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The role of natural exogenous foliar applications in alleviating salinity stress in *Lagerstroemia indica* L. seedlings

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Abstract

A pot experiment was conducted during 2014 and 2015 seasons in completely randomized factorial design to determine the effect of natural extracts foliar spray of *Moringa* leaves extract (1:30), humic acid (10%), seaweed (2%), Hogland nutrient solution and tap water as control on growth characteristics (plant height, stem diameter, number of leaves/plant, number of branches/plant, root length, and total dry weight of plant parts (roots, shoots and flowers), floral and chemical characteristics of *Lagerstroemia indica* grown at various sea salt concentrations (0, 3.12, 6.25 and, 9.37 dS/m) showed that by increasing sea salt concentrations, all growth characteristics, inflorescence number/plant and, inflorescence diameter decreased significantly, while, the number of days to inflorescence increased. Total chlorophylls, carotenoid contents, total carbohydrates and N, P, K%. Meanwhile, proline content, total soluble phenols, Na, Ca, and antioxidant enzymes (catalase, superoxide dismutase and peroxidase) increased at the high level of salinity (9.37 dS/m). The usage of the *Moringa* leaf extract was significantly improved growth, inflorescence, as well as chemical characteristics, but also, decreased significantly Na under the adverse conditions of the studied sea salt stress. *Moringa* leaf extract could promote and protect crape myrtle plants against injuries by sea salt stress can substitute inorganic or chemical fertilizer being safe and cheap.

Key words: *Lagerstroemia indica*, foliar applications, *moringa* leaves extract, sea salt stress, inflorescence characteristics, antioxidant enzymes.

Introduction

Lagerstroemia indica L. (Crape myrtle) is one of the most famous species of the *Lagerstroemia* genus, which belongs to the *Lythraceae* family (Conti *et al.*, 1997), includes 53 woody species (Tobe and Raven, 1990). It is a widely commercialized as a deciduous shrub or small tree (Anonymous, 1962) that has become naturalized and invasive in many tropical and subtropical regions of the world (Randall, 2012).

Crape myrtle is utilized as a versatile landscaping plant, decorative, an ornamental and attractive shrub in the world (Anderson, 2007). *L. indica* is used often around homes and in small parking as shade trees, also used for buffer strips along highways, decks, patios, and around parking (Moore and Walker, 2014). Its wood is hard and useful as a timber (Gamble, 1972). *L. indica* has a very dense and wide-spreading root system, so it used in erosion control. It is also grown as a boundary or barrier support plant in gardens and cultivated areas (Orwa *et al*., 2009).

It is also a medicinal shrub or small tree (Chopra *et al*., 1956). The leaves, flowers, roots and bark of *L. indica* are used as purgative, hydrogogue, febrifuge, stimulant and styptic. The leaves have great medicinal value, particularly in the treatment of diabetes. The roots are astringent and used as gargle and seeds contain a narcotic principle and are used in the production of pesticides. This plant feed as secondary food plants of non-mulberry Tasar silk worm. A paste of the flowers is applied externally to cuts and wounds, while, a decoction of the flowers is used in the treatment of colds (Yeung, 2010; Masharani and German, 2011). Yang *et al.* (2011) found that *L. indica* may be used as a valuable agent for treating allergic disease such as asthma due to its anti-inflammatory property. Diab *et al.* (2012) found that the methanol extracts of *L. indica*eaves exhibited antimicrobial activity against all pathogenic bacteria and yeast. In addition, Kotnala *et al.* (2013) concluded that particular silent disease can be treated by using extracts which has been made worldwide from different *Lagerstroemia* species.

L. indica in harsh urban conditions is effectively grown well. It is tolerant to fire, drought, cold conditions, and relatively free of disease and insect and thus has a potential to grow in new habitats, displacing and smothering native vegetation and makes it an excellent landscape plant (Foxcroft *et al.*, 2007; Oviedo *et al.*, 2012). However, the literature survey conducted to identify biotic stress (*i.e.* salinity) causing injury and affecting its nursery and landscape industries has indicated crape myrtle as a salt-sensitive species (till 3.0 dS/m) (Cabrera and Devereaux, 1999). Salinity is the most recognizable in semi-arid and arid regions of the world and responsible for decline in plant growth by osmotic stress, and specific ion toxicity. Therefore, many applications are made to minimize the effect of salt stress on plants, especially during the early development, to promote successful establishment and growth.

Foliar application with the exogenous plant growth regulators (PGRs) such as cytokinin or antioxidants (ascorbic acid) is the most effective approach involved in promoting plant growth and development by minimizing or alleviating the adverse effects of salt on plant growth and metabolism in many plants (Sadak and Dawood, 2014), but this application is expensive. Therefore, there is a need to explore the natural sources of PGRs for exogenous use which are not only organic, but also easily adapted, environmentally friendly, and inexpensive such

as extract obtained from *Moringa* (*Moringa oleifera*) leaves (Moussa and Hassan, 2016), humic acid (HA) (Rady *et al.*, 2016), seaweed extract (SE) (Battacharyya *et al.*, 2015).

Therefore, this investigation was undertaken to evaluate the promoting effects of foliar application of *Moringa* leaves extract, humic acid or seaweed extract to negate the negative effect of salinity on growth, floral and chemical characters of *L. indica*.

Materials and methods

The pot experiment was carried out at the Experimental Laboratory in the Natural Resources Department, Institute of African Research and Studies, and at the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during two successive seasons of 2014 to 2015. The soil texture of pot media was sandy with the following characteristics: coarse sand 30.82%, fine sand 62.61, silt 1.22%, clay 5.35%, pH 7.75, EC 1.15 dS/m, organic matter 0.08%, available N 6.9 ppm, available P 6.2 ppm, available K 64 ppm, $CaCO_3$ 0.26%, and water holding capacity 14.5%. The soil analysis was carried out according to the methods described by Chapman and Pratt (1961).

Plant material: Three years old seedlings of *L. indica* (Dark purple flower color) were obtained from a private nursery in El-Menoufia Governorate, Cairo, Egypt, on the 1st of April, in both seasons. The seedlings (15 cm tall) were transplanted into 25 cm diameter-plastic bags filled with 6 kg of the same sandy soil, and watered every 3 days. Recommended dose of chemical fertilizers were added at the rate of 5 g per pot ammonium nitrate (33.5% N), calcium superphosphate (15.5% P_2O_2) and potassium sulphate (48-50% K_2O) for plant maintenance.

***Moringa* leaves extract (MLE):** The fresh young *Moringa oleifera* leaves were air dried, ground and extracted according to Price (2007). Supernatant was diluted 30 times with water and used as a foliar spray (Table 1).

Seaweed extract (SW): Seaweed extract (powerful) was obtained from Cairochem for Agricultural Services Company, Alexandria, Egypt. The extract contains minerals, vitamins, enzymes, amino acids, sugars, and plant hormones (*i.e.*, auxins, cytokinins and gibberellins) (Table 2).

Humic acid extract (HA): Natural humic substances (Table 3) obtained from Soil, Water and Environment Research Institute, Agriculture Research Center (A.R.C).

Hoagland nutrient solution (HNS): Full strength Hoagland nutrient solution was prepared with tap water (Hoagland and Arnon, 1950), and the pH of the solution was monitored several times and maintained within a range of 6.0-6.5, which obtained from El-Gomhorya Company, Egypt, (Table, 4).

Treatments: After 15 days transplanting, the seedlings were subjected to following different levels of saline water irrigation: non saline water (the control; 0.5 dS/m)**,** saline water 3.12, 6.25 and 9.37 dS/m. Sea salt solutions for irrigation were prepared by adding the required amount of sea salt to tap water per liter, which is a mixture of synthetic seawater salt obtained from Sigma Company. Seedlings were irrigated with gradual increasing sea salt concentration weekly up to reaching the desired salinity levels of the experiment mentioned above. To maintain the required soil medium salt level the EC of the soil medium was measured periodically by portable EC meter.

Table 1. Chemical composition of *M. oleifera* leaves extract

Constituent	Content mg g^{-1} DW
K	29.00
P	6.15
Ca	9.02
Mg	6.04
Fe	1.90
Zn	0.49
Soluble phenols	2.13
Ascorbic acid	3.48
Amino acids	125.20
Proline	26.19
Total soluble sugars	157.80
Phytohormones	Content (µg g^{-1} DW)
Indole-3-acetic acid	0.91
Gibberellins	0.81
Zeatin	0.95
Abscisic acid	0.25

Table 2.Chemical composition of seaweed extract (powerful)

Constituent	Content (%)
Ascophyllum nodosum	75.00
N	1.50
P_2O_5	1.00
K_2O	1.50
S	1.60
Mg	1.60
Ca	1.60
Se	0.02
Mannitol	2
Topolin (6-[3-hydroxy benzyl-amino] purine)	0.01
O.M.	25
Free amino acid	10
Alginic acid	9

Seedlings grown under normal or saline conditions were sprayed four times at 30 day intervals at a rate of 5 mL/ pot, starting on 15th May, in both seasons, with each of the following: MLE (1:30), seaweed (2%), HA (10 %), full strength Hoagland nutrient solution as a chemical fertilization and distilled water only as a control. The spraying was done manually using a spraying bottle, on both sides of the leaves evenly and, it was carried out between 09:00 and 11:00 AM. The concentrations of MLE, HA and seaweed sprays were based on results from a preliminary pot trial (data not shown). After 125 days of treatments, the plants were harvested, in both seasons, measured and analyzed. The experiment was arranged as factorial including Randomized Complete Block Design with 20 treatments which were the

Table 3. Composition of natural humic acid

Chemical	Content (%)
Total Nitrogen (N)	1.40
Phosphorus (P)	0.76
Potassium (K)	2.80
Calcium (Ca)	1.00
Magnesium (Mg)	0.50
Sulfur (S)	1.30
Iron (Fe)	1.39
Boron (B)	0.07

Table 4. Composition of Hoagland nutrient solution

Chemical	Concentration
$CaNO_3$	4.0 mM
$MgSO_4$	2.0 mM
KNO_3	4.0 mM
$(NH_4)_2SO_4$	0.4 mM
$MnSO_4$	2.0 µM
$CuSO_4$	0.3 µM
$ZnSO_4$	0.8 µM
NaCl	30.0 µM
Na_2Mo_4	0.1 µM
KH_2PO_4	1.43 µM
H_3BO_3	10.0 µM
Fe-Na-EDTA	20.0 µM

combinations of four salinity levels (0, 3.12, 6.25 and 9.37 dS/m) and five foliar applications (MLE, seaweed, HA, Hoagland nutrient solution and the control). Each treatment was replicated six times with one seedling per unit.

Growth characteristics: After 60 days from sowing, plants from each replicate were harvested, and the following characters were recorded; plant height (cm), stem diameter (cm), number of leaves/plant, number of branches/plant, root length (cm), and total dry weight of the whole plants (roots, shoots and flowers) (g).

Floral characteristics: Number of days to flowering (day), inflorescence number /plant and, inflorescence diameter (cm) were recorded.

Chemical analysis

Photosynthetic pigments and total carbohydrates: Total chlorophylls and carotenoid contents (mg/g fresh weight) were measured by Spectrophotometer and calculated according to equation described by Moran (1982). The total carbohydrate percentage was determined according to the method of DuBois *et al.* (1956).

Proline content and total soluble phenols (TSP): The proline concentration was determined using fresh material according to Bates *et al.* (1973). In ethanol extract, total soluble phenols (mg/g f.w.) was estimated using the Folin-Ciocalteau calorimetric method (Swain and Hillis, 1959).

Macro-nutrient: Dried leaf samples were digested as described by Piper (1950) and the extract was analyzed to determine: nitrogen % by the modified micro Kieldahl method as described by Pregl (1945), phosphorus % as in Jackson (1967), potassium and sodium were determined according to the method described by (AL-Khayri, 2002) using a flame spectrophotometer, and Ca % was determined by atomic absorption (Allen *et al.*, 1984).

Antioxidant enzymes determination: Antioxidant enzymes extraction were carried out using fresh leaf tissues at 40 °C in a buffer solution (3: 1 buffer: fresh weight v/v) in a pastel. It was mortared with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 g for 30 min and then the supernatants were stored in separate aliquots at 8 °C (Vitoria *et al*., 2001). Antioxidant enzymes were assayed as follows; catalase (CAT) by measuring the decrease in absorbance due to disappearance of H_2O_2 at 240 nm according to Chance and Maely (1955), superoxide dismutase (SOD) by photochemical method as described by Giannopolitis and Ries (1977) and, Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined as suggested by Rao *et al.* (1996) and the optical density at 290 nm. Enzymes activities were expressed as units/min/mg protein.

Statistical analysis: The obtained results were subjected to statistical analysis of variance and the means were compared using the least significant difference (L.S.D.) test at the 5% level, as described by Little and Hills (1978).

Results and discussions

Growth characteristics: Growth characters [plant height, stem diameter, number of leaves/ plant, number of branches/ plant, root length, and total dry weight of the whole plant] of *L. indica* under different sea salt concentrations without any foliar application were reduced (Table 5). Total dry biomass of crape myrtle plants treated with sea salt solutions of 6.25 dS/m and 9.37 dS/m decreased to 27.93 and 23.14 g/plant, in both seasons, respectively, in comparison with non-saline (37.07 g/plant) in both seasons.

The reduction in growth characteristics under sea salt stress conditions may be due to ion toxicity and low water potential which may cause one or more of the following; water deficit, imbalance in absorption, uptake and transport of essential nutrients, turgor pressure reduction in expanding tissues, reduction in photosystem activity in leaf cells, reduced ability to produce and utilize assimilates to the growing regions, and direct effects of accumulated salts on metabolic steps in dividing and expanding cells (Tester and Davenport, 2003; Munns *et al*., 2006).

A significant increase in growth characteristics was observed in plants sprayed with full strength Hogland nutrient solution (HNS), seaweed (SW, 2%) or humic acid (HA, 10%), while the foliar spray of *Moringa* leaves extract (MLE) had significant effect, irrespective of growth characteristics under non saline (control) or different sea salt concentrations, which the sea salt concentration at 9.37 dS/m recorded the highest total dry weight (23.30 g/plant) compared to control (10.67g/plant) in the 1st season, while in the 2nd season (25 g/plant) compared to the control (12.74 g/plant). The effect of foliar application by MLE to improve the plant tolerance to abiotic stresses, including salinity

Table 5. Growth characteristics of *L indica* affected by exogenous foliar applications under different non-saline and sea salt concentrations during two seasons, 2014 (1st) and 2015 (2nd).

Treatments		Plant height (cm)		Stem diameter (cm)		Number of leaves/ plant		Number of branches/ plant		Root length (cm)		Total dry weight (g)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
S0	C	70.33	72.00	1.07	1.30	17.33	19.67	5.00	7.33	46.67	50.00	17.07	20.00
	HNS	84.33	85.67	1.77	1.93	27.67	28.33	11.67	14.33	56.67	59.67	28.03	28.73
	MLE	91.00	96.33	2.77	3.03	31.33	33.00	17.33	18.33	65.00	70.00	31.27	33.13
	SE	84.67	86.67	1.93	2.13	29.67	30.67	14.67	16.33	61.67	65.67	28.53	29.63
	HA	83.33	84.33	1.73	1.87	27.33	28.00	10.33	11.67	55.00	57.33	28.07	28.07
S1	C	68.33	69.67	0.97	1.23	15.67	18.33	4.33	6.00	44.00	48.33	16.23	18.63
	HNS	83.00	84.00	1.60	1.73	24.67	25.33	9.00	9.67	54.33	58.33	24.23	24.70
	MLE	90.00	95.00	2.53	2.73	29.33	31.67	16.00	17.33	62.33	67.67	29.70	31.13
	SE	83.33	85.33	1.70	2.07	27.00	29.67	14.00	14.33	58.33	63.00	27.53	29.27
	HA	81.67	82.33	1.57	1.87	25.00	26.33	9.33	10.33	54.00	59.00	25.47	26.23
S2	C	63.33	66.67	0.83	1.03	12.67	16.00	3.67	5.00	38.00	41.67	12.50	15.43
	HNS	77.67	80.00	1.07	1.20	16.33	22.33	8.00	8.67	47.33	51.67	21.23	22.60
	MLE	84.67	86.33	1.63	1.73	23.67	28.00	14.00	16.00	56.00	60.00	26.27	27.23
	SE	81.00	83.00	1.43	1.47	20.67	26.33	11.33	12.67	52.00	57.00	24.67	24.77
	HA	79.67	81.00	1.13	1.30	19.33	24.33	9.00	9.33	50.00	52.67	22.17	23.83
S3	C	51.67	58.00	0.57	0.83	7.67	11.67	3.00	4.67	32.67	36.67	10.67	12.47
	HNS	61.33	62.67	0.87	1.00	11.67	15.00	7.00	7.67	41.67	43.00	17.10	17.77
	MLE	77.33	79.67	1.30	1.47	17.00	22.67	11.67	12.67	51.00	54.00	23.30	25.00
	SE	74.67	75.67	1.10	1.17	14.67	19.67	10.67	11.00	47.67	49.33	21.40	21.97
	HA	71.67	73.33	0.97	1.07	12.33	16.67	8.00	8.67	43.00	45.00	17.97	18.77
LSD (*P*=0.05)		2.74	3.61	0.20	0.23	1.40	1.92	1.08	1.01	3.84	3.08	1.36	1.67
		2.06	1.60	0.15	0.14	1.73	1.85	1.04	0.96	2.49	2.82	1.11	1.24
		4.12	3.19	0.31	0.29	3.47	3.71	2.09	1.93	4.99	5.64	2.23	2.49

S0=control, S1= 3.12 dS/m, S2= 6.25 dS/m, S3= 9.37 dS/m.
C= foliar with distal water as control, HNS= foliar with Hoagland nutrient solution, MLE= foliar with *Moring* leaves extract, SE= foliar with seaweed, HA= foliar with humic acid.

has been reported by many researchers (Howladar, 2014; Rady and Mohamed, 2015).

The reason for growth acceleration of *L. indica* plants sprayed by *Moringa* leaves extract (MLE) under normal or saline conditions might be due to the presence of essential macro- and micro-nutrients such as Ca, K, and Zn, and enriched content of MLE of phytohormones (indole-3-acetic acid, gibberellins and zeatin as a cytokinin) in MLE that favored rapid division, multiplication and enlargement of plant cells, which encouraged plant growth and productivity under salt stress conditions. It is also rich in crude proteins which are essential for the protoplasm formation (Moyo *et al.*, 2011; Afzal *et al.*, 2012). In addition, Azra *et al.* (2012) and Rehman *et al.* (2014) concluded that MLE applications maintained optimum tissue water status and membranes stabilities, enhanced antioxidant levels and activated plant defense system, increased levels of plant secondary metabolites, reduced uptake of undesirable Na^+ and/or Cl^-, and enhanced leaf K^+. All these events lead to vigorous seedling growth, maximizing the crop performance.

Floral characteristics: Salt stress significantly affected the floral characteristics (number of days to flowering, inflorescence number /plant and, inflorescence diameter) of *L. indica* plants (Fig. 1). Increasing sea salt concentrations to 9.37 dS/m increased the number of days to flowering to 126 and 121 days, in the 1st and 2nd seasons, respectively, as compared with the non-saline treatment (control) (95 and 90 days, in the same order), while, it decreased the number of flowers/plant to 3 flowers/plant and an inflorescence diameter of 2.75 cm, in both seasons. Meanwhile, the number of flowers/plant and inflorescence diameter significantly increased in non-saline (control) to 9 flowers/plant and 4.15 cm, respectively, in both seasons. These results were similar to those of Nofal *et al.* (2015) and Shanan (2015), who indicated that value of flowering characteristics significantly decreased in *Calendula officinalis* and *Matthiola incana* plants with increasing salt concentrations. This reduction in value of flowering characteristics may be due to the effect of salinity which inhibits photosynthesis of plants via changes of chlorophyll contents and components and damage of the photosynthetic apparatus. It also inhibits the photochemical activities and decreases the activities of enzymes (Mazhar *et al.*, 2012).

As shown in (Fig. 1) *L. indica* plants sprayed with the *Moringa* leaves extract recorded the significantly highest inflorescence number /plant (15 flowers/plant) and, inflorescence diameter (7.02 cm) and decreased the number of days to flowering (77.5 days) in both seasons, followed by plants sprayed with seaweed extract as compared with humic acid and Hoagland nutrient solution or control treatments under non-saline (control) and also, in saline conditions which the highest sea salt concentration (9.37 dS/m) recorded highest significant inflorescence number / plant (8.50 flowers/plant) and, inflorescence diameter (5.08 cm) and decreased the number of days to flowering (92.5 days), in both seasons. This supports the report of Culver *et al.* (2012) and Bashir *et al.* (2014) who reported that, application of the *Moringa* leaf extract after planting significantly increased number of flowers of the tomato plants as a result of its rich contents of different nutrients and, plant hormone like auxin and cytokinin.

Chemical composition

Photosynthetic pigments: Total chlorophyll and carotenoid contents under control condition were higher as compared to sea salt stressed treatments (Table 6). Total chlorophyll and

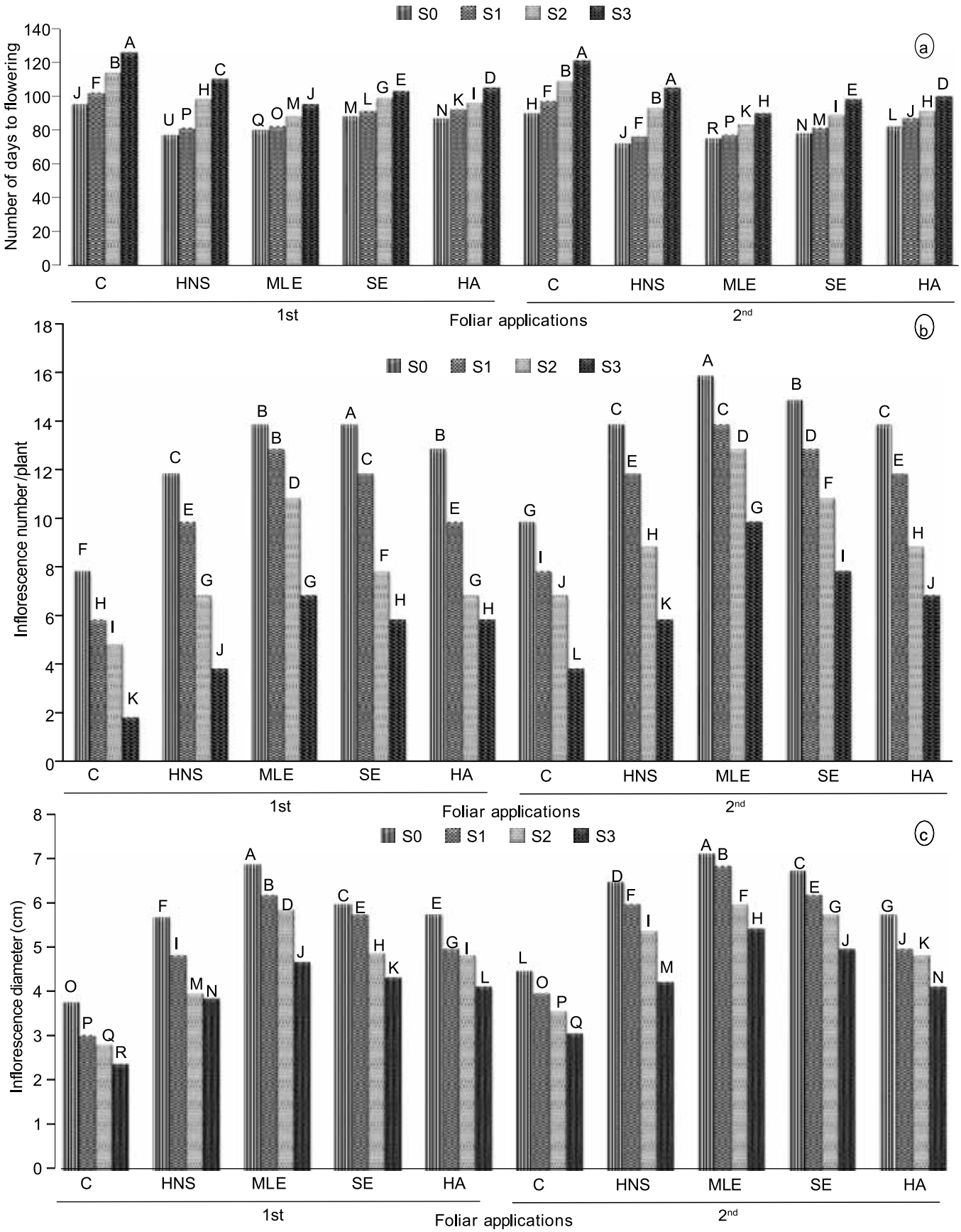

Fig. 1. Floral characters [number of days to flowering (a), inflorescence number /plant (b) and infloresence diameter (c) of *L. indica* as affected by natural exogenous foliar applications under non-saline and sea salt concentrations during two seasons, 2014 (1st) and 2015 (2nd). Columns are significantly different at P = 0.05 vertical bars represent ±SE. S0=control, S1= 3.12 dS/m, S2= 6.25 dS/m, S3= 9.37 dS/m. C= foliar with distal water as control, HNS= foliar with Hoagland nutrient solution, MLE= foliar with *Moring* leaves extract, SE= foliar with seaweed, HA= foliar with humic acid.

carotenoid contents decreased when plants were subjected to sea salt stress. However, the maximum reduction in total chlorophyll and carotenoid contents were observed at 9.37 dS/m. That reduction of photosynthesis under sea salt stress could be attributed to the formation of proteolytic enzymes such as chlorophyllase, destruction of chlorophyll molecules by ROS, decline in membrane permeability, reducing water availability and nutrients particularly magnesium (Sharma and Hall, 1992; Kalaji *et al*., 2011). Such inhibition in the photosynthesis after irrigation with sea water is also reported by Soliman *et al.* (2015) in *Moringa peregrine*.

Foliar spray of MLE led to the highest increment of total chlorophyll and carotenoid contents (2.56 and 1.29 mg/g f.w., in both seasons) in untreated plants (Table 6). However, total chlorophyll and carotenoid contents of crape myrtle plants significantly decreased with sea salt treatment at 9.37 dS/m and these increments were 1.70 and 0.86 mg/g f.w., in both seasons, as compared to non-saline (control) plants (0.79 and 0.40 mg/g f.w., in both seasons). The foliar spray MLE partially alleviates this reduction and minimized the reduction in chlorophyll contents. In addition, MLE maintained the chlorophyll in higher concentrations under salinity due to presence of zeatin-like cytokinin in MLE which prevents premature leaf senescence, induced cytokinin biosynthesis, and maintains higher leaf area for photosynthetic activity. It also contained calcium four times more than milk, potassium three times of banana and ascorbic acid seven times of orange and that led to reduce concentration of Na+ ions and increased concentrations of K^+ and Ca^{2+} ions coupled with the increased concentrations of carotenoids (Rady *et al*., 2013; Zaki and Rady, 2015). These results corroborate findings of Azra *et al.* (2013).

Total carbohydrates, proline content and total soluble phenols: It is obvious that total carbohydrates, proline content and total soluble phenols were significantly increased in relation to the increasing in the levels of applied sea salt treatments (Table 6). Total carbohydrates, proline content and total soluble phenols in leaves of *L. indica* plants reached its maximum, in both seasons, in the plants irrigated by 9.37 dS/m sea salt. Many plants under salt stress accumulated starch and soluble carbohydrates as a response to impaired carbohydrate utilization, low cell division and growth since carbohydrate is a source of energy and osmolyte so it might play an important role in increasing the internal osmotic pressure (Zidan and Al- Zahrani, 1994; Miandoab *et al*., 2015). Also, in response to salinity stress a large number of plant species accumulate proline, which may play a role in defense against salinity stress, cell osmotic adjustment, membrane stabilization and detoxification of injurious ions proline was considered as an indicator for some mechanism to resist the salinity stress (Ashraf and Foolad, 2007; Jampeetong and Brix, 2009). In addition, plants release phenolic metabolite which is induced in response to salinity and may strongly affect particularly leaf polyphenols and act as antioxidants to protect the plant against oxidative stress (Keutgen and Pawelzik, 2009; Radi *et al.*, 2013). These results are in harmony with many researches which concluded that the higher level of carbohydrate are associated with proline and total phenol (Deepika *et al.*, 2015; Mohsen *et al.*, 2015; Al- Hassan *et al.*, 2015) and might be a good strategy to increase salt tolerance.

Total carbohydrates, proline content and total soluble phenols significantly increased in salt-stressed crape myrtle plants by the exogenous applications of MLE (Table 6). This may be attributed

Table 6. Chemical compositions of *L. indica* affected by exogenous foliar applications under different sea salt concentrations during two seasons, 2014 (1st) and 2015 (2nd)

Treatments		Total chlorophyll (mg/g f.w.)		Carotenoids (mg/g f.w.)		Total carbohydrates (%)		Proline content (µ moles /g f.w.)		Total phenol content (mg/g f.w.)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
S0	C	1.48	1.50	0.75	0.77	15.00	17.67	17.00	19.33	0.52	0.55
	HNS	2.29	2.33	1.15	1.17	19.33	20.00	21.67	23.67	0.58	0.64
	MLE	2.54	2.58	1.28	1.29	25.00	28.00	26.33	28.00	0.64	0.70
	SE	2.42	2.49	1.21	1.26	22.67	24.67	25.67	27.00	0.59	0.69
	HA	2.29	2.34	1.14	1.18	20.33	22.00	24.00	26.00	0.56	0.62
S1	C	1.26	1.28	0.63	0.64	16.67	18.67	18.00	20.00	0.54	0.59
	HNS	2.11	2.16	1.06	1.08	20.00	21.00	22.33	25.00	0.58	0.66
	MLE	2.29	2.37	1.16	1.19	27.00	28.33	28.00	30.33	0.67	0.72
	SE	2.20	2.22	1.11	1.11	25.00	25.67	26.67	27.67	0.64	0.70
	HA	2.16	2.20	1.08	1.11	22.67	24.67	25.33	27.00	0.61	0.68
S2	C	1.16	1.19	0.58	0.60	21.33	25.00	21.67	24.67	0.59	0.63
	HNS	1.54	1.75	0.77	0.88	23.33	25.33	26.67	31.00	0.64	0.69
	MLE	1.96	2.18	0.99	1.08	31.67	34.00	34.67	37.33	0.72	0.78
	SE	1.83	2.10	0.92	1.05	28.33	31.33	34.00	36.00	0.71	0.75
	HA	1.70	1.91	0.86	0.96	27.33	29.00	31.33	35.00	0.65	0.72
S3	C	0.76	0.81	0.38	0.41	25.33	28.00	24.33	26.67	0.63	0.66
	HNS	1.22	1.30	0.61	0.65	28.33	32.33	31.00	32.67	0.69	0.72
	MLE	1.63	1.77	0.82	0.89	35.67	38.67	41.67	45.33	0.79	0.84
	SE	1.56	1.62	0.78	0.81	33.00	36.67	39.67	42.67	0.74	0.77
	HA	1.35	1.54	0.68	0.77	30.00	35.00	38.33	39.67	0.71	0.73
LSD (*P*=0.05)		0.06	0.06	0.03	0.03	1.71	1.16	1.90	1.83	0.03	0.04
		0.05	0.04	0.03	0.03	0.88	1.10	1.35	1.15	0.02	0.03
		0.11	0.09	0.05	0.06	1.77	2.20	2.70	2.30	0.05	0.07

S0=control, S1= 3.12 dS/m, S2= 6.25 dS/m, S3= 9.37 dS/m. C= foliar with distal water as control, HNS= foliar with Hoagland nutrient solution, MLE= foliar with *Moring* leaves extract, SE= foliar with seaweed, HA= foliar with humic acid.

Table 7. Macronutrients of *L. indica* affected by exogenous foliar applications under different sea salt concentrations during two seasons, 2014 (1st) and 2015 (2nd)

Treatments		N%		P%		K%		Na%		Ca%	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
S0	C	1.89	1.94	0.21	0.26	1.51	1.56	0.44	0.41	0.47	0.53
	HNS	2.29	2.36	0.23	0.29	1.57	1.59	0.38	0.37	0.53	0.58
	MLE	2.44	2.53	0.30	0.34	1.67	1.70	0.29	0.26	0.60	0.67
	SE	2.36	2.48	0.29	0.30	1.63	1.68	0.36	0.32	0.56	0.64
	HA	2.30	2.41	0.26	0.29	1.60	1.63	0.37	0.35	0.54	0.60
S1	C	1.82	1.85	0.17	0.22	1.48	1.54	0.52	0.47	0.70	0.78
	HNS	2.24	2.30	0.21	0.25	1.53	1.59	0.47	0.44	0.88	0.97
	MLE	2.41	2.45	0.28	0.31	1.64	1.68	0.39	0.35	0.99	1.17
	SE	2.31	2.41	0.27	0.28	1.62	1.65	0.41	0.39	0.96	1.08
	HA	2.28	2.32	0.22	0.26	1.58	1.61	0.44	0.43	0.92	1.01
S2	C	1.56	1.61	0.11	0.18	1.37	1.43	0.82	0.88	1.22	1.29
	HNS	1.60	1.72	0.16	0.19	1.46	1.51	0.80	0.76	1.51	1.57
	MLE	2.07	2.17	0.21	0.26	1.58	1.63	0.64	0.59	1.55	1.79
	SE	1.97	2.01	0.19	0.22	1.52	1.62	0.68	0.62	1.61	1.71
	HA	1.83	1.96	0.18	0.19	1.47	1.54	0.74	0.67	1.57	1.66
S3	C	1.19	1.23	0.07	0.11	1.25	1.30	1.13	1.06	1.54	1.63
	HNS	1.55	1.62	0.11	0.15	1.37	1.40	1.07	1.02	1.71	1.88
	MLE	1.94	2.05	0.17	0.20	1.42	1.50	0.96	0.90	1.95	2.03
	SE	1.87	1.95	0.15	0.19	1.44	1.47	0.99	0.96	1.91	3.00
	HA	1.79	1.84	0.13	0.16	1.40	1.45	1.04	1.00	1.55	1.90
LSD (*P*=0.05)		0.07	0.02	0.04	0.03	0.03	0.06	0.09	0.02	0.14	0.24
		0.05	0.04	0.03	0.02	0.05	0.05	0.04	0.06	0.10	0.35
		0.11	0.09	0.07	0.05	0.10	0.10	0.12	0.09	0.21	0.70

S0=control, S1= 3.12 dS/m, S2= 6.25 dS/m, S3= 9.37 dS/m. C= foliar with distal water as control, HNS= foliar with Hoagland nutrient solution, MLE= foliar with *Moring* leaves extract, SE= foliar with seaweed, HA= foliar with humic acid.

to that MLE is excellent source for minerals, soluble sugars, amino acids, some antioxidants and a high cytokinin (zeatin & zeatin riboside) which causes increases in the concentrations of proline and total soluble phenols, in turn, protect plants against the ROS generation, osmotic adjustment and membrane injury, or may result in the synthesis of other substances having a protective effect on plants grown under salt stress (Thakur and Sharma, 2005; Rady *et al*., 2015). In addition, Munoz *et al.* (2008) concluded that zeatin riboside affected mainly the mobilization of carbohydrates as well as distributed to the sink where more carbohydrates are needed to cater the needs of rapidly increasing growth. The protective role of *Moringa* leaf extract against ROS that are formed during salt stress was reported by Iqbal (2014).

Macro-nutrients: Sea salt stress had a significant effect on all macro-nutrients; N, P, K, Ca and, Na%; in both seasons. Shoot N (1.21), P (0.24) and K (1.54) % decreased significantly ($P \leq$ 0.01) as sea salt increased, in both seasons, than those of the control (1.92, 0.09 and 1.28%, in both seasons), while Na and Ca% increased with increasing sea salt concentrations. Maximum Na and Ca% was recorded at 9.37 dS/m, 1.10 and 1.59% in both seasons, as compared to control (0.43 and 0.50 %, in both seasons) (Table 7). Sea salt concentrations causing conflict in nutrient homeostasis by competing with the uptake of other essential nutrients. Also, the excessive Na+ decreases the uptake of other essential nutrients such as N, P, K^+ and Ca^{++} (Shahbaz *et al*., 2013; Parihar *et al*., 2014). These results are in harmony with those of Cornacchione and Suarez (2015) and Ferreira *et al.* (2015).

Application of MLE decreased the concentration of Na+ and increased concentration of N, P, K+ and, Ca in leaves against control, under normal as well as sea salt concentarations (Table 7). This result could be due to the important role of *Moringa* leaf extract which contain proteins, amino acids and various phenolics and zeatin, ascorbic acid, phenolic compounds, sugars, and minerals (*i.e.* calcium, magnesium, sodium, iron, phosphorus and potassium); hence, it is used as a natural plant growth enhancer (Anwar *et al*., 2007). Therefore, it has the ability to reduce the adverse effect of salinity and to protect the plant against the build-up of toxic ions by maintenance of the ionic homeostasis under salt stress and this makes N, P, K, Ca more available to the plant. These results are parallel with the finding of Abd El–Hamied and El-Amary (2015).

Enzyme activity: The activity of CAT, SOD and APX increased significantly with increasing sea salt concentrations (Fig. 2). Untreated plants (control) showed the lowest significant CAT (1.42), SOD (3.91) and, APX (2.12 units/min/mg protein), in both seasons. So increased activities of the antioxidative enzymes (CAT, SOD and, APX) in plants under salt stress to avoid the damage caused by oxidative stress that induced by the elevated levels of reactive oxygen species (ROS) such as hydrogen peroxide (Sharma *et al*., 2012). SOD constitutes the first line of defense against ROS58 by reducing the O_2- radical to H_2O_2 (Alscher *et al*., 2002). In addition, APX is considered as one of the most important enzymes in the reduction of this reactive molecule (Feierabend, 2005**)**. This was in conformity with the earlier findings of Muthulakshmi *et al.* (2015).

Moringa leaves extract had the superiority in increasing enzyme activities (CAT, SOD and APX) in sea salt stressed or unstressed plants, followed by seaweed extract, humic acid, Hogland nutrient solution which was more than the control. *Moringa* leaves extract increased enzyme activities at 9.37 dS/m by 3.14, 14.69, and 4.49, respectively, in both seasons, as compared with untreated plants under the same level of sea salt stress (1.89, 7.48, and

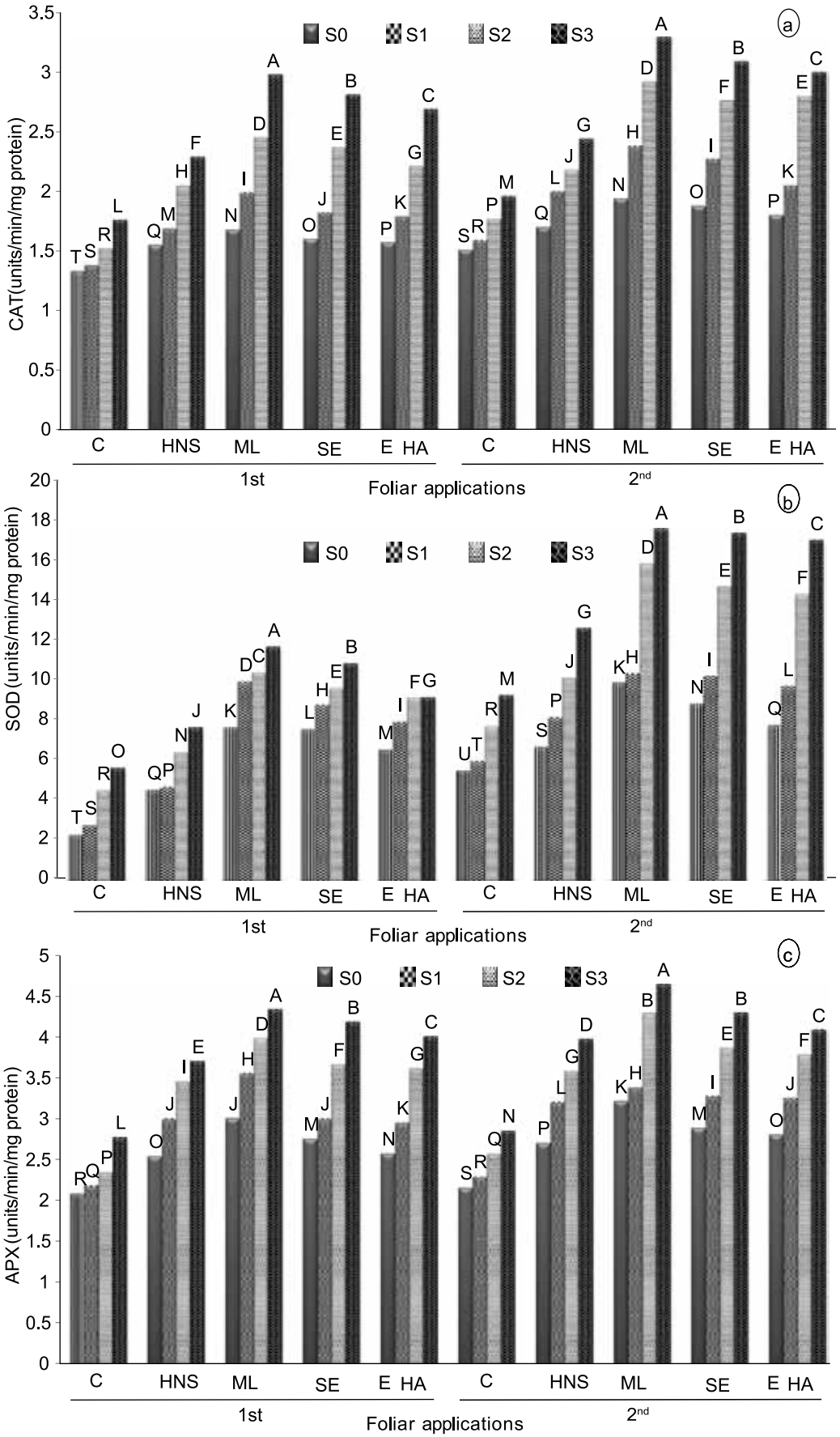

Fig. 2. Enzyme activity [CAT (a), SOD (b) and, APX (c)] of *L. indica* as affected by natural exogenous foliar applications under non-saline and sea salt concentrations during two seasons, 2014 (1st) and 2015 (2nd) Columns are significantly different at $P = 0.05$ vertical bars represent ±SE.
S0=control, S1= 3.12 dS/m, S2= 6.25 dS/m, S3= 9.37 dS/m. C= foliar with distal water as control, HNS= foliar with Hoagland nutrient solution, MLE= foliar with *Moring* leaves extract, SE= foliar with seaweed, HA= foliar with humic acid.

2.81 units/min/mg protein, respectively, in both seasons). This increase may be attributed to that *Moringa* leaves extract which contains several photochemicals, flavonoid pigments such as kaempferol, rhamnetin, isoquercitrin and kaempferitrin and, rich in zeatin and a group of the glycoside compounds, glucosinolates and isothiocyanates, which may enhance the formation of the photochemical compounds and that led to increase in the antioxidant activity. Moreover, to control the ROS production, repair and mitigate damage initiated by reactive oxygen, plants develop a complex antioxidant system enabling survival under abiotic stress, so MLE is proved to be the most effective PGR in reducing plant exposure to certain stress (Rajanandh *et al.*, 2012). Several studies have indicated such results (Azra *et al.*, 2013; Aslam *et al.*, 2015).

This study indicated that *Moringa* leaves extract (MLE) applied to *L. indica* plants at different concentrations of sea salt improved vegetative growth, floral characters and chemical compositions which enhanced the tolerance of sea salt stress by increasing the proline, total phenolic contents and activation of the antioxidant enzyme system of SOD, CAT and APX. *Moringa* leaves extract can be used as a possible supplement or substitute to inorganic or chemical fertilizer as growth enhancer and under different sea salt concentrations. Therefore, MLE can be recommend to bes used commercially to be used as environment friendly and economically cheap fertilizer especially in arid and semi-arid regions.

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