Plant Archives Vol. 20, Supplement 1, 2020 pp. 3599-3605 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ENZYMES ACTIVITY OF MICROPRAPAGATED *ANTIGONON LEPTOPUS* PLANT UNDER EFFECT OF SALINITY STRESS

Rasha A. El-Ziat[1]; Iman M.El-Sayed[2*];Lobna S. Taha[2] and Eman A. Ibrahim[3]
[1]Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
[2]Department of ornamental plants and woody trees, Agricultural and biological Research Division, National Research Centre (NRC), Egypt.
[3]Department of Plant Biochemistry, National Research Centre, 33 El Bohouth St. (former El Tahrirst.), Dokki, Giza, Egypt.
Corresponding author: imanelsayed6065@yahoo.com

Abstract

The micropropagation ability of *Antigonon leptopus* was investigated under different concentrations of NaCl (0, 1, 2, 4 and 8 g/L). Two levels of Iron Oxid Nanoparticles (5 and 10 mg/L) was examined to mitigate the effect of salt stress on micropropagated plants. The results showed that NaCl at different concentrations significantly reduced *in vitro* recorded shoots and roots parameters (survival%, number of shootlets, shootlet length, number of leaves, rooting%, number and length of roots). Moreover, proline content and enzymes (GSH, SOD and Catalase) were increased with increasing NaCl concentration in the culture medium. In addition, adding Iron Oxide NPs to the culture medium showed positive effect for alleviating salt stress on the micropropagated plants. Undertaking there are no reports on the *in vitro* growth behavior of this important medical plant under salinity stress. So, the aim of this study was to evaluate the adverse effect of salt stress on *Antigonon leptopus* plants and the possibility of using of Iron oxide nanoparticles to overcome these effects.
***Keywords*: *Antigonon leptopus,* micropropagation, salt stress, nanoparticles, *in vitro*.**

Introduction

Nowadays, nanoparticles are used in many sections, such as medicine, agriculture, and industry. Nanoparticles cause many morphological and physiological changes in plants depending on the properties of nanoparticles (Siddiqui *et al*., 2015). Synthesis of Nanoparticale (NPs) has gained an emerging interest as a result of their superior properties and potential application in almost every field (Shaban, 2012) such as catalysis (Shaban, 2012 and Ibrahim *et al*., 2018), magnetic (Ali *et al*., 2017), antimicrobial activity (El-Alfy *et al*., 2015).

Salinity is one of a wide range of environmental stresses which limit plant growth and productivity (Parida and Das, 2005). Salt stress of soil or water in arid and semi-arid regions is one of the major stresses (Parvaiz and Satyawati 2008). High concentrations of salt affect plants in several ways, such as water stress, ion toxicity, nutritional disorders, oxidative stress, and alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion and genotoxicity which reduce plant growth, development and survival (Carrillo *et al*., 2011).

Corallita (*Antigonon leptopus*) is a perennial plant belongs to family Polygonaceae, native to Mexico (Rajput *et al*., 2015). Corallita is an ornamental showy flowers and evergreen climber vine that hold via tendrils (Burke and DiTommaso, 2011). The aerial portion is used for cough and throat constriction (Vanisree *et al*., 2008). In Thailand, the Leaves and pink flowers are often eaten as cooked vegetables. A hot herbal tea prepared of *Antigonon leptopus* is made from the leaves and blossoms (Lim, 2013). The sexual propagation of *Antigonon leptopus* gave a small quantity of seeds (Ghareeb and Taha, 2018).

Iron oxide nanoparticles used for a variety of applications, such as soil groundwater treatments and photocatalytic reactions, Iron nanomaterial have been shown to promote plants to reduce the adverse effect of salt stress (Bombin *et al*., 2015).

Undertaking there are no reports on the growth behavior of this important medical plant under salinity stress, the aim of this study was to evaluate the adverse effect of salt stress on *Antigonon leptopus* plants and investigate the possibility of using Iron oxide nanoparticles to overcome these effects.

Materials and Methods

These investigations were conducted at Tissue Culture Technique Lab., Central laboratories, Department of Ornamental Plants and Woody Trees, National Research Centre (NRC), Department of Plant Biochemistry, National Research Center (NRC), and Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during years 2018-2019 to evaluate some morphological, chemical changes of *Antigonon leptopus* plants cultured *in vitro* under the effect of salt stress.

Explant source and surface sterilization

Stem nodal explants of *Antigonon liptopus* were collected from the unique climbing tree grown at Zohrya Botanical Garden, Zamalek, Cairo, Egypt, washed and sterilized in ethanol 70% (v/v) for 30 seconds. After that, the explants were immersed in 15% of sodium hypochlorite (Clorox) for 7 minutes then 1% of $HgCl_2$ (MC) solution (w/v) for 10 minutes and rinsed three times in sterile water.

Culture medium

After surface sterilization, nodal *Antigonon leptopus* explants (two nodes) were cultured for one month on MS free of hormones (Murashige and Skoog, 1962) supplemented with 0.2 ppm of 6- benzylamino-purine (BAP) and 0.1ppm indole butyric acid (IBA), 2.5% sucrose and 0.7% agar. The pH of the medium was adjusted to 5.6-5.8 then autoclaved at

121°C and 15 psi for 15 minutes. The *in vitro* obtained shootlets were used as explant source for two experiments:

First experiment: Various concentrations (0, 1, 2, 4 and 8 g/L) of NaCl were examined under *in vitro* conditions.

Second experiment: Testing two Iron oxide nanoparticles (5.0 and 10 mg/l) to alleviate salt stress on the micropropgated as following:

1-Control (MS free of salt)

2- NaCl (1 g/L) +5 mg/L Fe NPs

3- NaCl (1 g/L) +10 mg/L Fe NPs

4- NaCl (2g/L) +5 mg/L Fe NPs

5- NaCl (2 g/L) +10 mg/L Fe NPs

6- NaCl (4 g/L) +5 mg/L Fe NPs

7- NaCl (4 g/L) +10 mg/L Fe NPs

8- NaCl (8 g/L) +5 mg/L Fe NPs

9- NaCl (8 g/L) +10 mg/L Fe NPs

The specification of used Nanoparticles in the present experiment was indicated in Table (1) and Fig. (1)

Table 1 : Iron Oxide NPs specification

Specification	Test method	
Phase	hematite	XRD
Particle size	< 50 nm	TEM
Surface area	> 50m^2/gm	BET (P/Po: up to 0.35)

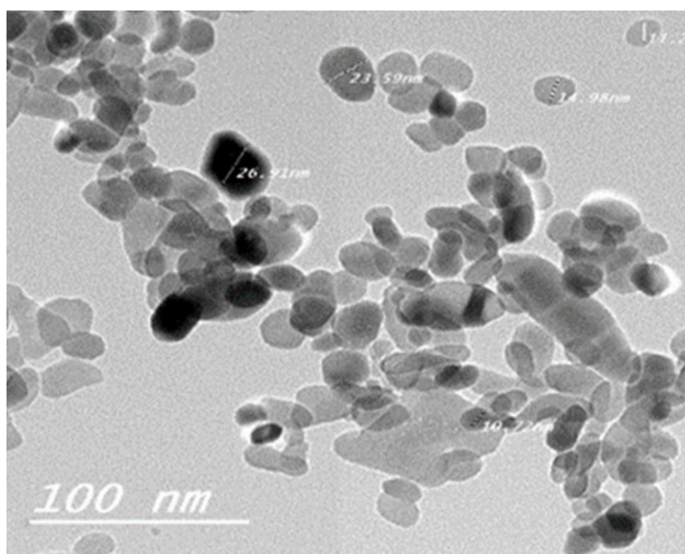

Fig. 1 : Scanning electron microscopy image of Iron oxide NPs

Culture conditions:

Cultures were incubated in growth chamber at 24 ± 1°C under white cool florescent lamps with light intensity of 3k lux at 16 hr photoperiod.

The culture period for each experiment (first and second) took two months after start of culture then, the following data were recorded:

Shooting behavior: Survival %, number of formed shootlets per explant, shootlet length (mm) and number of leaves per shootlet.

Rooting behavior: Percentage of roots formation (%), number of roots/shootlet and root length (mm).

Extraction and chemical analysis

1. Photosynthetic pigments

Photosynthetic pigments (chlorophylls a and b) as well as carotenoids were determined in shootlets tissues as mg/100g fresh weight using spectrophotometer, according to the procedure achieved by Saric *et al.*, 1967.

2. Proline

The proline content in dry leaves (mg/g F.W.) was determined using the method described by Bates *et al.*, 1973.

3. Antioxidant enzymes extraction

Fresh plant material (0.1g)was homogenized in 5 ml of ice-cold buffer phosphate (pH7.4).The homogenate was centrifuged at 10,000 rpm for 30 min and supernatant was collected. The resulting supernatant was used for determination of enzyme activities.

(a) Superoxide dismutase (SOD)

It is measured by (Marklund and Marklund, 1974) method. The degree of inhibition of autoxidation of Pyrogallol at alkaline pH by SOD was used as a measure of the enzyme activity. The buffer (2 ml) containing DEAPAC, Pyrogallol (0.5 ml of 2mM) and water (1.5 ml)were mixed for autoxidation.Initially,1-3 min interval needed for autoxidation of Pyrogallol. To give a final volume of 4 ml for the assay mixture of the enzyme, water was added to 2 ml of 0.05 MT Tris-HCl buffer, 0.5 ml pyrogallol, aliquots of the homogenate. The absorbance at 420 nm after addition of pyrogallol was inhibited by the presence of SOD.

(b) Glutathione peroxidase (GSP)

Glutathione peroxidase was estimated by the method of (Paglia and Valentine, 1967). The homogenate of tissue (0.2 ml) was added with the reaction mixture content (0.2 ml of 0.8 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of reduced glutathione, and 0.4 ml of 0.4 M) and was incubated at 37.8 °C for 10 min. The reaction was arrested by the addition of 0.5 ml of 10 % TCA and the tubes were centrifuged at 2000 rpm. The supernatant were mixed with disodium hydrogen phosphate (3.0 ml of 0.3 mM) and DTNB (1.0 ml of 0.04 %). The absorbance of samples was measured at 412 nm.

(c) Glutathione reductase (GSR)

The reduced glutathione content of supernatant was determined by the method of (Beutler and Kelly, 1983). The absorbance was spectrophotometrically recorded at 412 nm.

Statistical analysis

The data were analyzed as described by Snedecor and Cochran (1980) using a randomized complete block design with 3 replicates per treatments. Means of all characters were compared using L.S.D test at 0.05% level.

Results and Discussion

1. *In vitro* shooting behavior under effect of NaCl

The results in Table (2) showed that NaCl concentration had inversely proportional with the shoot parameters of *in vitro* propagated *Antigonon leptopus* plants. Subsequently, the lowest NaCl concentration in culture medium gave the

best values. It is recorded that, the high concentration of NaCl (8 g/L) in culture medium decreased the survival percentage to 77.70%, number of shootlets (4.00), shootlet length (36.25 mm), number of leaves (19.01) as compared to other salt levels (1, 2 and 4 g/l) and control. Similar results were found by (Shivanna *et al.*, 2013) on *Azadirachta indica* who found that increasing salinity up to 150 and 200 µM increased shoot growth. Also, (Teixeira and Retired, 2015) observed that NaCl concentrations ranging from 5 µM to 200 µM showed reduced of explant survival, fresh weight and dry weight on hybrid Cymbidium.

Table 2: *In vitro* shooting behaviors under effect of various concentrations of NaCl.

Characters / Treatment	Survival%	Number of shootlets/ explant	Shootlet length (mm)	Number of leaves /shootlet
Control	100	11.67	30.53	45.00
NaCl at 1g/L	100	10.00	53.80	39.50
NaCl at 2 g/L	100	8.66	44.43	31.67
NaCl at 4 g/L	88.80	7.16	39.24	39.00
NaCl at 8 g/L	77.70	4.00	36.25	19.01
$LSD_{0.05}$	9.73	2.38	5.89	5.18

2. *In vitro* rooting ability under effect of NaCl

Data in Table (3) demonstrated the effect of NaCl concentrations on rooting parameters of *Antigonon leptopus* formed *in vitro*. Variance analysis showed that, the lowest NaCl concentration (1 g/L) gave the best values of root parameters such as rooting percent (66.66%), number of roots (5.50) and length of roots (21.20 mm). On the other hand, NaCl at 8 g/L caused none rooting of forming shoots. Similarly, the reduction of root growth as a result of high salt concentration in culture medium was explained by Khenifi (Khenifi *et al.*, 2011), they suggested that the osmotic potential of the media was decreased by the addition of NaCl to the culture media so, inducing salinity stress affected the shoots and roots growth of *Antigonon leptopus* plants.

Table 3: *In vitro* rooting ability under effect of various concentrations of NaCl.

Characters / Treatment	Rooting%	Number of roots	Root length(mm)
Control	33.33	3.00	16.60
NaCl at 1g/L	66.66	5.50	21.20
NaCl at 2 g/L	44.44	1.30	18.80
NaCl at 4 g/L	33.3	1.00	10.50
NaCl at 8 g/L	---	---	---
$LSD_{0.05}$	4.40	1.60	5.30

3. Effect of NaCl on proline content

The results of the proline content in leaf samples that were taken from plantlets receiving different NaCl concentrations (Fig. 2) showed that, with increasing NaCl in culture medium, the proline content was generally increased. Accordingly, the highest salt concentration (8 g/l) had the highest proline content (0.64 mg/g F.w.). On the other hand, control plants and those were cultured on the lowest NaCl concentration had the lowest proline content (0.23 and 0.43 mg/g F.W., respectively). These results agreed with the results that were found (Ayala-Astorga G.I. and Alcaraz-Melendez, 2010 and Ayala-Astorga *et al.*, 2009) on *Paulownia imperialis*, (El-Sayed *et al.*, 2019) mentioned that proline content was increased with increasing salinity concentration in the culture medium on *Moringa oleifera* L. The considerable enhancement of proline accumulation in plants using high salt concentration in culture medium may lead to the conclusion that proline plays a role in plants tolerance to salinity. This role was explained by Greenway and Munna (Greenway and Munnus, 1980), who mentioned that proline can be considered as a stabilizer of osmotic pressure within the cell. Also, (Marcum and Murdoch, 1994) concluded that proline can make a substantial contribution to cytoplasmic osmotic adjustment.

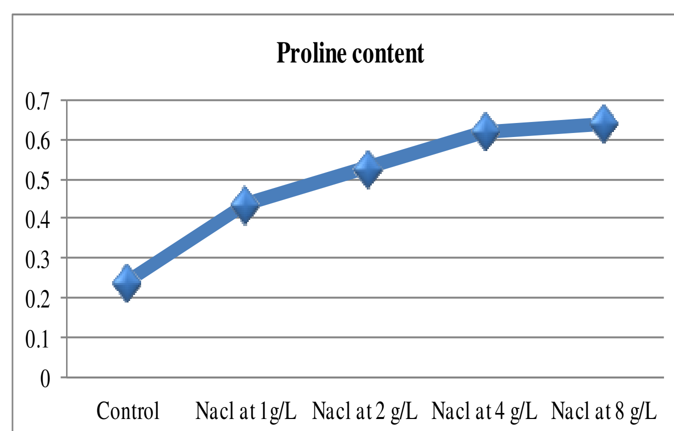

Fig. 2 : Effect of various concentrations of NaCl levels on proline content.

4. Effect of iron oxide NPs on alleviation of salt stress on shooting ability

The data in Table (4) observed that, control plant had the highest value of *in vitro* shoot parameters (survival 100%, number of shootlets/explant (11.6) and leaves/shootlet (45.00). Moreover, the iron oxide NPs decreased the effect of

salt stress on *in vitro* shooting ability of *Antigonon leptopus* plants. The data showed that, using iron oxide NPs at 10 mg/L with NaCl at 1g/L gave a higher value of survival (100%), number of shootlets/explant (10.19), shootlet length (45.20 mm), and number of leaves/shootlet (39.50) than using NaCl at 1 g/L only. On the other hand, using iron oxide NPs at high concentration (10 mg/L) with high salinity stress was favored than low concentration (5 mg/L) of iron oxide (survival 100 and 100 %, number of shootlets/explant (4.60 and 4.09), shootlet length (37.00) and (34.33 mm), and leaves/shootlet 19.30 and 18.00, respectively). These results agreed with those obtained by (Soliman *et al*., 2015) on *Moringa peregrina* which found that spraying Moringa plants with ZnO and Fe_2O_4 NPs increased growth parameters under salt stress.

5. Effect of salt stress and iron oxide NPs on rooting ability

Data in Table (5) showed that, all roots parameters (rooting %, number of roots/plantlet, and length of roots (mm)) were significantly affected by different concentrations of NaCl and iron oxide NPs in MS culture medium. These parameters were decreased gradually under salt stress levels as compared to control plants. Moreover, the application of iron oxide caused significant increase in root parameters. It is clearly noticed with the highest concentration of NaCl (8 g/L) and iron oxide NPs at 10 mg/L increased rooting by (11.10%), number of roots/plantlet (0.65), and length of roots (7.00 mm). These results agreed with (Soliman *et al*., 2015) on *Moringa peregrina* and (El-Kereti *et al*., 2013) on sweet basil plant.

6. Effect of salt stress and iron oxide NPs on proline content

Fig. (3) illustrated that proline content was increased gradually with increasing NaCl concentrations. The plantlets, which were treated with NaCl at 1 g/L in culture media, gave the lowest content of proline, but those which were treated with 8 g/L gave the highest value of proline. The application of iron oxide NPs (5 and 10 mg/L) gave promotion effect on alleviating salt stress with most concentrations of NaCl on the *in vitro* grown plants. Using 10 mg/L iron oxide was more effective than 5mg/L. Plants treated with 8g/L NaCl with 5 mg/L iron oxide NPs in culture media gave the highest proline content (1.27 mg/g F.W.). On the other hand, plants treated by 8 g/L NaCl with 10 mg/L iron oxide NPs gave proline content with less amount than that of the plantlets treated with 8 g/L of NaCl and 5 mg/L iron oxide NPs with a mean value (0.88 mg/g F.W). The data were confirmed by the same results were obtained by (Rezvani *et al*., 2012) on saffron plants.

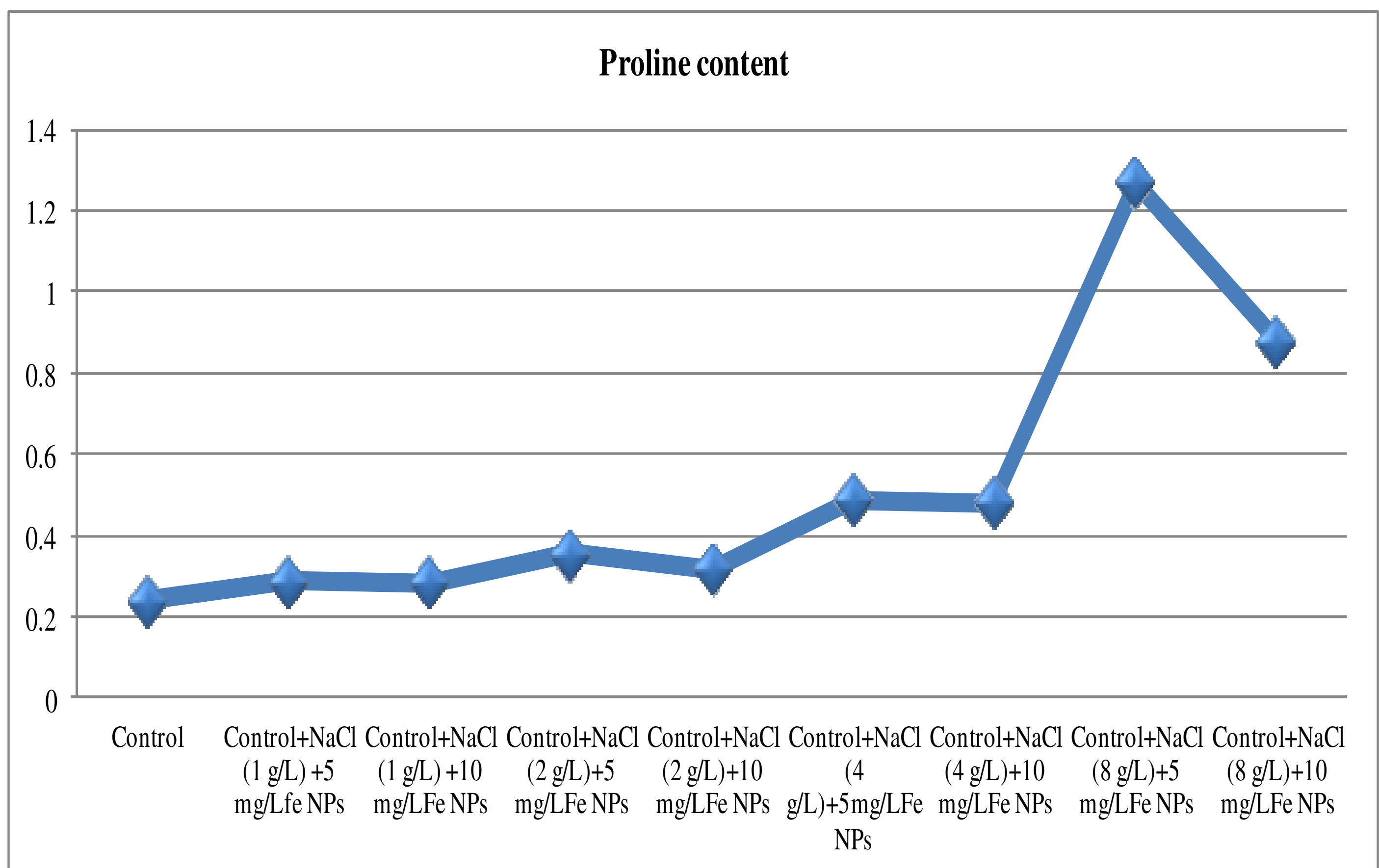

Fig. 3 : Proline content under effect of various concentrations of NaCl and iron oxide NPs.

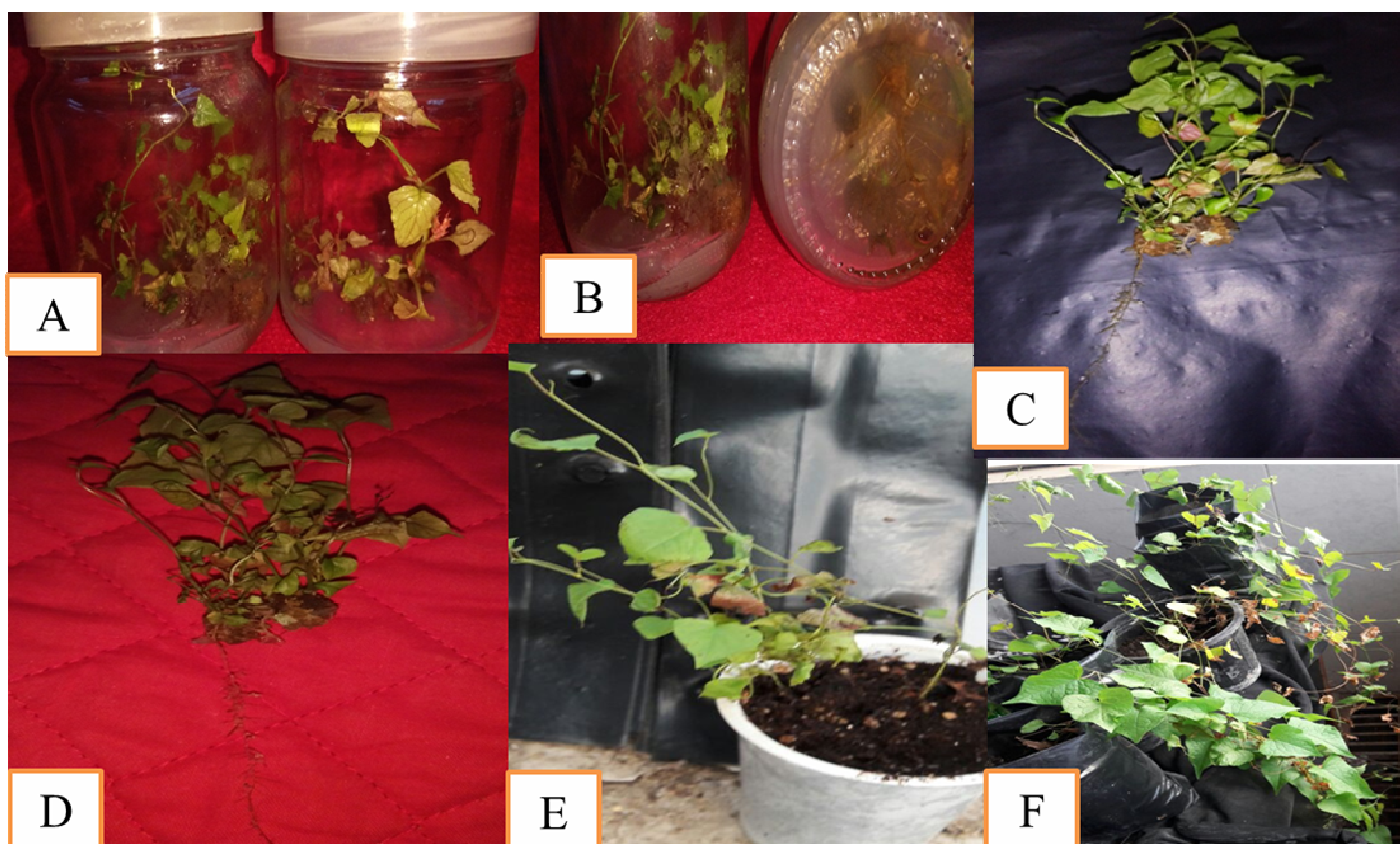

Fig. 4: Micropropagation of *Antigonon leptopus* A: *In vitro* culture establishment stage, B: Rooting of *in vitro* microshoots and C- F: *Ex vitro* acclimatization stage.

Table 4: *In vitro* shooting behavior under effect of various concentrations of NaCl and iron oxide NPs.

Treatment \ Characters	Number of shootlets/ explant	Shootlet length (mm)	Number of leaves /shootlet
Control	11.60	36.60	45.00
NaCl (1 g/L) + 5 mg/L Fe NPs	9.96	43.80	37.00
NaCl (1g/L) +10 mg/L Fe NPs	10.19	45.20	39.50
NaCl (2 g/L) +5 mg/L Fe NPs	7.78	42.20	36.00
NaCl (2 g/L) +10 mg/L Fe NPs	9.30	44.40	36.30
NaCl (4 g/L) +5 mg/L Fe NPs	6.69	40.80	20.00
NaCl (4 g/L) +10 mg/L Fe NPs	7.39	41.02	23.00
NaCl (8 g/L) +5 mg/L Fe NPs	4.09	34.33	18.00
NaCl (8 g/L) +10 mg/L Fe NPs	4.60	37.00	19.30
$LSD_{0.05}$	0.15	0.31	0.49

Table 5: *In vitro* rooting behavior under effect of various concentrations of NaCl and iron oxide NPs.

Treatments \ Characters	Rooting%	Number of roots/plantlet	Root length (mm)
Control	33.33	3.00	16.60
NaCl (1 g/L) + 5 mg/L Fe NPs	66.66	4.00	21.60
NaCl (1 g/L) +10 mg/L Fe NPs	66.66	5.90	22.40
NaCl (2 g/L) +5 mg/L Fe NPs	44.40	1.50	15.20
NaCl (2 g/L) +10 mg/L Fe NPs	66.66	2.00	18.10
NaCl (4 g/L) +5 mg/L Fe NPs	33.33	1.30	9.00
NaCl (4 g/L) +10 mg/L Fe NPs	44.40	3.00	12.00
NaCl (8 g/L) +5 mg/L Fe NPs	---	---	---
NaCl (8 g/L) +10 mg/L Fe NPs	11.10	0.65	7.00
$LSD_{0.05}$	0.05	0.17	0.39

7. Antioxidant and enzymes activity under salinity stress

Data presented in Table (6) showed the increase of SOD enzyme activity at concentration 1g/l salinity comparing with high concentration (8 g/l) of salinity which had also stimulation effect on the activity of catalase enzyme (25.52 U mg^{-1}) as compared to control. This might attributed to that salinity could change the physiological and biochemical activities by controlling the anabolic and

stimulating the catabolic processes (Corchete and Guerra, 1986). Peroxidase, catalase, glutathione reducatase and superoxide dismutase enzymes are mainly intracellular scavengers of reactive oxygen species (Uchida *et al*., 2002). Salt tolerance can be correlated with higher levels of antioxidant enzyme activities and these enzymes can be a defense team in protecting the cells from oxidative damage (Baby and Jini, 2010).

8. Effect of Salinity with iron oxide NPs on activity of antioxidant enzymes.

The highest activity of antioxidant enzymes in *Antigonon leptopus* (SOD and catalase) were observed at high concentration (8.0 g/L) of salinity plus 5.0 mg/l iron oxide NPs followed by the treatment NaCl 1g/l +10 mg/l iron oxide NPs. The *glutathione reductase* activity was increased in control to the highest value (22.01 mg/g) followed by these found with NaCl 8g/l +5mg/l iron oxide NPs and finally, the treatment of NaCl 1g/l +10 mg/l iron oxide NPs (Table 7). Earlier reports mentioned that Iron NPs plays a role in increasing the growth, development, and enhancement of the stress tolerance of plants and the provision of nutrients (Muhammad *et al*., 2018).

Table 6 : Effect of Salinity on antioxidant enzymes.

Treatments	GSR (mg g^{-1} tissue)	SOD (U mg^{-1} protein)	Catalase (µg^{-1} tissue)
Control	22.16	3.72	21.00
Nacl at 1g/L	20.91	4.80	22.63
Nacl at 8 g/L	21.39	4.25	25.52
$LSD_{0.05}$	0.20	0.69	1.48

Table 7 : Effect of Salinity with iron oxide NPs on activity of antioxidant enzymes.

Treatments	GSR (mg g^{-1} tissue)	SOD (U mg^{-1} protein)	Catalase (µg^{-1} tissue)
Control	22.01	3.72	21.00
NaCl (1 g/L) +10 mg/L Fe NPs	19.78	5.34	27.03
NaCl (8 g/L)+5.0 mg/L Fe NPs	20.27	6.05	30.18
$LSD_{0.05}$	2.17	0.80	2.34

Conclusion

In this study, the results showed that it could overcome the effect of salt stress by using iron oxide NPs. The micropropgated shoot and root parameters were increased by using iron oxide NPs under salt stress.

References

Ali, S.M.; Galal, A.; Atta, N.F. and Shammakh, Y. (2017). Toxic heavy metal ions removal from wastewater by nano-magnetite: Case study Nile river water. Egyptian Journal of Chemistry, 60(4): 601-612.

Ayala-Astorga, G.I. and Alcaraz-Melendez, L. (2010). Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold and Zuccarini) and *Paulownia fortunei* (Seemann and Hemsley) grown *in vitro*. Electronic Journal of Biotechnology, 13: 1-15,

Ayala-Astorga, G.I.; Alcaraz-Melendez, L.; Ayala, P.F. and Castellanos, A.E. (2009). Effect of sodium chloride stress in *Paulownia imperialis* (Siebold and Zuccarini) and *Paulownia fortunei* (Seemann and Hemsley) plant growing in vitro. Agrochimica, 53: 65-78.

Baby, J. and Jini, D. (2010). Insight into the Role of Antioxidant Enzymes for Salt Tolerance in Plants. International Journal of Botany, 6: 456-464.

Bates, L.S.; Suleiman, M.K.; Al-Menaia, H.; Al-Mulla, H.; Dcruz, G.; Ali, A. and George, P. (1973). Response of Zizyphus varieties to irrigation water salinity under arid climatic condition of Kuwait. European Journal of scientific research, 28: 543-548.

Beutler, E.D. and Kelly, M.B. (1983). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882–888.

Bombin, S.; Lefebvre, M.; Sherwood, J.; Xu, Y. and Bao, Y. (2015). Developmental and reproductive effects of Iron oxide nanopariticles in *Arbidopsis thaliana*. Int. J. Mol. Sci., 16: 24174-24193.

Burke, J.M. and DiTommaso, A. (2011). Corallita (*Antigonon leptopus*): intentional introduction of a plant with documented invasive capability. Invasive plant science and management., 4: 265-273.

Carrillo, P.; Annunziata, M.G.; Pontecorvo, G.; Fuggi, A. and Woodrow, P. (2011). Salinity stress and salt tolerance. Abiotic stress in plants-Mechanisms and Adaptations 21-38.

Corchete, P. and Guerra, H. (1986). Effect of NaCl and polyethylene glycol on solute content and glycosidase activities during germination of lentil seeds. Plant Cell Environ., 9: 589–93.

El-Alfy, E.A.; Attya, M. and Shaaban, M.F. (2015). Treatment of cotton fabrics to inhibit bacterial effect of some microorganisms part I: Using separate Nano antibacterial agents. Egyptian Journal of Chemistry, 58(6): 671-680.

El-Kereti, M.A.; El-Feky, S.A.; Khater, M.S.; Osman, Y.A. and El-Sherbini, E.A. (2013). ZnO Nanofertilizer and He Ne laster Irradiation for Promoting Growth and Yield of sweet Basil Plant. Recent Patents on Food Nutr. Agric., 5: 1-13.

El-Sayed, I.M.; Taha, L.S.; Mazhar, A.M.A. and Kandil, M.M. (2019). Iron oxide Nanoparticales role in micropropagation of *Moringa oleifera* L. under salinity stress. Middle East Journal of Agriculture Research. 8(4): 1123-1132.

Ghareeb, Z.F. and Taha, L.S. (2018). Micropropagation protocol for *Antigonon leptopus* an important ornamental and medicinal plant. Journal of Genetic Engineering and Biotechnology. In press.

Greenway, H. and Munnus, R. (1980). Mechanismus of salt tolerance in non-halophytes. Ann. Rev. of Plant Physiol., 31: 149-190.

Ibrahim, A.H.; Ismail, H.A.; El-Desouki, D.S.; Abdel-Azim, S.A. and Aboul-Gheit, N.A.K. (2018). Preparation of sulfated zirconia catalyst loaded by copper in 'nano-scale: Green application' to synthesis of biolubricant. Egyptian Journal of Chemistry, 61(3): 503-516.

Khenifi, M.L.; Booudjeniba, M. and Kameli, A. (2011). Effects of salt stress on micropropagation of potato (*Solanum tuberosum* L.). African journal of biotechnology, 10: 7840-7845.

Lim. T. K. (2013). Edible medicinal and non-medicinal. 8: 643-649.

Marcum, K. and Murdoch, C.L. (1994). Salinity tolerance mechanisms on six c4 turfgrasses. J. Amer. Soc. Hort. Sci., 119: 779-784.

Marklund, S.L. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem, 47: 469-474.

Muhammad, Z.R.; Asif, N.; Hinnan, K.; Muhammad, R.; Shafaqat, A. and Muhammad, A. (2018). Responses of Plants to Ir on Oxide NanoparticlesIn:- Nanomaterials in Plants, Algae, and Microorganisms . Concepts and Controversies, 1: 221-238.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Pagliam, D.E. and Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158–169.

Parida, A.K. and Das, A.B. (2005). Salt tolerance and salinity effects on plants:A review. Ecotoxicology and Environmental safety, 60: 324-349.

Parvaiz, A. and Satyawati, S. (2008). Salt stress and phyto-biochemical responses of plants –a review. Plant Soil Environment, 54: 89-99.

Rajput, K.S. (2015).Comparative study on secondary xylem and formation of successive cambia in stems and roots of *Antigonon leptopus* Hook. and Arn (Polygonaceae). Flora. Morphology, Distribution, Functional Ecology of Plants, 217: 131-137.

Rezvani, N.; Sorooshzadeh, A. and Farhadi, N. (2012). Effect of Nano-Silver on Growth of Saffron in Flooding Stress. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 6: 11-16.

Saric, M.; Kastrori, R.; Curic, R.; Cupina, T. and Gric, I. (1967). Chlorophyll determination. University of "Noveon Sadu Praktikum iz Fiziologize Biljaka", Belgrade, Serbia.

Shaban, S.A. (2012). Catalysis and nanotechnologies. Egyptian Journal of Chemistry, 55(5): 453-473 .

Shivanna, M.; Nagashree, B. and Gurumurthy, B. (2013). In vitro response of *Azadirachta indica* to salinity stress and its effect of certain osmoprotectants and antioxidative enzymes. International Journal of Pharma and Bio Sciences, 4: 591-602.

Siddiqui, M.H.; Al-Whaibi, M.H. and Mohammad, F. (2015). Nanotechnology and plant sciences: Nanoparticles and their impact on plants. Nanotechnology and Plant Sceinces: Nanoparticles and their Impact on Plants, 1-303.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods.7thed. Iowa Stat, Univ., Press, Ames. Iowa, USA. 11:1-42.

Soliman, A.; El-feky, S.A. and Darwish, E. (2015). Alleviation of salt stress on Moringa peregrina using foliar application of nanofertilizers. Journal of Horticulture and Forestry, 7: 36-47.

Teixeira, D.S.J. and Retired, S. (2015). Sensitivity of hybrid cymbidium to salt stress and induction of mild NaCl stress tolerance. Environmental and Experimental Biology, 13: 89–92.

Uchida, A.; Jagendorf, A.T.; Hibino, T.; Takabe, T. and Takabe, T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci., 163: 515-523.

Vanisree, M.; Alexander-Lindo, R.L.; DeWitt, D.L. and Nair, M.G. (2008). Functional food components of *Antigonon leptopous* tea. Food chemistry, 106: 487-492.