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Ocimum basilicum Leaf Extract induces Salinity Stress Tolerance in Faba Bean Plants

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THE PRESENT work investigated the possible protective role of aqueous *Ocimum basilicum* leaves extract (OLE) on *Vicia faba* plants irrigated with different leves of salinity (0.0, 50, 100 or 150 mM NaCl). The toxic effects of salinity on *Vicia faba* plants were assessed by determining oxidative stress such as lipid peroxidation. Furthermore, growth parameters, antioxidant enzymes activity [catalase (CAT), peroxidase (POD) and ascrobate peroxidase (APX)], organic solutes (soluble sugars, soluble proteins and proline) and ions content (Na^+, K^+, Ca^2 and Mg^{2+}) on *Vicia faba* plants grown under salinity stress in the presence or absence of aqueous extract of *O. basilicum* leaves were also measured. The obtained results revealed that salinity stress resulted in a significant increase in the activity of antioxidant enzymes, organic solutes, lipid peroxidation and ions content, which were associated with a significant reduction in growth parameters compared with control plants. OLE significantly alleviated the harmful effect of salinity on *Vicia faba* plants and increased the activity of antioxidant enzymes activity, osmolytes (soluble sugar and soluble protein), and ions content (K^+, Ca^{2+} and Mg^{2+}), which could be an induced defensive mechanism against salinity stress.

Keywords: Antioxidant enzymes, Malondialdehyde, *Ocimum basilicum*, Proline, Salinity.

Introduction

Faba bean (*Vicia faba* L.) is one of the famous cultivated legumes crops and a major source of protein for both human and animal diet (Crépon et al., 2010; Dawood et al., 2019; Loutfy et al., 2019). In Egypt, faba bean is considered the first legume crop (Mohsen et al., 2013). Mature faba bean seeds are a sources of vitamin C, cellulose, starch, protein and minerals (Hamilton, 2005). The cultivation of faba bean leads to rising N compounds in the soil (Hungria & Vargas, 2000). Salinity gives plant growth two primary intimidations: osmotic stress and ionic stress (Flower & Colmer, 2008). In addition, it generats oxidative stress, leading to an increase in the release of reactive oxygen species (ROS) or free radicals such as hydrogen peroxide, (H_2O_2), single oxygen ($^1O_2$) and superoxide (O_2^-) (Noctor &Foyer, 1998). These ROS are extremely reactive and can influence regular cellular metabolism by destroying lipids, proteins and oxidizing nucleic acids (Imlay, 2003; Rahdari et al., 2012). Munns (2002) indicated that, in relation to modifications in plant metabolic processes, salinity decreases the capacity of crops to use water and creates a reduction in growth level. The NaCl in pepper induces a reduction in root length, new and dry root mass, number of leaves, and leaf area (Zhani et al., 2012). Plants have created a complicated defense to reduce the oxidative injury caused by ROS, including antioxidant enzymes, such as ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD) (Yan et al. 2017; Hamed & AbdElgawad, 2018). SOD is the major O_2^- scavenger and outcomes in H_2O and O_2 formation due to its enzymatic action. CAT and several groups of peroxidases scavenge the generated H_2O_2. CAT, located in peroxisomes, cytosol and mitochondria, decomposes H_2O_2 to H_2O and O_2 (McKersie & Leshem, 1994). Peroxidases (GPX and APX) are distributed throughout the cell, catalyzing H_2O_2 to H_2O reduction. In the first step of the ascorbate – glutathione cycle, APX uses ascorbate as an

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electron donor and is considered to be the most major plant peroxidase in H_2O_2 detoxification (Noctor & Foyer, 1998). Malondialdehyde (MDA), a consequence of lipid peroxidation, has been assessed as an expression of oxidative damage (Meloni et al., 2003; Kumari et al., 2013; Hassan et al., 2017). Plants can tolerate stress caused by soil salinity by producing antioxidant agents. Salt tolerance in plants can occur through mechanisms such as coordination between water, K^+, and some compatible cellular solutes and osmotic adjustment (Al Kharusi et al., 2019). The accumulation of high levels of salt by the plant causes an increase in the osmotic pressure of the cytosol. Under these circumstances, cell homeostasis is maintained by an osmotic adjustment mechanism consisting of the sequestration of massive amounts of salt ions in the vacuole and/or organic osmolyte synthesis (Munns, 2002). Compatible osmoprotectants, such as proline and soluble sugars, are generated in many species to protect the cells from adverse effects of salinity. High accumulation of proline is correlated with stress tolerance (Sairam & Tyagi, 2004; Hokmabadi et al., 2005). The K^+/Na^+ ratio in leaves is often seen as a salt-tolerant marker (Azooz et al., 2004). The calcium content under salinity stress depends on the particular physiology of the plant, its organs and its duration. It has been indicated that the role of Ca^{2+} in ameliorating salt toxicity is linked to the impact of K^+/Na^+ selectivity by managing the inflow of Na^+ via non-selective ion channels (Gucci & Tattini, 1997; Melgar et al., 2006). It is reported that salt stress increases Na^+, Ca^{2+}, Mn^{2+}, Cu^{2+} and Fe^{2+} but causes a decrease in K^+ (Erdal et al., 2000). *Ocimum basilicum* L. is a common herb belonging to the Lamiaceae family (Mohamed, 2015; Chenni et al., 2016). The *O. basilicum* plants have been investigated as potential allelopathic plants (Baličević et al., 2015).

Allelopathy refers to direct or indirect negative effects of one plant on another through the release of chemical compounds into the environment (Delabays et al., 2004; Ahmed & Slima, 2020). These biochemicals are known as allelochemicals (Singh & Chaundharv, 2011). Allelochemicals include secondary metabolites, flavonoids and phenolic acids (Pandey et al., 2016). Elemental analysis and phytochemical screening of aqueous extract of *O. basilicum* showed the presence of cardiac glycosides, tannins, saponins, calcium, sodium, potassium and magnesium (Khair-ul-Bariyah et al., 2012). The known phenolic compounds identified in *O. basilicum* are flavonol-glycosides and phenolic acids (Kivilompolo & Hyötyläinen, 2007). Kaurinovic et al. (2011) stated that the amount of total phenolic content and total flavonoids of *O. basilicum* in H_2O extract was more than its amount in the other extracts [ether (Et_2O), chloroform ($CHCl_3$), ethylacetate (EtOAc) and n-butanol (n-BuOH)]. This study investigated the ability of OLE to protect *Vicia faba* against salinity stress and highlighted the underlying defense mechanisms behind the aqueous extraction of *O. basilicum* leaves in induced tolerance.

Materials and Methods

Preparation of O. basilicum leaf extract (OLE)

Ocimum basilicum leaves were obtained from the local region of South Valley University, Qena, Egypt. Fresh leaves of *O. basilicum* were washed under running tap water, then air-dried and homogenized to obtain a fine powder. Air-dried leaves powder (20g) were added to 300mL of distilled water, put for 24hrs on low heat and then filtered. The filtrate was concentrated to make the final volume one-third of the original volume using water bath and this 20% (w/ v) of this concentrated solution was used as the *O. basilicum* extract (Pandey et al., 2016).

Pot experiment

The present research was carried out in the experimental farm of the South Valley University in Qena, Egypt. Faba bean seeds were sterilized in 5% sodium hypochlorite (NaOCl) solution for 5min to prevent contamination. The same number of seeds (7 seeds/ pot) of faba bean were planted in plastic pots (30cm in diameter) filled with 2kg of dried soil and arranged in a fully randomized model. Seedlings were thinned to four in each pot after seven days of germination, the seeds were irrigated at field capacity with different salinity levels (0.0, 50, 100 or 150mM NaCl). The salt solution was added once and leaching was avoided by maintaining soil water below field capacity at all times. The pots were then irrigated at field capacity with normal water through the whole experimental period. NaCl treated seedlings were sprayed with the *O. basilicum* leaf extract solution after 7 and 10 days of NaCl treatment and the control plants were sprayed with distilled water. The concentration of *O. basilicum* leaf extract and NaCl was selected based on our preliminary study. Hoagland's solution (1/2 strength) was added once every week in all pots. Plant samples were

collected after four weeks of sowing.

Growth measurement

Harvested seedlings were washed 3 times with double distilled water, then the fresh weight (FW) of each plant sample was measured. The harvested plant materials were quickly frozen and stored at –20ºC for further biochemical investigation. Some plant samples were dried in an oven at 80ºC to estimate the constant weight of the dry weight (DW).

Determination of antioxidant enzyme activities

Approximately 500mg of plant tissue were homogenized in 1mL K-phosphate buffer pH. 7, involving 0.1mM sodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) and 1% polyvinylpyrrolidone (PVP), and then centrifuged at 10,000 rpm at 4^0C for 20min. The supernatant was used for enzyme assay. The activity of catalase was determined using the technique mentioned by Aebi (1984). Peroxidase (POX) activity was determined according to MacAdam et al. (1992). Ascorbate peroxidase was determined using the technique outlined by Nakano & Asada (1981).

Determination of malondialdehyde (MDA)

The MDA was evaluated in new leaf samples according to the technique followed by Heath & Packer (1968).

Determination of organic solutes

The content of soluble sugars was estimated by the anthrone sulphuric acid technique as explained by Badour (1959). The Bradford (1976) technique was used to determine the soluble protein content. Proline content was estimated in accordance with Bates et al. (1973) method.

Determination of mineral ions

Sodium and potassium were determined using flame photometry according to Williams & Twine (1960). Calcium and magnesium were determined volumetrically as described by Schwarzenbach & Biedermann (1948).

Statistical analysis

The data were statistically analyzed by ANOVA and compared using the least significant difference (LSD) test at 5% (*) and 1% (**) levels (Snedecor & Cochran, 1980). Values in the tables and figures indicate the mean values ± SD based on three independent determinations (n= 3).

Results

Growth attributes

Vicia faba subjected to NaCl stress exhibited a considerable decrease in the growth aspects like fresh weight (FW) and dry weight (DW) of plants (Figs 1 and 2). Relative to control, 150 mM NaCl decreased FW (35.29%) and DW (43.57%). However, foliar spraying of OLE significantly improved these aspects and also alleviated the negative influence of salt stress. The percent increase in FW and DW by treatment of OLE was 76.47% and 31.25%, respectively as compared to control (Figs. 1, 2). Under the influence of 150 mM NaCl, treatment with OLE increased the FW and DW by 65.15 % and 11.11 %, respectively as compared to salt stressed plants.

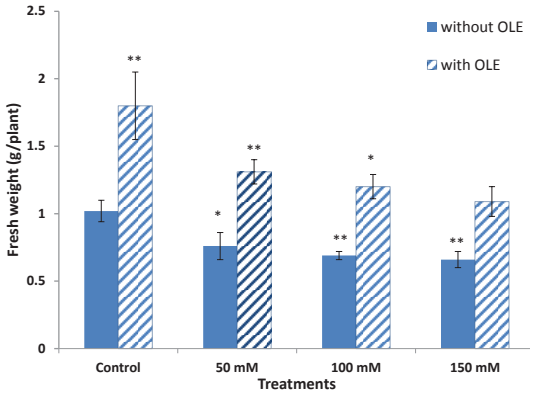

Fig. 1. Effect of salinity and OLE on fresh weight (g/plant) and dry weight (g/plant) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P<0.05; ** significant at P< 0.01].

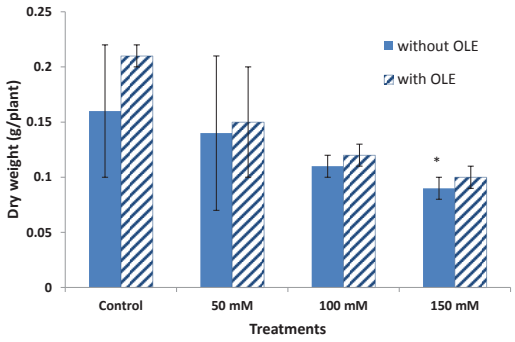

Fig. 2. Effect of salinity and OLE on dry weight (g/plant) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P< 0.05; ** significant at P<0.01].

Organic solutes

Exposure of faba bean to salinity stress resulted in a significant increase in the contents of soluble sugars, soluble proteins and proline. The concentration 150 mM NaCl recorded the highest increase in soluble sugars (14.45%), soluble proteins (108.36%) and proline (17.33%) as compared to the control (Figs 3-5). Treatment with OLE alone or in combination with salt significantly elevated the content of soluble sugars, soluble proteins over those of either control or salt stressed plants. On the other side, an opposite pattern was noticed in proline content. The content of proline decreased under the effect of OLE or salt stressed-OLE treatments.

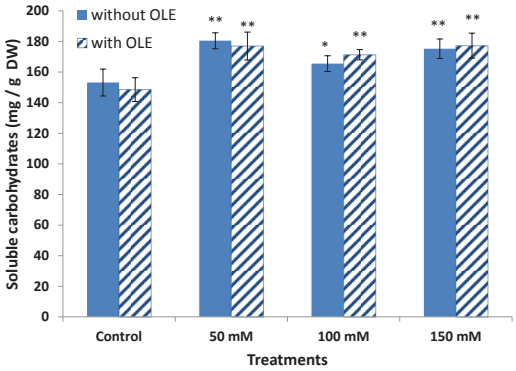

Fig. 3. Effect of salinity and OLE on soluble carbohydrate (mg/g^{-1} DW)) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P< 0.05; ** significant at P< 0.01].

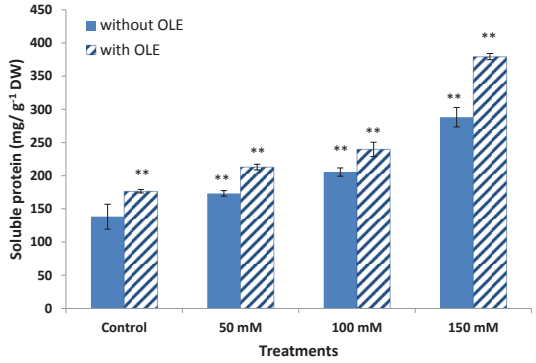

Fig. 4. Effect of salinity and OLE on soluble protein (mg/g^{-1} DW)) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P< 0.05; ** significant at P< 0.01].

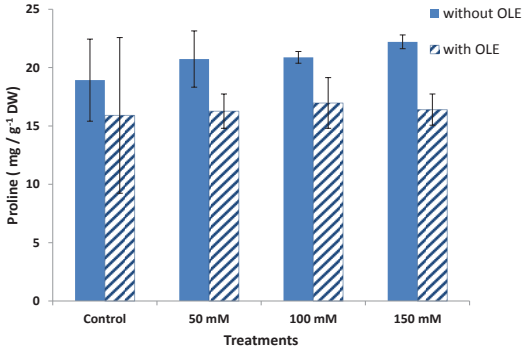

Fig. 5. Effect of salinity and OLE on proline (mg/g^{-1} DW)) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P< 0.05; **significant at P< 0.01].

Activity of antioxidant enzymes

Generally, activities of all studied enzymes (CAT, POD and APX) increased with the increase of NaCl concentrations (Fig. 6). In comparison with control plants, under the influence of 150 mM NaCl, the increase in CAT, POD and APX activity were 82.50%, 28.16% and 58.11%, respectively. The data present in Fig. 6 indicated that there was a significant increase in CAT, POD and APX activity under the salt stressed-OLE treatment, compared to salt stressed seedlings. In comparison to the corresponding salt stressed plants, the maximum increase in the activity of POD was 16.13% under the influence of 150mM NaCl, while the maximum increase in the activity of APX and CAT were 14.23% and 15.53%, respectively, under the influence of 50mM NaCl.

Mineral ions

The data in Fig. 7 illuminated that, the contents of Na^+, K^+, $Mg^{2+}$and Ca^{2+} were positively affected by increasing NaCl levels. On the other hand, foliar application of OLE reduced the levels of Na^+, and increased the content of K^+, Mg^{2+} and Ca^{2+} compared with salt stressed plants.

Malondialdehyde (MDA) content

The content of MDA was increased with increasing salinity levels (2.14%, 31.33% and 126.04% at 50, 100 and 150mM NaCl, respectively) compared with control (Fig. 8). In comparison to the corresponding salt stressed plants, the treatment of OLE decreased MDA content.

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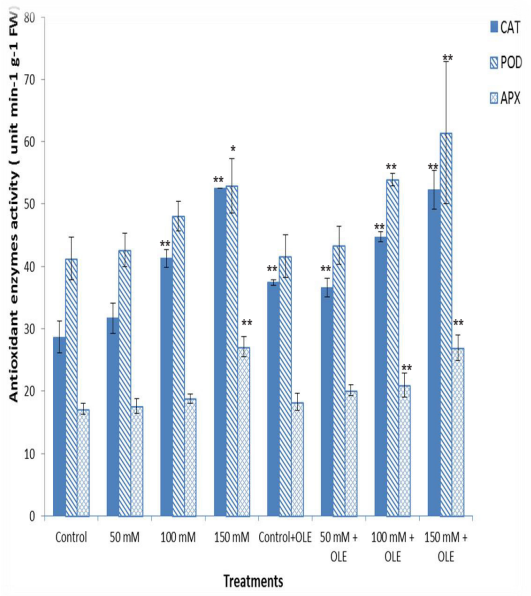

Fig. 6. Effect of salinity and OLE on CAT, POD and APX activities (unit min^{-1} g^{-1} FW)) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P< 0.05; **significant at P< 001].

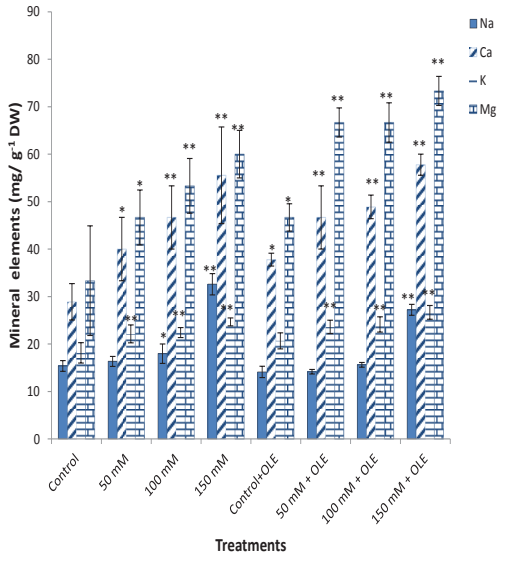

Fig. 7. Effect of salinity and OLE on mineral elements (mg/ g^{-1} DW)) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P<0.05; ** significant at P<0.01].

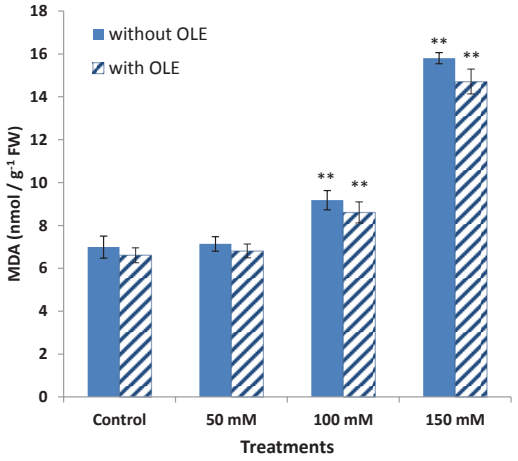

Fig. 8. Effect of salinity and OLE on MDA (nmol/ g^{-1} FW)) of *Vicia faba* plants [Values are means of three replicates ± standard deviation (SD), statistical significance of differences compared to the control: *significant at P<0.05; ** significant at P<0.01].

Discussion

The use of plant extracts and phyto-products to reduce several stresses is actually receiving attention due to their accessibility, cost-effectiveness and biodegradability (Pandey et al., 2016; Hanafy, 2017; Hassanein et al., 2019). The aqueous *O. basilicum* leaves extract (OLE) includes phenolic compounds (Kaurinovic et al., 2011). Phenols have antioxidant properties and ability to extinguish free radical reactions (Bozin et al., 2008). The inhibition of membrane lipid peroxidation by "capturing" alkoxyl radicals is another mechanism underlying the antioxidant characteristics of phenolic compounds. Phenols such as flavonoids stabilize membranes by reducing their fluidity, which in turn limits the diffusion of free radicals and decreases the peroxidation of membrane lipids. Membrane stabilization is due to phenolic ability (particularly flavonoids) to bind to some of the essential phospholipids and membrane proteins (Michalak, 2006). The impact of OLE on different levels of NaCl associated parameters in *Vicia faba* was evaluated in the present study. Growth reduction is the most common response of plants to stress such as salinity exposure. A massive reduction in the growth parameters (fresh and dry weights) of faba bean was detected in regard to salinity stress compared to non-stressed control plants. This might be due to inhibition of cell division and cell elongation exerted by the existence of

high levels of salt (Rady & Mohamed, 2015; Abdel Latef et al., 2017). In addition, growth inhibition under salinity stress has been related to a decrease in carbon assimilation due to stomatal limitation and/or metabolic injury (Hajiboland et al., 2014). Application of OLE not only mitigated the toxic effect of salinity on the development of faba bean crops, but also considerably improved the characteristics when implemented alone. This outcome retained the stimulating impacts of moringa extracts as stated by Rady et al. (2013) and Rady & Mohamed (2015). In addition, *Ocimum basilicum* therapy was considerably shown to keep greater complete dry mass after harvesting under drought stress, as compared to control (Pandey et al., 2016).

In this study, APX, POD and CAT in *Vicia faba* were improved under salinity stress, and these enzymes may be important for the defense of *Vicia faba* crops when cultivated under salinity stress. De Azevedo Neto et al. (2006) reported that the actions of CAT, APX and GPX play a key protective position in the $O_2^{\bullet-}$ and H_2O_2 scavenging process, and that the active participation of these enzymes is linked, at least in part, to salt-induced oxidative stress tolerance in maize plants. Treatment with OLE produced an increase in CAT, POD and APX activity giving that strength to the antioxidant protection system in elimination of toxic ROS. The increased activities of CAT, POD and APX by foliar application with OLE might also help in growth maintenance through scavenging of H_2O_2. In the present work, it could be decided that application of OLE supported the antioxidant potential of *Vicia faba*. Kahilo et al. (2015) showed that foliar treatment of tomato plants with 30% aqueous extract of *Ocimum sanctum* increased the antioxidant enzymes.

Salinity stress caused an enhancement in lipid peroxidation in faba bean plants, which is supported by the results of Abdel Latef et al. (2017). Upchurch (2008) revealed that salt stress alters the composition and structure of the plasma membrane lipids. This change influences the degree of saturation of free fatty acids and free sterols, which may ultimately lead to a decrease in the fluidity of the cell membrane. Treatment of OLE decreased the content of MDA. This reduction may demonstrate membrane establishment and thus protects the biological membrane from the oxidative impacts of toxic free radicals. *Ocimum basilicum* treatment noticeably decreased the MDA content of dry-stressed rice flag leaf as compared to untreated control (Pandey et al., 2016). In the present study, the reduction in growth, especially at severe salinity, was associated with an enhancement in the accumulation of soluble sugars, soluble protein and proline. This increase in organic solutes under NaCl stress might be useful in osmoregulation and membrane adjustment (Azooz et al., 2015). Also, when salt-stressed tested *Vicia faba* plants were sprayed with OLE, proline content was decreased compared with stressed plants alone. While there is a noticeable enhancement in other organic solutes (soluble sugars and soluble proteins) indicating an altering impact and other strategies by which OLE could adjust the osmotic potential of *Vicia faba* plants and hence stimulate their tolerance to salinity stress. Salinity stress exposure augmented the uptake of essential mineral elements in *Vicia faba* plants. High concentrations of Na^+ ions are toxic to the plant (Iqbal et al., 2015). There was a massive increase in the uptake of K^+, Ca^{2+} and Mg^{2+} of the studied plants under salt stress, as K^+ may play an important function in maintaining osmotic adjustment and cell turgor (Marschner, 1995). Calcium has been shown to enhance salt stress by increasing the selective absorption of potassium in plants with high sodium levels and allowing plants to execute osmotic adjustment by improving ion uptake (Epstein, 1998; Schachtman & Liu, 1999).Karimi & Maleki Kuhbanani (2015) indicated that salinity stress rises Ca^{2+} in the shoot of *Badami zarand* plants. OLE treatment not only alleviated the deleterious impact of excess sodium by restricting its uptake, but also resulted in a substantial rise in the uptake of essential mineral elements, including K^+ and Ca^{2+}. Improved K^+ uptake has favorable control over growth performance through active involvement in several metabolic mechanisms such as enzyme activation, osmoregulation, and selective sodium accumulation (Tomar & Agarwal, 2013; Ahmad et al., 2014; Ahanger et al., 2015). These results suggest a positive effect of OLE on the uptake of K^+ and Ca^{2+} and hampering Na^+ uptake in faba bean plants.

Conclusion

NaCl stress caused changes in the growth of *Vicia faba* by affecting the physological and biochemical behaviors. Treatment with OLE amended growth by enhancing organic solutes (soluble sugars, soluble proteins, and proline)

and essential minerals (K^+, Ca^{2+}, Mg^{2+}) and the activity of antioxidant enzymes (CAT, POD, and APX), in addition to reducing increased Na^+ and MDA contents. Consequently, it could be decided that OLE has a role in the alleviation of deleterious influences of NaCl stress. Therefore, we recommend that foliar spray of OLE might be a useful strategy for improving *Vicia faba* plants tolerance when exposed to NaCl stress. Furthermore, this method is easy to apply and is eco-friendly and could be accepted by farmers to be used in the field to increase the growth and yield of plants.

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: This work was carried out in collaboration between all authors. Naglaa Loutfy designed the study, performed the statistical analysis and wrote the protocol. Mona F. Abou Alhamed managed the analysis of the study and the literature searches. All authors read and approved the final manuscript

Ethical approval: Not applicable.

References

Abdel Latef, A.A., Abu Alhmad, M.F., Hammad, S.A. (2017) Foliar application of fresh moringa leaf extract overcomes salt stress in fenugreek (*Trigonella foenum-graecum*) plants. *Egyptian Journal of Botany*, **57**(1), 157-179.

Aebi, H. (1984) Catalase *in vitro*. *Methods in Enzymology*, **105**, 121-126.

Ahanger, M.A., Agarwal, R.M., Tomar, N.S., Shrivastava, M. (2015) Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L cultivar Kent). *Journal of Plant Interaction*, **10**, 211-223.

Ahmad, P., Ashraf, M., Azooz, M.M., Rasool, S., Akram, N.A. (2014) Potassium starvation induced oxidative stress and antioxidant defense responses in *Brassica juncea. Journal of Plant Interaction*, **9**, 1-9.

Ahmed, D.A., Slima, D.F. (2020) Effect of aqueous extract of *Trianthema portulacastrum* L. on the growth of *Zea mays* L. and its associated weeds. *Egyptian Journal of Botany*, **60**(1), 169-183.

Al Kharusi, L., Al Yahyai, R., Yaish, M.W. (2019) Antioxidant response to salinity in salt-tolerant and salt-susceptible cultivars of date Palm. *Agriculture*, **9**(8), 1-17.

Azooz, M.M., Metwally, A., Abou-Elhamd, M.F. (2015) Jasmonate-induced tolerance of *Hassawi okra* seedlings to salinity in brackish water. *Acta Physiologiae Plantarum*, **37**, 1-13.

Azooz, M.M., Shaddad, M.A., Abdel-latef, A.A. (2004) Leaf growth and K^+ /Na^+ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. *Acta Agronomica Hungarica*, **52**(3), 287-296.

Badour, S.S.A. (1959) Analytisch-chemische untersuchung des kaliummangels bei Chlorella im vergleich mit anderen mangelzustanden. P*h.D. Dissertation*, Gottingen.

Baličević, R., Ravlić, M., Ravlić, I. (2015). Allelopathic Effect of aromatic and medicinal plants on *Tripleurosper mumidorum* (L.) *C. H. Herbologia*, **15**(2), 41-53.

Bates, L.S., Wladren, P.R., Tear, D.T. (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39**, 205-207.

Bozin, B., Mimica-Dukic, N., Samojlik, I., Goran, A., Igic, R. (2008) Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chemistry*, **111**, 925-929.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. *Analytical Biochemistry*, **72**, 248-254.

Chenni, M., El Abed, D., Rakotomanomana, N., Fernandez, Z., Chemat, F. (2016) Comparative study of essential oils extracted from Egyptian basil leaves (*Ocimum basilicum* L.) using hydro-distillation and solvent-free microwave extraction. *Molecules*, **21**, 113-116.

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P., Duc, G. (2010) Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crop Reserch*, **115**, 329-339.

Dawood, M.G., Abdel-Baky, Y.R., El-Awadi1, M.E., Bakhoum, G.S. (2019) Enhancement quality and

quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application. *Bulletin of the National Research Centre*, **43**(1), 43-28.

De Azevedo Neto, A.D., Prisco, J.T., Enéas-Filho, J., De Abreu, C.E.B., Gomes-Filho, E. (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, **56**, 87-94.

Delabays, N., Mermillod, G., De Joffrey, J.P., Bohren, C. (2004). Demonstration, in cultivated fields, of the reality of the phenomenon of allelopathy. *International Conference on Weed Biology*, **2004**, 97-104

Epstein, E. (1998) How calcium enhances plant salt tolerance. *Science*, **280**, 1906-1907.

Erdal, İ., Türkmen, O., Yıldız, M. (2000) Tuz stresi altında yetiştirilen hıyar (*Cucumis sativus* L.) fidelerinin gelişimi ve kimi besin maddeleri içeriğindeki değişimler üzerine potasyumlu gübrelemenin etkisi. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi. *Journal of Agricultural Science*, **10**(1), 25-29.

Flowers, T.J., Colmer, T.D. (2008) Salinity tolerance in halophytes. *New Phytologist*, **179**, 945-963.

Gucci, R., Tattini, M. (1997) Salinity tolerance in olive. In: "*Horticultural Reviews*", Janik. J. (Ed.), 21, pp. 177–214. John Wiley & sons, Inc., USA.

Hajiboland, R., Norouzi, F., Poschenrieder, C. (2014) Growth, physiological, biochemical and ionic responses of pistachio seedlings to mild and high salinity. *Trees*, **28**, 1065-1078.

Hamed, B.A., AbdElgawad, H. (2018) Differential Effects of Some Ascorbates on *Phaseolus vulgaris* L. In Response to Salinity Stress. *Egyptian Journal of Botany*, **58**(1), 23-36.

Hamilton, D. (2005) Broad bean. Available from http:// www.selfsufficientish. com/bean.htm.

Hanafy, R.S. (2017) Using *Moringa olifera* leaf extract as a bio-fertilizer for drought stress mitigation of *Glycine max* L. plants. *Egyptian Journal of Botany*, **57**(2), 281-292.

Hassan, Y.M., Hamed, B.A., Khodary, S.A., Hamouda, O.H.I. (2017) Salt stress mitigation by seed priming with olive waste of *Phaseolus vulgaris* L. *Nature and Science,* **15**(6),145-154.

Hassanein, R.A., Abdelkader, A.F., Faramawy, H.M. (2019) Moringa leaf extracts as biostimulants-inducing salinity tolerance in the sweet basil plant. *Egyptian Journal of Botany*, **59**(2), 303-318.

Heath, R.L., Packer, L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125**,189-198.

Hokmabadi, H., Arzani, K., Grierson, P.F. (2005) Growth, chemical composition, and carbon isotope discrimination of pistachio (*Pistacia vera* L.) rootstock seedlings in response to salinity. *Australian Journal of Agricultural Research*, **56**, 135-144.

Hungria, M., Vargas, M.A.T. (2000) Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crop Research*, **65**,151-164.

Imlay, J.A. (2003) Pathways of oxidative damage. *Annual Review of Microbiology*, **57**, 395-418.

Iqbal, N., Umar, S., Khan, N.A. (2015) Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). *Plant Physiology*, **178**, 84-91.

Kahilo, K., Elkany, H., Sadek, K., Kheir-Eldeen, A. (2015) Antioxidant and immunostimulant effects of basil (*Ocimum basilicum*) against gibberllic acid and auxin supplemention in broilers ration. *Global Veterinaria*, **15**(3), 289-95.

Karimi, H.R., Maleki Kuhbanani, A. (2015) The evaluation of inter-specific hybrid of P. atlantica × P. vera cv. 'Badami Zarand as a pistachio rootstock to salinity stress. *Journal of Nuts*, **6**(2), 113-122.

Kaurinovic, B., Popovic, M., Vlaisavljevic, S., Trivic, S. (2011) Antioxidant capacity of *Ocimum basilicum* L. and *Origanum vulgare* L. extracts. *Molecules*, **16**, 7401-7414.

Khair-ul-Bariyah, S., Ahmed, D., Ikram, M. (2012) *Ocimum Basilicum*: A review on hytochemical and pharmacological studies. *Pakistan Journal of Chemistry*, **2**(2), 78-85.

Kivilompolo, M., Hyötyläinen, T. (2007) Comprehensive two-dimensional liquid chromatography in analysis of Lamiaceae herbs: Characterization and quantification of antioxidant phenolic acids. *Journal of Chromatography A*, **1145**, 155-164.

Kumari, N., Jain, V., Talwar, G. (2013) Salinity induced changes in ascorbic acid, hydrogen peroxide, lipid peroxidation and glutathione content in leaves of salt tolerant and salt-susceptible cultivars of cotton (*Gossypium hirsutum* L.). *Research in Plant Biology*, **3**, 6-11

Loutfy, N., Azooz, M.M., Hassanein, A.M., Bassiony, A. (2019) Potassium synergize the positive effect of ascorbic acid on some morpho-physiological parameters of salt stressed faba bean cultivars. *Egyptian Journal of Botany*, **59**(3), 735-751.

MacAdam, J.W., Nelson, C.J., Sharp, R. (1992). Peroxidase activity in the leaf elongation zone of tall fescue (I). *Plant Physiology*, **99**, 872-878.

Marschner, H. (1995) "*Mineral Nutrition of Higher Plants*", 2nd ed. London: Academic Press.

McKersie, B.D., Leshem, Y.Y. (1994) "*Stress and Stress Coping in Cultivated Plants*". Kluwer Academic Publishes, Dordrecht.

Melgar, J.C., Benlloch, M., Fernandez-Escobar, R. (2006) Calcium increases sodium exclusion in olive plants. *Scientia Horticulturae*, **109**, 303-305.

Meloni, D.A., Oliva, M.A., Martinez, C.A., Cambraia, J. (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*, **49**, 69-76.

Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, **15**, 523-530.

Mohamed, G.S. (2015) Toxicity of Basil (*Ocimum basilicum* L.) and Rosemary (*Rosmarinus officinali* L.) extracts on *Tribolum confusum* (DuVal) (Coleoptera:Teneberionidae). *Journal of Phytopathology and Pest Management*, **2**(2), 27-33.

Mohsen, A.A., Ebrahim, M.K.H., Ghoraba, W.F.S. (2013) Effect of salinity stress on *Vicia faba* productivity with respect to ascorbic acid treatment. *Iranian Journal of Plant Physiology*, **3**(3), 725-736.

Munns, R. (2002) Comparative physiology of salt and water stress. *Plant Cell and Environment*, **25**, 239-250.

Nakano, Y., Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, **22**, 867-880.

Noctor, G., Foyer, C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**, 249-279.

Pandey, V., Ansarib, M.W., Tulac, S., Sahooc, R.K., Bainsa, G., Kumard, J., Tutejae, N., Shuklaa, A. (2016) *Ocimum sanctum* leaf extract induces drought stress tolerance in rice. *Plant Signaling Behavior*, **11**(5), (9 pages).

Rady, M.M., Mohamed, G.F. (2015) Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Scientia Horticulturae*, **193**, 105-113.

Rady, M.M., BhavyaVarma, C., Howladar, S.M. (2013) Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress a result of presoaking in *Moringa oleifera* leaf extract. *Scientia Horticulturae*, **162**, 63-70.

Rahdari, P., Tavakoli, S., Hosseini, S.M. (2012) Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleraceae* L.) leaves. *Journal of Stress Physiology & Biochemistry,* **8**(1), 182-193.

Sairam, R.K., Tyagi, A. (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*, **86**, 407-421.

Schwarzenbach, G., Biedermann, W. (1948) Komplexonex. Erdalkalikomplexe van, o,6-Doxyazofarbstoffen He\v. *Chemical Acta*, **31**, 678-687.

Schachtman, D., Liu, W. (1999) Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Trends in Plant Science*, **4**, 281-287.

Singh, P.A., Chaudharv, B.R. (2011) Allelopathic potential of algae weed Pithophoraoedogonia (Mont.) ittrock on the germination and seedling growth of *Oryza sativa* L. *Botany Research International*, **4**(2), 36-40.

Snedecor, G.W., Cochran, W.G. (1980) "*Statistical Methods*", 7th ed. Iowa StateUniversity Press, Ames, Iowa, USA.

Tomar, N.S., Agarwal, R.M. (2013) Influence of treatment of *Jatropha curcas* L. leachates and potassium on growth and phytochemical constituents of wheat (*Triticum aestivum* L.). .*American Journal of Plant Science*, **4**, 1134-1150

Upchurch, R.G. (2008) Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters*, **30**, 967-977.

Williams, C.H., Twine, M.E. (1960) Flame photometric method for sodium, potassium and calcium. In: "*Modern Methods of Plant Analysis*", Paech, K. and Tracey, M.V. (Eds.), Vol. 5, 535p. Springer-Verlag, Berlin.

Yan, K., Shijie Zhao, S., Bian, L., Chen, X. (2017) Saline stress enhanced accumulation of leaf phenolics in honeysuckle (*Lonicera japonica* Thunb.) without induction of oxidative stress. *Plant Physiology and Biochemistry,* **112,** 326-334.

Zhani, K., Mariem, B.F., Fardaous M., Cherif H. (2012) Impact of salt stress (NaCl) on growth, chlorophyll content and fluorescence of Tunisian cultivars of chili pepper (*Capsicum frutescens* L.). *Journal of Stress Physiology & Biochemistry*, **8**(4), 236-252.

المستخلص الورقي لنبات الريحان يحفز تحمل الاجهاد الملحي في نبات الفول

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تناول البحث الحالي الدور الوقائي المحتمل للمستخلص المائي لأوراق الريحان علي نباتات الفول البلدي المروية بتركيزات مختلفة من الملوحة (0، 50، 100 أو 150 مل مول كلوريد صوديوم). تم تقييم التأثيرات السامة للملوحة علي نبات الفول البلدي من خلال دراسة الإجهاد التأكسدي مثل فوق أكسدة الدهون. علاوة على ذلك، تم قياس معاملات النمو، نشاط الإنزيمات المضادة للأكسدة (مثل إنزيم الكاتاليز، إنزيم البيرأوكسيديز وإنزيم فوق أوكسيد الأسكوربيت)، الذائبات العضوية (مثل السكريات الذائبة، البروتينات الذائبة والبرولين) ومحتوي الأيونات (مثل أيونات الصوديوم، البوتاسيوم، الكالسيوم والمغنسيوم) علي نباتات الفول البلدي النامى تحت تأثير الملوحة في وجود أو غياب المستخلص المائي لأوراق نبات الريحان. أظهرت النتائج التي تم الحصول عليها أن الإجهاد الملحي أدي إلي زيادة ملحوظة في نشاط الإنزيمات المضادة للأكسدة، الذائبات العضوية، الدهون فوق المؤكسدة ومحتوي الأيونات والتي كانت مصحوبة بإنخفاض ملحوظ في معاملات النمو مقارنة بالعينات الضابطة (النبات الكنترول). أدى استخدام المستخلص المائي لأوراق الريحان إلى تخفيف التأثير الضار للملوحة علي نباتات الفول البلدي بشكل ملحوظ كما أنه زود نشاط الإنزيمات المضادة للأكسدة، الأسمولات (السكريات الذائبة والبروتنات الذائبة)، ومحتوي الأيونات (البوتاسيوم، الكالسيوم والمغنسيوم)، والتي يمكن أن تكون آلية دفاعية مستحثة بواسطة مستخلص أوراق الريحان ضد إجهاد الملوحة.