend.	C. Foliar applications	Leaf area/plant (cm^2)		Fresh weight/plant (g)		Dry weight/plant (g)	
			2018	2019	2018	2019	2018	2019
Area 1 (EC 5.0)	Control	Without	58.33 ^{m}	62.33 ^{l}	41.67 ^{j}	44.00 ^{j}	8.37 ^{ij}	8.33 ^{ij}
		silicon (200ppm)	69.67 ^{j}	73.67 ^{i}	48.67 ^{h}	51.67 ^{f}	8.77 ^{fgh}	9.00 ^{def}
		Yeast extract (50ml/l)	76.00 ^{gh}	79.67 ^{fg}	53.33 ^{g}	55.67 ^{e}	9.37 ^{bcd}	9.17 ^{de}
		Chitosan (200ppm)	80.33 ^{ef}	82.00 ^{e}	58.00 ^{ef}	58.33 ^{d}	9.23 ^{cd}	9.30 ^{cd}
	Sulfuric acid	Without	65.67 ^{k}	69.67 ^{j}	46.33 ^{h}	48.33 ^{hi}	8.70 ^{fg}	8.83 ^{fg}
		silicon (200ppm)	88.00 ^{d}	87.00 ^{d}	59.33 ^{de}	61.67 ^{c}	9.23 ^{cd}	9.30 ^{cd}
		Yeast extract (50ml/l)	93.33 ^{c}	90.67 ^{c}	61.00 ^{b}	62.67 ^{bc}	9.40 ^{bc}	9.53 ^{bc}
		Chitosan (200ppm)	96.67 ^{ab}	95.67 ^{ab}	63.53 ^{ab}	64.67 ^{ab}	9.60 ^{ab}	9.77 ^{ab}
	Sulphur	Without	67.00 ^{k}	61.00 ^{j}	49.00 ^{h}	50.67 ^{fg}	8.90 ^{ef}	9.03 ^{cd}
		silicon (200ppm)	94.67 ^{bc}	95.00 ^{b}	59.67 ^{de}	62.67 ^{bc}	9.37 ^{bcd}	9.50 ^{bc}
		Yeast extract (50ml/l)	96.33 ^{ab}	98.33 ^{a}	61.53 ^{ab}	64.33 ^{ah}	9.53 ^{ab}	9.67 ^{ab}
		Chitosan (200ppm)	98.00 ^{a}	99.33 ^{a}	64.67 ^{a}	66.00 ^{a}	9.77 ^{a}	9.90 ^{a}
Area 2 (EC7.0)	Control	Without	46.33 ^{n}	50.33 ^{m}	35.00 ^{m}	38.33 ^{m}	6.50 ^{l}	6.57 ^{l}
		silicon (200ppm)	58.00 ^{m}	62.00 ^{l}	39.00 ^{k}	41.00 ^{kl}	8.20 ^{jk}	8.27 ^{j}
		Yeast extract (50ml/l)	65.33 ^{k}	69.00 ^{j}	41.00 ^{j}	43.00 ^{jk}	8.40 ^{hij}	8.50 ^{hij}
		Chitosan (200ppm)	67.00 ^{k}	71.00 ^{i}	45.67 ^{i}	47.67 ^{i}	8.53 ^{ghi}	8.63 ^{ghi}
	Sulfuric acid	Without	60.00 ^{lm}	64.00 ^{kl}	38.00 ^{h}	40.00 ^{lm}	8.00 ^{k}	7.83 ^{k}
		silicon (200ppm)	71.33 ^{ij}	75.33 ^{hi}	45.67 ^{i}	47.67 ^{i}	8.53 ^{ghi}	8.20 ^{j}
		Yeast extract (50ml/l)	72.33 ^{i}	77.33 ^{g}	48.00 ^{h}	49.33 ^{ghi}	8.67 ^{fgh}	8.87 ^{fg}
		Chitosan (200ppm)	75.33 ^{h}	79.33 ^{fg}	52.33 ^{g}	53.67 ^{e}	8.90 ^{ef}	8.87 ^{efg}
	Sulphur	Without	61.67 ^{l}	65.67 ^{k}	39.67 ^{kl}	41.67 ^{kl}	8.27 ^{ij}	8.23 ^{j}
		silicon (200ppm)	74.33 ^{gh}	78.3 ^{efg}	48.33 ^{h}	50.33 ^{fgh}	8.70 ^{fg}	8.63 ^{ghi}
		Yeast extract (50ml/l)	78.33 ^{fg}	80.33 ^{e}	53.33 ^{g}	55.33 ^{e}	8.90 ^{ef}	8.8 ^{fgh}
		Chitosan (200ppm)	81.67 ^{e}	85.33 ^{d}	58.67 ^{ef}	59.67 ^{d}	9.10 ^{de}	9.03 ^{def}

Means followed by the same letter within each column do not significantly differed using Duncan's Multiple Rang Test at the level of 5%.

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تفادي تأثير الإجهاد الملحي الطبيعي علي إنبات ونمو نباتات اللوبيا باستخدام بعض المعالجات المختلفة

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أجريت هذه الدراسة بهدف تفادى آثار الملوحة الضارة على إنبات البذور والنمو الخضري للنباتات باستخدام بعض المعالجات المختلفة. فمعمليا، تم تهيئة البذور للإنبات (Seed priming) تحت ظروف الإجهاد الملحى (5 and 7dS/m) عن طريق نقع البذور بالشيتوزان (ppm200) والسليكون (ppm200) ومستخلص الخميرة (50مل/لتر) بالمقارنة بالكنترول لمدة 3½ ساعات. كما تم زراعة البذور السابقة المتهيئة للإنبات تحت نفس مستويي الإجهاد الملحى الطبيعى فى حقلين تابعيين لمركز البحوث الزراعية بالسرو محافظة دمياط، خلال موسمين متتاليين 2018-2019 وتم استخدام نوعين من المعالجة أولهما : معاملات أرضية , حيث تم إضافة الكبريت (0.4 طن/فدان) وحمض الكبريتيك (10 لتر/فدان) وثانيهما : معاملات بالرش هي نفس معاملات الرش المستخدمة فى نقع البذور. وقد أوضحت النتائج أن ارتفاع الإجهاد الملحى له تأثيره السلبى على صفات الإنبات وصفات النمو الخضري، وقد أدت معاملات البذور السابقة إلي حدوث تحسن معنوى واضح في نمو البذرة . كما أظهرت النتائج أن هناك زيادة معنوية في صفات النمو الخضرى نتيجة التفاعل بين المعاملات الأرضية وخصوصا الكبريت (0.4 طن/فدان) يليها حمض الكبريتيك (10 لتر/فدان) مع الرش بالشيتوزان (ppm200) اوالرش بمستخلص الخميرة (50مل/لتر) تحت ظروف الإجهاد الملحى الطبيعى. لذا نوصي بإضافة الكبريت للتربة (0.4 طن/فدان) قبل الزراعة ونقع بذور اللوبيا (صنف قها 1) لتهيئتها للإنبات (Seed priming) وكذلك رش النباتات اثناء نموها بعد 20 يوم من الزراعة 3 مرات على فترات 10 يوم، بالشيتوزان (ppm200) او الرش بمستخلص الخميرة (50مل/لتر) لزيادة تحمل النبات لملوحة التربة الطبيعية للحصول على أفضل نمو خضرى وبالتالي محصول جيد تحت نفس الظروف.