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Mitigation of Salinity Stress in *Vicia faba* by Natural and Synthetic Growth Enhancers

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Abstract: Soil salinity decreases plant growth, photosynthetic activity and productivity besides resulting in nutrient imbalance in plants. Coping with salinity is a global issue to ensure sustainable crop production. This study evaluated the efficacy of foliar applied growth enhancers, *Moringa oleifera* leaf extract (MLE; 20 times diluted), amongst naturally occurring plant growth stimulants, and for evaluating the efficacy of sulphate of potash as a source of potassium (SOP, 3%) in reducing the salinity effects in faba bean plants. The experiment was conducted with two levels of salinity in the nutrient solution, 0.0 (Control) and 135 mM of NaCl (S) which are equivalent to13 dS m^{-1}. Water spray treatment was taken as a control. The plants grown at 13 dS m^{-1} show a decreased in the chlorophyll (a+b), carotenoids, total protein contents, weight of 100 grains, enzyme activity of ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase), photosynthetic activity ($^{14}CO_2$-fixation) and macronutrient level (N, P, and K) in shoot and root. Foliar application of MLE+S and SOP+S increased these parameters as compared to salt stressed plants. In contrast, salt stressed faba bean plant considerably increased proline content, Na^+ and Cl^- levels and transpiration rate as compared to control and plants treated with MLE+S and SOP+S. Meanwhile, foliar application of MLE+S and SOP+S decreased Na^+ and Cl^- levels and proline content below that of the control plants. Foliar applied growth enhancers (MLE+S and SOP+S) increased the macronutrient contents N, P, and K in shoot and root of faba bean plants as compared to salt stressed and control plants. Nonetheless, foliar applied growth enhancers particularly moringa leaf extracts (MLE) and sulphate of potash as a source of potassium (SOP, 2%), ameliorated the adverse effects of salinity stress by enhancing carboxylating enzyme activity of RuBPCase, total protein level, macronutrient contents (N, P, and K), photosynthetic activity ($^{14}CO_2$-assimilation), total yield and plant secondary metabolites. Moringa leaf extract was more effective than sulphate of potash.

Key words: *Chlorophyll, salinity stress, Moringa oleifera, Growth enhancers.*

1. Introduction

Among various environmental stresses soil salinity limits plant growth and production in many parts of the world, particularly in arid and semi arid areas [1]. Salt stress causes various effects on plant physiology such as increased respiration rate, ion toxicity, changes in plant growth, mineral distribution, and membrane instability resulting from calcium displacement by sodium [2], and decreased photosynthetic rate [3, 4, 5, 6, 7, 8]. The salinity damage was associated with the accumulation of Na^+ in leaf tissues followed by reduction of enzymatic processes and protein synthesis [9]. Faba bean, *Vicia faba* L., is one of the world's most important legume crops. In Egypt it represents a major food crop which used in the human diet, as animal feed, and for industrial purposes [10]. Exogenous application of plant growth regulators, osmoprotectants and fertilizers have been successfully employed to mitigate the salt-induced losses [11]. An earlier study indicated that sulphate of potash (SOP) has positively influenced yield and quality of bush pepper [12]. As SOP is manufactured through physical separation from natural deposits, it can very well fit into organic production package. In this context, fertilizers containing sulphur such as sulphate of potash (SOP), having K and S would be more useful [13, 14]. *Moringa oleifera* is one of the 13 species of genus Moringa and family Moringnance. It is well known vegetable in Africa, Arabia, India, America and Pakistan [15]. Moringa has attained enormous attention being a rich source of cytokinin, potassium and antioxidants [16]. Recently, *Moringa oleifera* leaf extract (MLE) is being introduced as a natural plant growth enhancer, which not only improves the plant growth and biomass production but also induces tolerance under salinity stresses [17, 18].

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The present work was undertaken to investigate whether the injurious effects induced by salinity stress could be mitigated using exogenous application of MLE as a natural tools and SOP as a synthetic growth stimulators.

2. Materials and Methods

Plant material and experimental conditions. Uniform-sized faba bean seeds (*Vicia faba* L.*)* cv. Giza 2 was purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt. Grains were surface sterilized in 0.1% (w/v) sodium dodecyl sulphate solution and then thoroughly rinsed with sterile deionized water. The seeds were germinated in pots (40 cm high × 35 cm diameter), each filled with 15 kg sandy loam soil with 2.5% organic matter and available N, P and K concentrations of 170, 80 and 200 mg kg^{-1}, respectively. Ten days after emergence four uniform seedlings were maintained in each pot. The pots were irrigated with full-Hoagland's nutrient solution [19]. Plants were grown in a controlled environment growth chamber with 15-h photoperiod; 65%–75% relative humidity; and day and night temperatures of 22 and 20°C. Photosynthetic photon flux density at maximum plant height was about 440 μM $m^{-2}s^{-1}$. Cultural practices, such as weed control and irrigation, were performed as needed. The experimental design was randomized complete block design with three replicates. After 20 days of seedling emergence salt stress treatments were imposed. The seedlings were irrigated with full Hoagland's solution containing two levels of salinity, 0.0 (Control) and 135 mM of NaCl (S) which are equivalent to13 dS m^{-1}. The pots were rinsed with water once a week to avoid salt accumulation. Growth enhancers, MLE (diluted 20 times) and sulphate of potash as a source of potassium (SOP, 3%) was added to Tween 20 to fix solutions to the plant leaf surface. Water spray treatment was taken as a control. The solutions were sprayed once into the leaves in the morning at the flowering stage (50-day-old) using a manual pump. All determinations were carried out at the harvest stage.

Preparation of moringa leaf extracts (MLE). Moringa leaf extracts was extracted from young fresh leaves and tender branches with little distilled water (8 kg L^{-1} fresh material) according to [16,18]. After purification, the extract was centrifuged at 8,000 rpm for 15 min. thereafter, the supernatant was taken and diluted 20 times (MLE 20) by adding distilled water for foliar spray.

Photosynthetic activity ($^{14}CO_2$-fixation). Photosynthetic activity was measured in the Atomic Energy Authority, Radioisotope Department, Cairo, Egypt, with the method of [8].

Chemical analysis. Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase, EC 4.1.1.39) was determined as suggested by [20]. The transpiration rate was determined as described by [21]. Proline was determined according to the method described by [22]. Protein content was quantified according to [23]. The pigment content was determined according to the method of [24].

Elemental analysis. Determination of Na^+, K^+ and Cl^- in shoot and root of faba bean was done by flam photometer (Jenway, PFP-7). The method of [25] was applied for phosphorus estimation. Total nitrogen was determined in the dry seeds using the Kjeldahal method according to [26].

Statistical Analysis. Data were analyzed using ANOVA (SPSS10.0, SPSS Inc., Chicago, IL).Means were separated with Duncan's Multiple Range Test.

3. Results and Discussion

The present study, showed that on overall basis the increase in salinity level decreased the photosynthetic pigment contents (chlorophyll *a+b* and carotenoids) of the faba bean plants. Whilst foliar application of potassium containing materials (MLE+S and SOP+S) improved the pigment contents as compared to the salt stressed plants (Table.1). These results are in accordance with that of [27, 28]. It has been noticed that salinity stress decreased the rate of CO_2 assimilation, chlorophyll and carotenoids contents [8, 29]. Nevertheless, the foliar spray of growth stimulators (MLE+S and SOP+S) alleviates this reduction by improving the chlorophyll contents, RuBPCase activity and photosynthetic activity [30, 31, 19, 32, 33]. It is apparent that, application of salinity at 13 dS m^{-1} to faba bean plants induced remarkable inhibition (38.6%) in their photosynthetic activities as compared with the control samples (Table. 1). Foliar applied growth enhancers (MLE+S and SOP+S) induced a significant acceleration in $^{14}CO_2$-fixation rate by 7.1% and 1.3% respectively, as compared with the control plants. Yield attributes showed a decreasing trend with increasing salinity. However, foliar applied growth enhancers (MLE+S and SOP+S) caused maximum increase in weight of 100 grain of faba bean as compared to salt stressed plants (Table.1), these results are in agreement with that of [16, 24] who investigated that this may be attributed to hormonal influence especially rich zeatin contents of moringa leaves.

Table 1. Changes in chlorophyll *a+b* (mg g^{-1}FW), carotenoids (mg g^{-1}FW), weight of 100 grain (g), photosynthetic activity (*KBq mg^{-1}FW) and transpiration rate ($mMH_2O\ m^{-2}s^{-1}$) of *Vicia faba* grown with or without NaCl and foliar applied by MLE and SOP.

Treatment	*Chlorophyll (a+ b)*	*Carotenoids*	*Weights of 100 grain*	*Photosynthetic activity*	*Transpiration rate*
C	3.59 ^{a}	1.67 ^{a}	65 ^{a}	13.720 ^{a}	4.3 ^{a}
S	2.01 ^{b}	0.92 ^{b}	40 ^{b}	8.427 ^{b}	7.1 ^{b}
S + MLE	3.42 ^{c}	1.42 ^{c}	62 ^{c}	14.694 ^{c}	4.2 ^{c}
S + SOP	3.18 ^{d}	1.10 ^{d}	56 ^{d}	13.895 ^{d}	4.0 ^{d}

Data are means of three replicates.
Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01). **C:** control;**S:** addition of 135 mM NaCl into nutrient solution; **MLE:** *Moringa oleifera* leaf extract and **SOP:** sulphate of potash, respectively. * Kilo Becquerel (10^3 Bq).

Also, [18] found that, the moringa leaf extract application produces 20–35 % increase in yield of peanut, maize, soybean and sugarcane. Salt stress caused a remarkable increase in proline content (Table. 2). Nonetheless, MLE+S and SOP+S application caused a significant decrease in proline level in salt stressed plants as compared to the control.

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Table 2. Changes in proline (μmol g^{-1}DW), enzyme activity of RuBPCase (μmol CO_2 $min^{-1}mg^{-1}$protein) and total protein (mg g^{-1}DW) of *Vicia faba* grown with or without NaCl and foliar applied by MLE and SOP.

Treatment	*Proline*	*Total protein*	*RuBPCase*
C	7.7 ^{a}	88^{a}	33.8 ^{a}
S	12.9 ^{b}	71 ^{b}	21.5 ^{b}
S + MLE	9.6 ^{c}	103^{c}	31.7 ^{c}
S + SOP	8.7 ^{c}	97^{d}	28.1 ^{d}

Data are means of three replicates.
Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01). **C:** control; **S:** addition of 135mM NaCl into nutrient solution; **MLE:** *Moringa oleifera* leaf extract and **SOP:** sulphate of potash, respectively.

Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger [34]. In the present study, foliar applied growth enhancers (MLE+S and SOP+S) increased total protein content as compared with the control and salt stressed plants [14]. However, treatment with MLE was more effective than SOP in this regard (Table. 2). Sulphur application increased the yield since it is a constituent of amino acid and protein production [35, 36]. The salinity damage is also associated with increased

transpiration rate [4]. Whilst foliar application of potassium containing materials (MLE+S and SOP+S) decreased the transpiration rate as compared to that of the control and salt stressed plants [37]. Maximum contents of Na^+ and Cl^- were observed at 135 mM of NaCl, while least contents of Na^+ and Cl^- at same salinity level was obtained with exogenous application of MLE+S and SOP+S as compared to the control plants (Table. 3). Foliar application of MLE+S and SOP+S increased concentration of N, P, and K in shoot and root of salt stressed and control plants (Table. 3).

Table 3. Ion contents of Na, Cl, N, P, and K (mg g^{-1}DW) in shoot and root of *Vicia faba* grown with or without NaCl and foliar applied by MLE and SOP.

Treatment	*Na*		*Cl*		*N*		*P*		*K*	
	Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
C	65 ^{a}	26 ^{a}	78 ^{a}	23 ^{a}	334 ^{a}	167 ^{a}	208 ^{a}	78 ^{a}	267 ^{a}	103 ^{a}
S	83 ^{b}	38 ^{b}	98 ^{b}	45 ^{b}	267 ^{b}	112 ^{b}	170 ^{b}	49 ^{b}	205 ^{b}	73 ^{b}
S + MLE	62 ^{a}	24 ^{a}	76 ^{a}	22 ^{a}	370 ^{c}	198 ^{c}	256 ^{c}	108 ^{c}	288 ^{c}	139 ^{c}
S + SOP	56 ^{c}	16 ^{c}	60 ^{c}	15 ^{c}	349 ^{d}	177 ^{d}	223 ^{d}	80 ^{d}	238 ^{d}	118 ^{d}

Data are means of three replicates.
Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01). **C:** control; **S:** addition of 135 mM NaCl into nutrient solution; **MLE:** *Moringa oleifera* leaf extract and **SOP:** sulphate of potash, respectively.

These results are in agreement with that of [38]. Also, [17] reported increase in potassium contents of wheat leaves when MLE was exogenously applied to induce tolerance against salinity stress conditions. Sulphur can increase the absorption of potassium or it can react with nitrogen and potassium [39]. Finally, MLE and SOP partially offset the negative impacts of NaCl stress.

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