Alleviation of Salt Stress in *Nigella Sativa* L. By Gibberellic Acid and Rhizobacteria

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ABSTRACT

Salinity is one of the extremely serious abiotic stresses for plants, affecting other subsequent consequences such as oxidative stress, which finally leads to cell death. A pot experiment was performed during 2014 / 2015 and 2015/ 2016 at Sakha Agricultural Research Station, to elucidate the alleviation of salinity effects by spraying gibberellic acid (GA_3), *Azospirillum sp*. and *Azotobacter sp*. *Rhizobacteria* (PGPR) and the combination between GA_3+(PGPR) and their effects on the vegetative growth, yield characters, chemical composition and fixed oil percentage of black cumin plant. Salinity concentrations were 1000, 2000, 3000 and 4000 ppm sea water diluted compared with fresh water as control, GA_3 was used at 100 ppm and PGPR at10%. Salinity treatments significantly decreased plant height, number of branches, plant dry weight, number of capsules, number of roots per plant, root volume, roots fresh and dry weights, capsules yield, seed yield /plant and 1000 seeds weight compared with control. Salinity also decreased chlorophyll content, fixed oil percentage and relative water content. However, proline content, peroxidase and catalase activities, membrane permeability and total soil soluble salts were increased relative to the control. GA_3 or PGPR treatments alleviated the above mentioned undesirable effects of salinity. The increment of enzymes activities and proline accumulation due to GA_3 or PGPR treatments are suggested to involve as part of the defense versus salinity on *Nigella sativa* L plants. To reduce the unfavorable salinity influences, treatment of GA_3 at 100 ppm or PGPR at10% was recommended.

Keywords: Salinity, *Nigella sativa* L., gibberellic acid, *Rhizobacteria*, Seed yield, Fixed oil.

INTRODUCTION

Nigella sativa L. (black cumin) is an aromatic and medicinal plant from Ranunculaceae family. This plant is customarily utilized as a flavor and as a characteristic cure in the treatment of a few diseases (Cheikh-Rouhou *et al*., 2007). It exhibited an extensive pharmacological actions (Bourgou *et al.*, 2008, 2010) which due to its abundance in a few secondary metabolites including seed volatile oil (Bourgou *et al.*, 2010), seed fixed oil which, contain linoleic acid (40.3–58.9%), oleic (18.7–28.1%), palmitic (10.1–12.5%) and stearic (2.6–3.1%) acids (Ramadan, 2007 and Matthaus and Ozcan, 2011) and phenolic compounds in the shoots and the roots (Bourgou *et al*., 2008). The previous both organs are chiefly rich in vanillic acid. This plant has been expanded as a usual remedy for illnesses for example, asthma, irritation, diabetes, tumor, gastrointestinal unsettling influences, hypertension, and gynecological disorders for over several years (Ramadan, 2007).

Egyptian economy depends on a great degree on agriculture. The rapidly rising population and variations in the way of life require judicious advancement in agricultural production. Thus, the prominent goal of the Egyptian policy is to rise the land production through better land usage, improvement of agricultural techniques and bring new land areas to cultivation. The Egyptian budget of the Nile freshwater is low and its quantity approximately 55.5 milliard m^3. Looking at the upcoming of stressing water demands, it is quite obvious, that a very careful use of accessible water sources and expansion of new resources such as drainage, well, sewage and sea water should be contemplated. Irrigation by saline water may decrease crops yield. Although, using sea water in irrigation may save the fresh water resources for the other usages but, what about the effect of using sea water in agriculture?

Plants grown in farming systems are subjected to numerous abiotic and biotic stresses which reduce their quality and revenue potential. Salinity remains the basic reason which, decreasing plant growth then productivity worldwide. It influences around 7% of the world's whole land area (Flowers *et al*., 1997 and Zhu, 2002). Salinity stress influences growth besides metabolic activities of plant species (Baghalian *et al.*, 2008 and Oueslati *et al*., 2010). Upon observing environmental stresses plants enact a range of resistance mechanisms which might also be made artificially or boosted by

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using specific chemicals (Rajasekaran and Blake, 1999). These days consideration has been directed to practical and environment-friendly substitutes for example, biological ways to improve and encourage plant growth. Beneficial bacteria, particularly in plants rhizosphere have been examined and established to have growth-promoting activities. The impact of PGPR on the alleviation of salinity has been stated (Weyens *et al*., 2009 and Yang *et al*., 2009)

Also, other attempts have been made to alleviate deleterious effects of salinity; different types of phytohormons are being used. Of these, gibberellic acid is an essential phytohormone able to impart stress tolerance involving salinity, in several plants (Hoque and Haque, 2002). The GA_3 has significantly impact the procedures of seed germination, leaf extension, stem stretching, bloom and trichome origination, and fruit development (Yamaguchi, 2008). Through their impact on photosynthetic enzymes, GA_3 is identified to increase the photosynthetic efficacy of plants, leaf area index, light capture, the efficiency of nutrients and assume an essential part in regulating various processes through plant development (Khan, *et al*. 2010). GA_3 has been accounted to reduce the undesirable consequences of salinity plant water relationships in addition water use efficiency (Yamaguchi, 2008). The effect of GA_3 on salinity mitigation has been previously reported (Maggio *et al.*, 2010) on tomato plants, (Saeidi-Sar *et al*., 2013) on (*Phaseolus vulgaris* L. cv. Naz) otherwise (Khan, *et al.* 2010) on (*Linum usitatissimum* L.).

Under stress, plants established compound mechanisms to combat against these oxidative stresses *via* the synchronous activity of various antioxidants. Of these, superoxide dismutase (SOD) which changes superoxide to H_2O_2, peroxidase (POD) which changes H_2O_2 to water and catalase (CAT) eliminates H_2O_2. Also, plants adjust osmotic stress by gathering some compatible solutes for example, proline, glycinebetaine, polyols and trehalose (Ghoulam *et al*., 2002 and Sakamoto and Murata, 2002). Proline plays a key role in keeping plants from osmotic stress. Thus, antioxidants besides compatible solutes could supply approach to boost plants salt tolerance. Concerning *Nigella sativa* L. the effect of salinity on leaves fatty acid content has been studied by (Bourgou *et al*., 2012) . However, no data have been collected regarding seeds fatty acid (fixed oil) content under saline sea water. Nevertheless, to the best of our insight, no published literature exists about sea water salinity effects on fatty acid content. Therefore, this study attempted to investigate for the first time the effect of PGPR and GA_3 on growth attributes and biochemical characters under different concentrations of saline sea water, in order to use of *Nigella sativa* L.as an economic substitute for field crops and to save freshwater.

MATERIALS AND METHODS

Field site description

A pot experiment was carried out at Sakha Agricultural Research Station (31° 07' N Latitude, 30° 05' E Longitude), Kafr El-Sheikh Governorate, North Nile Delta of Egypt during 2014 / 2015 and 2015/ 2016 growing seasons to study the impact of irrigation with sea-fresh mixed water, of increased salinity levels. The experiment was performed using complete randomized blocks design with four replications. Plastic pots with a top diameter of 30 cm and a depth of 18 cm were filled with 5-kilogram clayey soil. Physical and chemical soil properties of the experimental site was showed in Table (A). Black cumin seeds were acquired from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Egypt. Ten seeds were sown on December 1st, 2014 and 2015 in every pot and after six weeks then they were thinned to five healthy seedlings per pot.

Experimental treatments:

Salinity treatments

The plants irrigated with freshwater 289 ppm (0.45ds/m) from sowing until 60 days, then salinity treatments were applied after the seedlings started their growth and development until harvesting on May 1st for both seasons. The plants were given water requirements plus 20% as leaching requirements for all treatments through all seasons until harvest. The salinity levels were obtained by addition of appropriate quantity of sea water to freshwater and were adjusted through a portable Ec meter instrument.

Table A. Some physical and chemical soil properties of the used medium as mean values of the two experimental growth season

Field capacity (%)	Wilting point (%)	Bulk density (mg m^{-3})	Total porosity (%)	Sand (%)	Silt (%)	Clay (%)	Texture class	pH
44.62	22.83	1.16	56.23	19.16	26.52	54.32	Clayey	8.21
EC_e	Inions concentration meq/L				Cations concentration meq/L			
(dS m^{-1})	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
3.73	---	4.60	16.48	17.18	7.35	8.11	22.47	0.33

The treatments were as the following

1-Control (0.45ds/m). 2- 1000 ppm (1.562ds/m). 3- 2000 ppm(3.125ds/m). 4- 3000 ppm (4.69ds/m).

5- 4000 ppm (6.25ds/m).

Spraying treatments

Gibberellic acid (GA_3)

Gibberellin 100 ppm was sprayed twice in the morning during the vegetative stage, the first one was on February 15th and 17th in both seasons, respectively. So, this means that after one week from the beginning of the saline irrigation treatments, while the second spray was on March 4th and 6th in both seasons, respectively

Rhizobacteria inoculants (PGPR)

Selected strains of Rhizobacteria (PGPR) were *Azospirillum* sp. and *Azotobacter* sp. both cultures were kindly supplemented by Microbiology Department, Sakha Agricultural Research Station in modified tryptone yeast extract and glucose (TYG) (Jensen, 1951 and Bashan *et al.*, 2002) both media with cell density 4 x 10^{11} and 9 x10^{9} for *Azospirillum* sp and *Azotobacter* sp respectively. Rhizobacteria were sprayed twice as mentioned in GA_3 at a concentration of 10%.

Gibberellic acid (GA_3) plus Rhizobacteria inoculants(PGPR)

GA_3 and PGPR were dissolved separately in distilled water as a solution and added as a foliar application using a sprayer and conical bowl which was putted on top of the pots to concentrate spraying on treated plants and prevents spraying other plants. Gibberellic acid was sprayed on the early morning then after two hours, the microbial (PGPR) was sprayed twice in the meantime of the previous treatments (GA_3).

Freshwater

Tap water (0.45ds/m) was sprayed twice in the meantime of the previous treatments as a control.

Harvest time

The plants were gathered at full maturity stage on May 1st in the two seasons.

Collected data

Growth and yield characters

Vegetative growth and yield characters

The height of plants was measured in centimeters of the main stem from ground level to the plant top by measuring tape. The number of branches/plant was determined by counting the number of reproductive branches that appeared within growing season from each plant. Plant dry weight (g): The plants mentioned above were cut, then placed in an envelope and dried naturally in the lap. Capsules were picked randomly from plants, put in a small envelope bag and weighted (g), then determined average capsules number and weight per plant. Seeds yield /plant (g) capsules were picked randomly from plants, shelling the seeds from capsules after physically, drying in the lap their seeds were added to their respective seeds of the capsules in the small bags and weighted, and dry weight of 1000 seeds (g) was estimated by counting 1000-seeds randomly from each pot five times and weighted using a sensitive balance for both seasons.

Root characters

Roots length, roots number, fresh and dry weight and root volume. Plant samples of each plant were taken at harvesting time, washed with distilled water, then spread roots and shoots. Main roots were counted as a number and the length of the main root was measured using scale ruler. Root weighted as fresh using sensitive balance, oven dried at 70 ^{o}C even weight stability, dry weight was recorded, ground and kept for analyses**.** Root volume was determined by water relocation methods, the measuring was prepared in a unique container with an overflow spout. This container is loaded with water until it floods from the spout. Then fresh washed roots which have been carefully dried with a soft cloth are immersed and the flood water volume is measured in a graduated cylinder (Bohm, 2012).

Biochemical characters

Chlorophyll Content

Randomly samples of new leaves April1st were grabbed from the central part of the stem for chlorophyll determination. Chlorophyll a and b mg/g F.W were determined by the method defined by (Moran, 1982) by using spectrophotometer (Pharmacia, LKB-Novaspec II)

Relative Water Content of Leaves (RWC)

The relative water content of leaves (RWC) was estimated by the method of (Whetherley, 1950). Leaf material was balanced (0.5 g) to establish fresh weight (FW) and located in double-distilled water for 4 h, subsequently this time turgid weight (TW) was recorded. Subsequently, the samples were saved in a hot air oven at 65 °C for 48 h and their dry weights (DW) were recorded. RWC was calculated as:

$$(W_{fresh} - W_{dry}) / (W_{turgid} - W_{dry}) \times 100.$$

Membrane permeability (Mb)

Membrane permeability of the excised leaves was measured at the completion of the experiment (Yan *et al*., 1996) fresh portion from the center of leaves was

balanced into a glass beaker comprising reverse osmosis water. The beakers were dipped at 30 ± 1°C for 3 h, and subsequently the conductivity of the solution was calculated with a conductivity meter. The conductivity was determined again next boiling the samples for 2 min. once the solution was air-conditioned to room temperature. The percentage of electrolyte leakage was considered by implementation of the formula, EC % = (C1/C2) X 100, since C1 and C2 are the electrolyte conductivities evaluated before and after boiling, respectively.

Fixed oil content

The air dried seeds balanced (50 g) were powdered mechanically and extracted with light petroleum ether (60 – 80 °C) for 4h in a Soxhlet apparatus. Removal of the solvent was done under reduced pressure gave the fixed oils (Horwitz *et al*., 1970).

Proline

The free proline content was determined according to (Bates *et al.*, 1973). Frozen leaf tissue (0.5g) was homogenized with 10 ml of 3% sulfosalicylic acid at 4 °C. Then, the acquired extract was clarified with Whatman No. 2. A mixture of 2 ml of the filtrate, 2 mL from acid-ninhydrin, and 2 mL of glacial acetic acid was mixed inside a test tube and incubated at 100 °C for 1 h. The reaction was done on the ice, and the reaction combination was then separated with 4 mL of toluene. The chromophore-containing toluene was removed from the hydrated stage. The absorbance at 520 nm was spectrophotometrically defined with toluene as the blank. The proline concentration was calculated established on a standard curve and was communicated as µmol g^{-1} F.W.

Antioxidant Enzyme Activity

To obtain the enzyme extract for antioxidant enzymes determination, the method formerly described by (Hassan and Mahfouz, 2012) was used. The subsequent supernatant was consumed as an enzyme extract to determine peroxidase (POX) and catalase (CAT) activities. Soluble protein contents of the enzyme extract were assessed according to (Bradford, 1976).

Peroxidase activity

was tested according to (Shannon *et al.*, 1966). Sodium acetate buffer (0.1M) and 0.5% guaiacol were added to the enzyme extract. The reaction was commenced with 0.1% H_2O_2 . The rate of variation in absorbance was spectrophotometrically measured at 470 nm and quantity of enzyme activity was communicated as µ mol min^{-1} mg^{-1} protein.

Catalase activity

was spectrophotometrically evaluated by (Claiborne, 1985) following the disappearance of H_2O_2 at 240 nm. The amount of enzyme activity was stated as µmol min^{-1} mg^{-1} protein.

Total soluble salts

At the completion of the experiments soil samples were taken from every pot and chemically analyzed, total soluble salts were measured by electrical conductivity (EC) apparatus in the saturated soil paste extract (Page *et al*., 1982).

Statistical analysis

Data for each season were evaluated by the method defined by (Steel *et al*., 1980) and differences between the means were investigated by Duncan's Multiple Range Test (Snedecor and Cochran, 1980) using COSTAT computer program.

RESULTS

Growth and yield characteristics

Vegetative growth characteristics

Salinity treatments adversely affected on plant height, branches number, plant dry weight and capsules number compared to control in both seasons (Table 1). Plants received freshwater (without salinity) gave the highest significant mean values for plant height, branches number, plant dry weight and capsules number for both seasons. Expanding salinity levels gradually decreased all previously mentioned characters. Generally, the overall mean values for this characters can be descended in order 1000ppm >2000ppm >3000ppm > 4000ppm in most cases for the two seasons. The decline in plant height, branches number, plant dry weight and capsules number by salinity was alleviated when GA_3 or PGPR or GA_3 + PGPR were applied. Application of GA_3 positively improved plant height better than PGPR or GA_3 + PGPR. Moreover, applying PGPR enhanced the branches number which led to increasing plant dry weight and capsules number for both seasons. The interaction among different saline water and exogenous GA_3 application recorded the highest plants under different saline water concentrations. Among all treatments applied, the tallest black cumin plants were recorded by GA_3 with 1000 ppm or control treatment without significant variations among them in the first season and GA_3 with 2000 ppm in the second season. Applied PGPR with salinity at 2000 ppm in the first season and PGPR with salinity at 1000ppm in the subsequent season recorded the highest branches number per *Nigella sativa* L. plant. Additionally, using PGPR with 1000 ppm saline water achieved the heaviest dry weight plus the highest capsules number for both seasons.

Root growth characteristics

All root characters (root length, roots number, root volume, root fresh and dry weight) were significantly influenced by salinity and salinity alleviators treatments

Table 1. Effect of saline irrigation water levels and spraying by GA_3 and PGPR on plant height, branches number, plant dry weight and capsules number of *Nigella sativa* L. plants

Seasons	1st Season 2015					2nd Season 2016				
Spraying / Salinity	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean
	Plant height (cm)					Plant height (cm)				
Control	44.67f-j	67.00a	49.33d-f	56.67bc	54.41a	37.67fg	50.00ab	43.00de	48.33b	44.75a
1000ppm	42.67h-j	65.33a	46.67e-i	52.67cd	51.83b	35.67g	49.33b	43.33d	47.67bc	44.00ab
2000ppm	48.00de-g	58.67b	42.00h-j	51.33de	50.00bc	44.33d	52.67a	36.00g	44.33d	44.33ab
3000ppm	40.67j	56.67bc	47.33e-h	48.67d-g	48.33cd	36.33g	50.67ab	40.00ef	44.33d	42.83b
4000ppm	40.67j	51.67cde	43.67g-j	51.67c-e	46.92d	36.00g	44.67cd	38.33fg	43.00de	40.50c
Mean	43.33d	59.87a	45.80c	52.2b		38.00d	49.47a	40.13c	45.53b	
	Branches number					Branches number				
Control	5.00b	6.00a	6.00a	5.00b	5.75a	7.00ab	6.67a-c	7.00ab	5.33ef	6.5a
1000ppm	6.00a	4.67cd	6.00a	4.00ef	4.92b	7.00ab	6.33b-d	7.33a	5.00fg	6.42ab
2000ppm	5.00b	5.00b	6.00a	3.67f	4.92b	6.67a-c	5.67d-f	7.00ab	5.33ef	6.17bc
3000ppm	6.00a	4.33de	5.33b	4.00ef	4.92b	6.00cde	6.33b-d	7.00ab	4.33g	5.92c
4000ppm	4.00ef	6.00a	6.00a	4.00ef	5.00b	5.00fg	7.00ab	7.00ab	5.00fg	6.00c
Mean	5.20b	5.20b	5.87a	4.13c		6.33b	6.40b	7.07a	5.00c	
	Plant dry weight (g /plant)					Plant dry weight (g /plant)				
Control	6.49cd	7.28b	6.89bc	6.45cd	6.78a	5.61a	5.89a	5.63a	5.75a	5.72a
1000ppm	6.91bc	4.94fg	8.11a	4.28hi	6.06b	5.28a	3.51a	6.15a	3.65a	4.64b
2000ppm	5.21ef	4.82fgh	6.26d	5.55e	5.46c	4.63a	4.07a	5.42a	4.60a	4.68b
3000ppm	4.52gh	3.81i	5.66e	4.70fgh	4.67d	3.82a	3.10a	4.87a	3.61a	3.85c
4000ppm	3.89i	3.70i	7.42b	4.71fgh	4.93d	3.30a	3.12a	6.39a	3.49a	4.08c
Mean	5.41b	4.91d	6.87a	5.14c		4.53b	3.94d	5.69a	4.22c	
	Capsules number					Capsules number				
Control	12.67bc	13.33b	17.67a	12.00bc	13.92a	11.00c	10.00d-f	13.33a	8.33h	10.67a
1000ppm	13.00bc	12.00bc	17.00a	12.00bc	13.50a	10.00d-f	10.33c-e	13.33a	9.33fg	10.67a
2000ppm	12.67bc	12.00bc	16.33a	11.00cd	13.00ab	11.00c	11.00c	10.67cd	6.33i	9.75c
3000ppm	9.33de	13.33b	13.67b	13.00bc	12.33bc	9.67e-g	9.00g	12.67b	9.00g	10.17b
4000ppm	12.67bc	12.67bc	13.33b	8.67e	11.83c	6.00i	10.67cd	11.00c	6.33i	8.50d
Mean	12.07bc	12.67b	15.60a	11.33c		9.53c	10.20b	12.20a	7.87d	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range tests.

(Table 2). While, salinity treatments significantly decreased (root length, root number, root volume, root fresh and dry weight). Using GA_3 or PGPR or GA_3 + PGPR significantly increased them when applied or minimized the reduction occurred by salinity. The most efficient treatment in this concern was GA_3 which promoted root length, root volume, root fresh and dry weight for both seasons. Otherwise, GA_3 + PGPR significantly boosted roots number. Moreover, a combination between GA_3 with salinity mostly caused a noticeable root length increment and root volume in the two seasons. Also, the inhibitory impact of salinity stress was completely ameliorated generally at low salinity level (1000 ppm) with GA_3 especially in root fresh and dry weight for both seasons.

Yield characteristics

From Table 3 and Fig 1, a gradual reduction in capsules dry weight, seeds yield/plant and 1000 seeds weight with increasing of salinity levels could be noticed; the least values in this concern were obtained from the elevated level (4000ppm) for all parameters in both seasons. Otherwise, salinity treatments caused more reduction in seeds yield/plant reached 67.39 and 80.37% at 4000ppm for both seasons, respectively. Although GA_3, PGPR and GA_3+ PGPR alleviated the adverse salinity influences on capsules dry weight, seeds yield/plant and 1000 seeds weight. Applying PGPR enhanced capsules dry weight and seeds yield/plant. Moreover,

increasing of seeds yield/plant by applying PGPR reached to 46.88 and 60.40% in both seasons, respectively while, using GA_3 increased 1000 seeds weight. The highest capsules dry weight was obtained

from plants treated with GA_3+ PGPR without salinity for both growth seasons. A promotion effect on seeds yield/plant and 1000 seeds weight was also noticed

Table 2. Effect of saline irrigation water levels and treatment with GA_3 or PGPR on root length, root number, root volume, root fresh and dry weights of *N. sativa* L. plants

Seasons	1st Season 2015					2nd Season 2016				
Spraying / Salinity	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean
	Root length (cm)					Root length (cm)				
Control	13.00e	17.00a	14.00d	14.00d	14.50a	11.00e	15.00a	13.00c	12.00d	12.75a
1000ppm	14.00d	17.00a	14.00d	13.00e	14.50a	13.00c	13.00c	14.00b	11.00e	12.75a
2000ppm	16.00b	12.00f	15.00c	12.33f	13.83b	11.00e	11.00e	14.00b	12.00d	12.00b
3000ppm	12.67e	14.00d	13.00d	13.00e	13.42b	13.00c	12.00d	11.00e	10.67e	11.67c
4000ppm	13.00e	12.00f	13.00e	13.00e	12.75c	12.00d	11.70d	10.00f	11.00e	11.18d
Mean	13.73b	14.40a	13.80b	13.07c		12.00b	12.54a	12.40a	11.33c	
		Root Number					Root Number			
Control	5.00d	6.00c	5.00d	8.00a	6.00a	6.00b	6.00b	5.00c	7.00a	6.00a
1000ppm	5.00d	6.00c	5.00d	7.00b	5.75b	6.00b	5.00c	5.00c	5.00c	5.25b
2000ppm	6.00c	6.00c	5.00d	6.00c	5.75b	6.00b	5.00c	5.00c	5.00c	5.25b
3000ppm	5.00d	6.00c	6.00c	6.00c	5.75b	4.00d	5.00c	5.00c	5.00c	4.75c
4000ppm	6.00c	5.00d	5.00d	5.00d	5.25c	5.00c	5.00c	5.00c	4.00d	4.75c
Mean	5.40c	580b	5.20c	6.40a		4.80c	5.20b	5.00b	5.40a	
		Root volume(cm^3)					Root volume (cm^3)			
Control	3.6cde	4.5a	4bc	4bc	4.03a	2.27b-e	3.27a	2.9ab	2.5bcd	2.73a
1000ppm	3fg	3fg	4bc	4.17ab	3.54b	2.43bcd	2.17cde	2.90ab	2.67abc	2.54ab
2000ppm	3.27ef	4.5a	3fg	2.5h	3.32b	2.27b-e	3.17a	2.4bcd	1.73ef	2.39b
3000ppm	3fg	3.27ef	2.67gh	3.50de	3.11c	2.17c-e	2.43bcd	2def	2.5bcd	2.28b
4000ppm	3.70cd	4bc	3fg	2i	3.18c	2def	2.7abc	1.67ef	1.5f	1.97c
Mean	3.31b	3.85a	3.33b	3.23b		2.23bc	2.75a	2.37b	2.18c	
		Root fresh weight (g/ plant)					Root fresh weight (g/ plant)			
Control	2.43b-d	2.17cde	2.9ab	2.67a-c	2.54ab	2jk	2.06ijk	3.06b-d	2.78c-e	2.48b
1000ppm	2.27b-e	3.27a	2.9ab	2.5b-d	2.73a	2.54e-i	3.66a	2.66c-g	3.29ab	3.04a
2000ppm	2.27b-e	3.17a	2.4b-d	1.73ef	2.39b	1.93jk	2.77cde	1.73k	2.19f-k	2.16c
3000ppm	2.17c-e	2.43b-d	2def	2.5b-d	2.28b	2.17g-k	2.69c-f	2.39e-j	2.56d-i	2.45b
4000ppm	2def	2.7a-c	1.67ef	1.5f	1.97c	2.39e-j	3.07bc	2.58c-h	2.13h-k	2.54b
Mean	2.22bc	2.75a	2.37b	2.18c		2.21c	2.85a	2.49b	2.59b	
		Root dry weight (g/ plant)					Root dry weight (g/ plant)			
Control	1.53g-i	2.76a	2.17b-d	1.92d-f	2.10a	1.28fg	1.18g-i	1.85a	1.71c	1.51b
1000ppm	1.97c-e	2.82a	1.77efg	2.32b	2.22a	1.50d	1.86a	1.28fg	1.80abc	1.61a
2000ppm	1.55g-i	2.85a	1.37i	1.93d-f	1.93b	1.16g-i	1.37ef	1.01jk	1.85ab	1.35c
3000ppm	1.44hi	1.75e-g	1.73e-g	2.22bc	1.79c	1.07ij	1.47de	1.36ef	1.28fg	1.29cd
4000ppm	1.77e-g	1.34i	1.67f-h	1.87ef	1.66c	1.13hi	1.73bc	1.22gh	0.94k	1.25d
Mean	1.65d	2.31a	1.74c	2.05b		1.23c	1.52a	1.34b	1.52a	

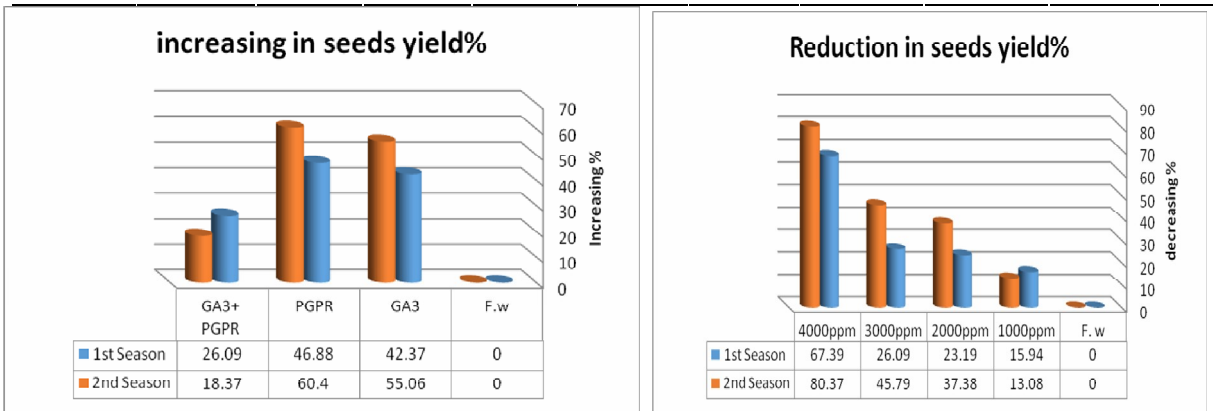
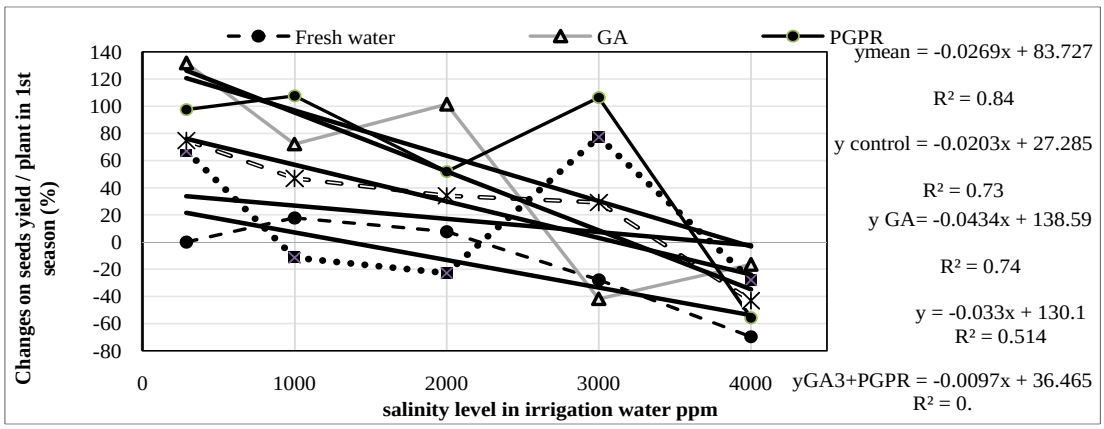


Figure.1. Reduction and increasing % in seeds yield /plant as affected by salinity irrigation water levels and foliar application in the two seasons

Table 3. Effect of saline irrigation water levels and spraying by GA_3 and PGPR on capsules dry weight, seeds yield/plant (g) and weight of 1000 seed of *Nigella sativa* L. plants

Seasons	1st Season 2015					2nd Season 2016				
Spraying	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean
Salinity	Capsules dry weight /plant (g)					Capsules dry weight /plant (g)				
Control	2.45g	3.03de	3.54a-c	4.02a	3.26a	1.95de	2.46bc	2.44bc	3.01a	2.47a
1000ppm	1.65h	2.77d-g	3.74ab	3.26b-d	2.85bc	1.09h	1.64e-g	3.03a	2.63ab	2.10bc
2000ppm	1.83h	2.67e-g	2.92d-g	3.75ab	2.79c	1.49f-h	1.56e-g	2.19cd	2.77ab	2.00cd
3000ppm	2.50g	3.02d-f	3.58a-c	3.09c-e	3.05ab	1.89d-f	1.71e-g	2.82ab	2.44bc	2.21b
4000ppm	2.51fg	2.48g	3.72ab	2.42g	2.78c	1.60e-g	1.61e-g	2.72ab	1.45gh	1.85d
Mean	2.19d	2.79c	3.50a	3.31b		1.60d	1.80c	2.64a	2.46b	
	Seeds yield/plant (g)					Seeds yield/plant (g)				
Control	0.79h	1.83a	1.56c	1.32e	1.38a	0.58ef	1.46a	1.35b	0.88d	1.07a
1000ppm	0.93g	1.36de	1.64b	0.70i	1.16b	0.61e	1.21c	1.41ab	0.50fg	0.93b
2000ppm	0.85h	1.59bc	1.20f	0.61jk	1.06c	0.42gh	1.15c	0.87d	0.23jk	0.67c
3000ppm	0.57	0.46l	1.63bc	1.40d	1.02d	0.28ij	0.29ij	1.17c	0.58ef	0.58d
4000ppm	0.24n	0.66ij	0.35m	0.57k	0.45e	0.12l	0.34-i	0.23jk	0.24jk	0.21e
Mean	0.68d	1.18b	1.28a	0.92c		0.40d	0.89b	1.01a	0.49c	
	Weight of 1000 seed (g)					Weight of 1000 seed (g)				
Control	3.07b	3.59a	2.61c	2.34d-g	2.90a	2.65b	2.83a	2.22c	2.23c	2.48a
1000ppm	2.45	2.47c-e	2.49cd	2.25f-h	2.42b	2.17cd	2.22c	2.17cd	2.13cd	2.17b
2000ppm	2.37d-g	2.44de	2.33e-g	2.14h	2.32c	2.11cd	2.14cd	2.13cd	2.08d	2.11c
3000ppm	2.23gh	2.39d-f	2.18h	2.15h	2.24d	2.06d	2.13cd	2.08d	1.91e	2.05d
4000ppm	1.12k	1.53j	1.73i	1.83i	1.56e	1.12h	1.42g	1.46g	1.59f	1.40e
Mean	2.25b	2.49a	2.27b	2.15c		2.02b	2.15a	2.01b	1.99b	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range tests.



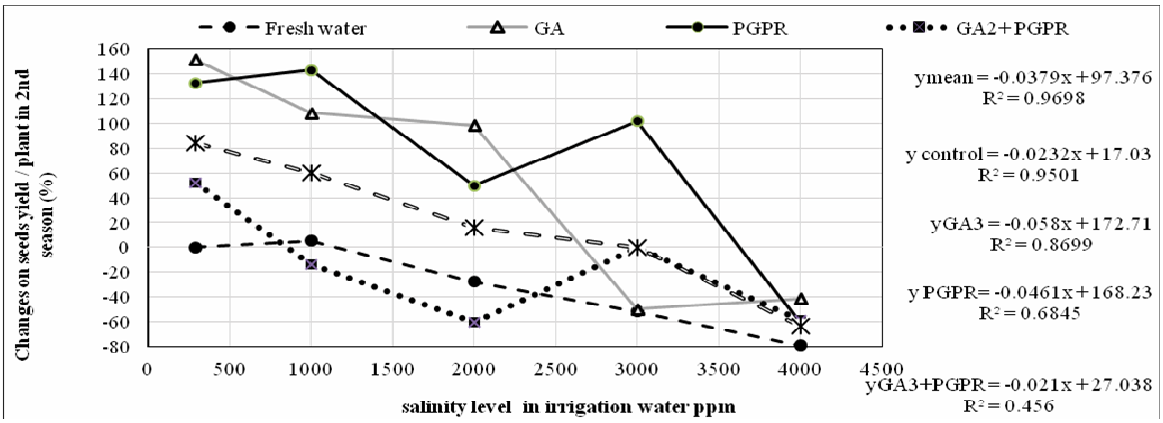


Figure 2. Correlation of seeds yield /plant of black cumin as affected by salinity irrigation water levels and foliar application of GA_3 and PGPR

Table 4. Effect of saline irrigation water levels and spraying by GA_3 and PGPR on chlorophyll (a) mg/g F. W., chlorophyll (b) mg/g F. W, relative water content and membrane permeability % of *Nigella sativa* L. plants

Seasons	1st Season 2015					2nd Season 2016				
Spraying Salinity	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean	Fresh water	GA_3	PGPR	GA_3+ PGPR	Mean
	Chlorophyll(a) mg/g F. W					Chlorophyll(a) mg/g F. W				
Control	1.50f	1.70a	1.51fg	1.60cd	1.58a	1.46ef	1.32i	1.45ef	1.53a	1.44a
1000ppm	1.40k	1.60cd	1.52f	1.55e	1.52b	1.47de	1.35i	1.48cd	1.51ab	1.45a
2000ppm	1.57d	1.41jk	1.55e	1.61bc	1.54b	1.42gh	1.44fg	1.44fg	1.40h	1.42b
3000ppm	1.44ij	1.52f	1.63b	1.55e	1.53c	1.33i	1.51ab	1.34i	1.50bc	1.42b
4000ppm	1.49g	1.51fg	1.50fg	1.46hi	1.49d	1.33i	1.49bc	1.45ef	1.35i	1.41c
Mean	1.48c	1.55ab	1.54b	1.55a		1.40c	1.42b	1.43b	1.46a	
	Chlorophyll(b) mg/g F. W					Chlorophyll(b) mg/g F. W				
Control	0.71e	0.90a	0.75cd	0.76c	0.78a	0.68de	0.77a	0.63g	0.74b	0.71a
1000ppm	0.73de	0.66f	0.65f	0.80b	0.71b	0.76a	0.67ef	0.40m	0.71c	0.64b
2000ppm	0.82b	0.76c	0.47i	0.81b	0.71b	0.69d	0.46l	0.56i	0.74b	0.61c
3000ppm	0.44j	0.74cd	0.61g	0.80b	0.65c	0.67f	0.40m	0.50k	0.72c	0.57d
4000ppm	0.71e	0.43j	0.58h	0.81b	0.63d	0.39m	0.61h	0.53j	0.71c	0.56e
Mean	0.68c	0.70b	0.61d	0.80a		0.64b	0.58c	0.52d	0.72a	
	Relative water content (RWC)%					Relative water content (RWC)%				
Control	77.66e	86.56b	81.38c	90.46a	84.02a	76.52e	85.44b	80.59c	89.41a	82.99a
1000ppm	66.45l	76.34f	78.42d	75.33g	74.14b	65.30l	75.40e	77.44d	74.41g	73.14b
2000ppm	65.46m	74.10h	77.70e	73.33i	72.64c	64.41m	73.15h	76.41e	72.48i	71.61c
3000ppm	63.61o	72.60j	78.42d	64.54n	69.74d	62.52o	71.26j	77.41d	63.40n	68.65d
4000ppm	56.48q	68.58k	77.46e	62.57p	66.27e	55.55q	67.45k	76.37e	61.33p	65.18e
Mean	65.89d	75.64b	78.68a	73.25c		64.86d	74.54b	77.65a	72.21c	
	Membrane permeability (MP) %					Membrane permeability(MP)%				
Control	68.28j	73.81h	43.31l	32.05m	54.37e	56.96j	70.34f	40.47l	32.11m	49.97d
1000ppm	72.79h	91.21b	63.61k	77.01g	76.15d	60.93i	80.04d	60.19i	69.85f	67.76c
2000ppm	89.03c	91.63b	73.99h	88.57c	85.81b	63.70h	89.89a	67.92g	86.28b	76.95b
3000ppm	70.84i	86.02d	86.00d	94.64a	84.38d	54.07k	80.28d	82.68c	90.41a	76.86b
4000ppm	81.97e	95.16a	79.09f	92.16b	87.10a	57.20j	91.85a	72.61e	90.50a	78.04a
Mean	76.58b	87.57a	69.20c	76.89b		58.57d	82.48a	64.77c	73.83b	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range tests.

when GA_3 was used without salinity treatment in both growth seasons.

The relationship between saline irrigation water levels and spraying GA_3 and PGPR on changes in seeds yield in both seasons.

A positive linear relationship was obtained between saline irrigation water levels and spraying GA_3 and PGPR on seeds yield changes in both seasons (Figure 2). The correlation coefficient values r^2 0.74, 0.74, 0.51, and 0.08, respectively in the first season and 0.95, 0.87, 0.68, and 0.46, respectively in the second season. The positive relationship indicated that there is a high reduction in seeds yield with increasing salinity level, this reduction reduced by spraying treatments especially GA_3 in both seasons. At low saline irrigation level, there is an increase in seeds yield reached 131.65 and 151.72 % with using GA_3 in both seasons, successively.

Biochemical characteristics

Increasing salinity levels from 0 to 4000ppm caused a gradual decrease in chlorophyll a, chlorophyll b, fixed oil and RWC in *Nigella sativa* L. Table (4 and 5). The most elevated salinity level recorded the least values in this respect. Furthermore, membrane permeability (Mp), proline accumulation, (CAT) and (POX) enzymes activities were gradually increased by increasing salinity concentrations. Spraying GA_3 + PGPR noticeably increased chlorophyll a and b content, fixed oil%, proline accumulation, CAT and POX enzymes activity in both seasons, while using GA_3 increased membrane permeability in both seasons. The promotion effect was observed when salinity levels were combined with GA_3 or GA_3 + PGPR treatments. The greatest

chlorophyll a and b were recorded by GA_3 without salinity for both seasons.

Furthermore, spraying GA_3 + PGPR without salinity recorded the maximum fixed oil percentage. Proline accumulation, CAT and POX enzymes activities

were pronounced when salinity treatments were combined with GA_3 + PGPR treatments and the greatest values were noted by 4000 ppm salinity level with GA_3 + PGPR. However, when salinity treatments combined with GA_3 or PGPR treatments, the lessening in RWC was retarded and the highest membrane permeability recorded at 3000 and 4000 ppm together with GA_3 or GA_3 +PGPR treatments in both seasons.

Total soluble salts

Fig (3) show a positive linear relationship obtained between used saline water and spraying GA_3, PGPR and GA_3+PGPR on values of soil EC. There are highly significant (with correlation coefficient values, r^2 =0.95, 0.97, 0.92, and 0.97 for spraying with GA_3, PGPR, and GA_3+PGPR, respectively). Moreover, significant variations in the values of soil EC after using different saline irrigation water, which increased significantly in excess of salinity concentrations of 2000 ppm, 3000 ppm and 4000 ppm diluted sea water for irrigation.

Table 5. Effect of saline irrigation water levels and treatment with GA_3 or PGPR on fixed oil%, proline and antioxidant enzyme activities of *Nigella sativa* L. plants

Seasons	**1st Season 2015**					**2nd Season 2016**				
Spraying / Salinity	**Fresh water**	**GA_3**	**PGPR**	**GA_3+ PGPR**	**Mean**	**Fresh water**	**GA_3**	**PGPR**	**GA_3+ PGPR**	**Mean**
			Fixed oil%					Fixed oil%		
Control	30.86h	34.11e	39.53b	40.13a	36.16a	29.00fg	32.74d	37.04b	38.33a	34.28a
1000ppm	31.20g	31.94g	39.05c	39.72b	35.48b	30.34e	29.69ef	35.56c	36.22bc	32.95b
2000ppm	29.58i	26.38l	28.51j	36.28d	30.19c	28.52g	24.00j	27.23h	30.37e	27.53c
3000ppm	17.56p	23.48n	27.51k	32.88f	25.36d	16.50n	21.56l	25.89i	30.34e	23.57d
4000ppm	13.49q	22.92o	24.52m	29.35i	22.57e	12.73o	19.56m	22.71k	27.48h	20.62e
Mean	24.54d	27.76c	31.82b	35.67a		23.42d	25.51c	29.68b	32.55a	
			Proline(?mol/g^{-1} FW)					Proline(?mol/g^{-1} FW)		
Control	1.71h	2.34f	2.56e	2.67de	2.32d	1.49jk	1.88gh	2.2def	2.49a-c	2.02b
1000ppm	1.72h	2.09g	2.77d	3.12c	2.42c	1.61ij	1.89gh	2.1fg	2.33c-e	1.98bc
2000ppm	1.82h	2.14g	2.7de	3.2bc	2.47bc	1.3k	1.60ij	2.1fg	2.59ab	1.90c
3000ppm	1.66h	2.09g	3.29ab	3.11c	2.53b	1.38k	1.89gh	2.08fg	2.39b-d	1.93bc
4000ppm	1.76h	2.16g	3.36ab	3.39a	2.67a	1.76hi	1.88gh	2.15ef	2.70a	2.12a
Mean	1.73d	2.16c	2.93b	3.09a		1.51d	1.83c	2.13b	2.50a	
				Antioxidant enzyme activities						
			CAT ?mol min^{-1} mg^{-1} protein					CAT ?mol min^{-1} mg^{-1} protein		
Control	0.87l	0.93k	1.16h	1.44f	1.10e	0.77j	0.87hi	0.97fg	1.18d	0.95d
1000ppm	0.96jk	1.21h	1.58de	1.86b	1.40cd	0.80ij	0.92gh	1.32c	1.47b	1.13c
2000ppm	0.98ij	1.26g	1.59de	1.90ab	1.43c	0.87hi	1.01ef	1.32c	1.59a	1.19b
3000ppm	1.03i	1.46f	1.64d	1.88b	1.50b	0.92gh	1.07e	1.29c	1.48b	1.19b
4000ppm	1.31g	1.57e	1.74c	1.95a	1.64a	1.04ef	1.24cd	1.46b	1.62a	1.34a
Mean	1.03d	1.28c	1.54b	1.81a		0.88d	1.02c	1.27b	1.47a	
			POX ?mol min^{-1} mg^{-1} protein					POX ?mol min^{-1} mg^{-1} protein		
Control	11.67l	14.05jk	15.19h-j	17.88fg	14.69e	11.07l	13.52i-k	14.23h-j	16.23f-h	13.76d
1000ppm	13.25k	15.47hi	18.48e-g	19.56de	16.69d	12.14kl	13.71i-k	17.34d-f	18.29de	15.37c
2000ppm	14.30i-k	17.61g	20.52d	21.97c	18.60c	13.19jk	16.15f-h	18.56d	20.84c	17.19b
3000ppm	15.61h	18.00fg	22.48c	25.45b	20.39b	14.34h-j	16.52e-g	23.16ab	22.59bc	19.16a
4000ppm	17.41g	19.05ef	24.86b	27.89a	22.30a	15.30g-i	17.26d-g	22.59bc	24.66a	19.95a
Mean	14.44d	16.84c	20.31b	22.55a		13.21d	15.43c	19.18b	20.52a	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range tests.

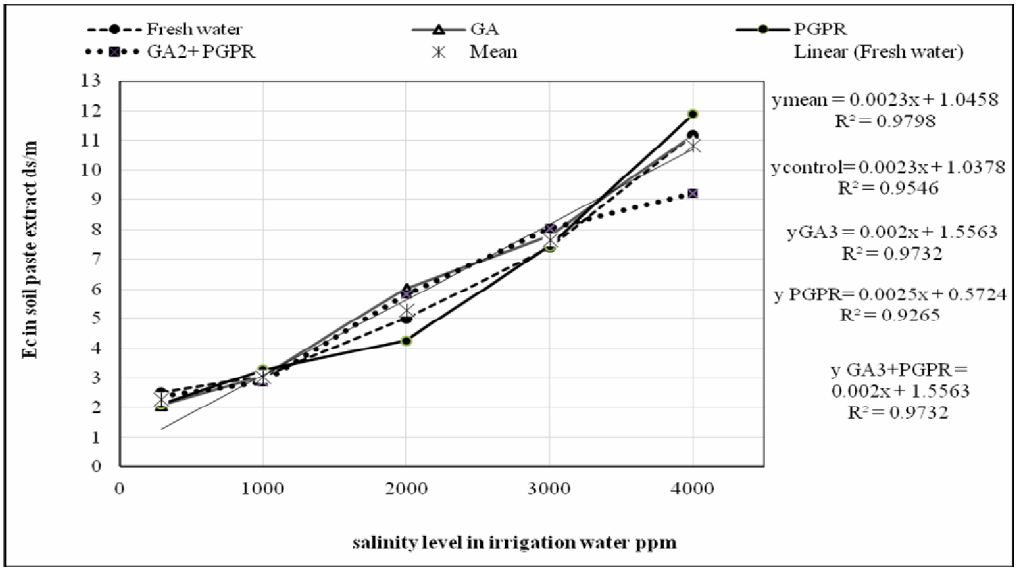


Figure.3. Correlation of Soil salinity at the end of experiment affected by saline irrigation water levels and foliar applications

DISCUSSION

The results reported in the present study displayed a general decline in the growth of *Nigella sativa* L. plants as far as plant height, the number of branches, plant dry weight, number of capsules, weight of capsules, 1000 seeds weight and seed yield (Tables 1, 2 and 3). The inhibition in growth parameters by salinity stress was previously reported by (Khan *et al*., 2010) on *Linum usitatissimum* L., (Bourgou *et al*., 2012) on *Nigella sativa* L. and (Saeidi-Sar *et al*., 2013) on *Phaseolus vulgaris* seedlings. Salinity can hamper plant growth by altering the water potential, increasing the ion toxicity, impeding the cell division besides cell expansion, or causing an ion imbalance (Arshi *et al.*, 2005). In this perspective, (Younis *et al*., 2010) stated that the growth reduction initiated by salinity stress due to inhibiting apical growth in plants in addition to an endogenous hormonal imbalance. In both situations, the reduction could have been produced by the lethal effects of ions (Na^+ and Cl^-) on metabolism or from adverse water relations.

In our study, the growth features of black cumin under salinity stress was effectively enhanced with GA_3 supplement, however, the harmful influences of salinity was strong enough to hamper plant growth because of a decrease in gibberellin production. Therefore, the addition of gibberellic acid might increase seedling growth by enhancing endogenous gibberellin content as mentioned by (Rodr?guez *et al.*, 2006). Furthermore, the improvement of growth rate by gibberellin might result in an enlargement of leaf area, the motivation of cell division and/or cell elongation, stimulation of photosynthetic rate, modified partitioning of photosynthates, or in their combination. The GA_3-mediated invertase activity in elongating shoots could result in an accumulation of hexoses which considered important for the primary cell wall biosynthesis, accordingly enhancing seedling growth beneath stress condition (Saeidi-Sar *et al*., 2007). Enhancing plant growth under salinity stress by GA_3 has formerly been reported by (Ashraf *et al*., 2002, Khan *et al.*, 2010) on wheat and *Linum usitatissimum* L plants.

Increasing levels of saline irrigation water produced a clear reduction in branches number, capsules number, dry weight of plants, seed yield, capsules yield and RWC of black cumin; nevertheless, the decline in the aforementioned organs was partially overcome by PGPR . The positive effect of PGPR on plant growth may be attributed to that PGPR motivation plant in growth and productivity *via* direct or indirect mechanisms. Direct mechanisms include plant hormone creation, improved iron accessibility, phosphorus solubilization and nutrient mobilization are a portion of the direct methods of growth development by PGPR . Indirect growth promotion happens when PGPR encourage plant growth by improving growth-restricting conditions.

Production of antagonistic materials to eliminate specific destructive microbes from roots vicinity and initiation of systemic resistance provides fortification against pathogens so improving growth-promoting conditions as reported by (Pierson and Thomashow, 1992 and Weller *et al*., 2002). Our results are in harmony with data from other researchers. They reported that the foliar treatment of PGPR had a considerable effect on alleviation salt stress, PGPR from medicinal plants such *Withania somnifera*,

Catharanthus roseus, *Coleus forskohlii*, *Ocimum sanctum* and *Aloe vera* have been stated to increase growth and yield (Attia and Saad, 2001; Karthikeyan *et al.*, 2008).

The decrease in *Nigella Sativa* L. root parameters (Table 2) such weight decline as a result of salinity stress is formerly documented on *Phaseolus vulgaris* L., (Saeidi-Sar *et al.*, 2013) on linseed (Khan *et al*., 2010)

Ashraf *et al*., (2002) reported that fresh and dry weights of roots, were decreased with increasing salt amount for *Triticum aestivum* L. Applying GA_3 clearly improved black cumin root growth this confirms earlier reports on various plant species. Saeidi-Sar *et al*., (2013) found that common bean seedlings were less affected as a result of GA_3 applications and almost exhibited no root growth reduction under salty conditions, but GA_3 increased linseed root dry weight under salt stress (Khan *et al*., 2010). Moreover, the gibberellic acid treatment caused a significant effect on fresh and dry weight of both spring wheat cultivars (Ashraf *et al*., 2002). In addition applying PGPR generally resulted in an obvious increase in *Nigella sativa* L. root growth and the inhibitory impact of salinity stress was fully ameliorated particularly at low salinity level. Our findings are in harmony with many authors who revealed that PGPR had a significant impact on alleviation of salt stress. Egamberdieva *et al*., (2013) stated that PGPR significantly improved root length, shoot length and total biomass of *Silybum marianum* (milk thistle) plants subjected to salt stress after using *Pseudomonas extremorientalis* TSAU20 by producing auxin, exopolysaccharide, biofilm creation, as well as Saravanakumar and Samiyappan, (2007) found that applying PGPR increased salt tolerance of *Arachis hypogaea* through lowering ethylene production, auxin production, exopolysaccharide. On the same plant (Nautiyal *et al*., 2013) showed an increment in fresh biomass, total length and root length over control under salt stress by using PGPR through the manufacture of NH_3, siderophore, chitinase, HCN, IAA production and phosphorus solubilization.

As our data revealed, a decrease in chlorophyll content in relation to the undesirable effect of prolonged saline water stress (Table 4) which might be anticipated to a reduction in the uptake of minerals such as Mg and N required for chlorophyll biosynthesis or membrane deterioration (Sheng *et al*., 2008). In addition to the uncertainty of protein complexes and damage of chlorophyll by the raised activity of chlorophyll-degrading enzyme chlorophyllase under stress circumstances (Reddy and Vora, 1986). Numerous reports proved that leaves total chlorophyll content was lessened by rising salinity level (Tuna *et al*., 2008; Shoresh *et al.*, 2011; Celik and Atak, 2012). The results also, indicated that PGPR treatment support greater chlorophyll concentration under saline situation and these findings were consistent with several authors who reported that PGPR increase chlorophyll content in mung bean plants (Dutta *et al*., 2005) and maize (Nadeem *et al.*, 2007), (Nabti *et al*., 2010) reported that inoculation durum wheat (*Triticum durum* var. Waha) with the rhizosphere bacterium *azospirillum brasilense* under saline environments increased chlorophyll content. Moreover, the obtained results showed the beneficial effect of GA_3 treatment on chlorophyll content under saline situations which is in agreement with (Misratia *et al*., 2013) who mentioned that GA_3 increased photosynthetic capacity an essential feature for greater dry matter synthesis in rice salt-stressed plants. Also, applying GA_3 increased chlorophyll levels for both spring wheat cultivars (*Triticum aestivum* L.) exposed to salinity circumstances (Ashraf *et al.*, 2002). On chamomile plant, chlorophyll degradation occurred by salinity was prohibited by using GA_3 (Ali and Hassan, 2014). Furthermore, spraying the vegetative parts of maize, wheat, cotton, broad and parsley plants with GA_3 increased pigments content (Abd El-Samad and Shaddad, 2014). This because the role of GA_3 for the inhibition of pigment degradation or motivation of protochlorophyllide synthesis by phytohormons (Pazuki *et al*., 2013) and this may be a vital part of a defense versus salinity stress.

Our results display that salinity stress induces membrane permeability changes (Table 4) which are in agreement with results achieved by NaCl application (Ali and Hassan, 2014) on chamomile plant. Additionally, when salt-stressed maize inoculated with PGPR, ACC deaminase comprising *Pseudomonas syringae*, *Enterobacter aerogenes* and *P. fluorescens* caused high relative water content (Nadeem *et al*., 2007) . Highest leaf (RWC) and least (MP) have been certified in wheat and barley treated with PGPR strains of *Bacillus* and *Azospirillum* (Turan *et al*., 2012). Greater cell wall flexibility and the capability to modify plant hormones are particular mechanisms induced by *Azospirillum* to combat with salinity and osmotic stress (Creus *et al*., 1998 and Bashan *et al*., 2004). On the other hand, GA_3 counteracts with salinity stress by rising membrane permeability and nutrient amounts in leaves which finally leads to superior seedling growth, shoot, root and whole biomass (Iqbal *et al*., 2012). Also, (Ali and Hassan, 2014) found that membrane stability index for chamomile plants was prevented when salinity treatments were combined with GA_3.

Proline accumulation in plant tissues is a valuable physiological reaction to counterbalance saline stress.

Proline performs a defensive function against salinity disorders in plants (Verbruggen and Hermans, 2008). The significantly improved proline levels located in *Nigella Sativa* L. plants during harsh salt stress (Table 5) reflect this response. Such proline accumulation in consequence of salt stress is well documented (Nabti *et al*., 2010; Ali and Hassan, 2014 and Shao *et al*., 2015). As our data indicated, salinity mitigation by GA_3 may occur through its effect on proline metabolism *via* regulating N accumulation (Iqbal and Ashraf, 2013). In addition (Tuna *et al*., 2008) reported that foliar treatment of GA_3 improved proline content which lessened antagonistic impacts of salinity by maintaining membrane permeability, increasing macro and micronutrient levels. This superior gathering of proline could characterize a major biochemical adaptation in plants osmotic adjustment (Siddiqui *et al*., 2008).

An increment in CAT and POX enzymes activities we noticed with rising salinity levels (Table 5). Also, a secondary aspect of salinity in plants is the stress-induced creation of reactive oxygen species (ROS) (Manchanda and Garg, 2008). The enriched production of (ROS) through salinity stress lead to the advanced oxidative damage and finally cell death and growth suppression (Ruiz-Lozano *et al.*, 2012). Thus, to keep metabolic tasks under stress, the scavenging of ROS is required. ROS scavenging depends on detoxification method offered by antioxidant enzymes (CATand POX).Under salt stress, plants displayed the enhanced amount of enzymes activities (CAT and POX), contrasted with their control. GA_3 may likewise enhance salinity tolerance by keeping up enzyme activities. It is, accordingly, possible that foliar utilization of GA_3 could be a helpful tool in supporting great seedling growth and establishment under salty conditions. Moreover, exogenous treatment with growth hormones may possibly be beneficial to return metabolic activities toward their regular levels (Iqbal *et al.*, 2012). Also, (Tuna *et al*., 2008) stated a similar impact of GA_3 on the antioxidant levels .The findings showed that foliar treatment of GA_3 was observed to be efficient in lightening the unfavorable impact of salt stress by improving antioxidants activity which is consistent with (Ali and Hassan, 2014) on chamomile and (Saeidi-Sar *et al*., 2013) on *Phaseolus vulgaris* L. seedlings. PGPR is also reported to protect the plants from saline disorders by decreasing membrane destabilizing activity in the cell (Khan and Panda, 2008). Moreover, PGPR improves ROS-scavenging enzymes such as catalase and ascorbate peroxidase (Kohler *et al*.,2010 and Gururani *et al*., 2013).

The increase in total soluble salts (Figure 3) may be proportional to the salts from saline irrigation water these results agree with those obtained by (Mostafazadeh-fard *et al*., 2007 and Noufal *et al*., 2008). However, utilizing freshwater and 1000 ppm sea water decreased soil EC by 39 and 18%, respectively compared to soil EC before planting this anticipated by addition of leaching requirements (Mostafazadeh-fard *et al*., 2007 and Mostafazadeh-Fard *et al*., 2008). The values of EC differ between spraying treatments; the greatest values of soil EC were recorded after using GA_3 spraying treatments.

As a conclusion, salinity treatments negatively influenced the growth and yield characters of *Nigella sativa* plants. Under salinity treatments relative water content, chlorophyll content and fixed oil percentage were decreased. However, proline, membrane permeability, enzyme activities and total soil salts were increased. Meanwhile, GA_3 or PGPR treatments lightened the harmful impacts of salinity on the formerly declared parameters. GA_3 or PGPR treatment increased proline content and activities of CAT and POX which may consider promising mechanisms for salinity alleviation in *Nigella sativa* L. plant.

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الملخص العربي

تخفيف الاجهاد الملحى لحبة البركه بالجبريللين والريزوبكتريا

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تعتبر الملوحة احد اهم الاجهادات الخطيرة للنباتات مما يؤثر على العمليات الاخرى مثل الاجهادات التاكسدية والتى تؤدى فى النهاية لموت الخلايا. تم اجراء تجربة أصص خلال موسمى ٢٠١٤/٢٠١٥ ؛ ٢٠١٥/٢٠١٦ في المزرعه التجريبيه بمحطة بحوث البساتين بسخا لدراسة امكانية تقليل التأثيرات الضاره للملوحه باستخدام الرش بالجبريللين والازوسبيريلم والازوتوباكتر على حدة او باضافه الجبريللين والازوسبيريلم والازوتوباكتر معا على النمو الخضرى والمحصول والتركيب الكيماوى والنسبه المئويه للزيت الثابت لنبات حبة البركه وكانت معاملات الملوحه عباره عن ماء عادى كنترول وماء بحر مخفف الى ١٠٠٠، ٢٠٠٠ ، ٣٠٠٠، ٤٠٠٠ جزء في المليون وتم استخدام الجبريللين بتركيز ١٠٠ جزء فى المليون والريزوبكتريا بتركيز ١٠ % وقد أدت معاملات الملوحه الى تقليل طول النبات وعدد الأفرع والوزن الجاف للنبات وعدد الكبسولات وعدد الجذور لكل نبات و الوزن الطازج والجاف للجذور ومحصول الكبسولات والبذور ووزن ١٠٠٠ بذره بالمقارنه بالكنترول كما أدت الملوحه لتقليل تركيز الكلوروفيل والنسبه المئويه للزيت الثابت والمحتوى النسبى للماء ومع ذلك ازداد محتوى البرولين ونشاط انزيم البيروكسيديز والكتاليز ونفاذية الغشاء والمحتوى الكلى للأملاح الذائبه في التربه بالمقارنه بالكنترول . وقد أدى استخدام الجبريللين والريزوبكتيريا لتخفيف التأثيرات الضاره للملوحه. الزياده في نشاط الانزيمات وتراكم البرولين يرجع الى الجبريللين والريزوبكتريا والذى اقترح كجزء من دفاع النبات ضد الملوحه فى نبات حبة البركه ولتخفيف التأثيرات غير المرغوبه توصى الدراسة بالرش بالجبريللين بتركيز ١٠٠ جزء فى المليون أوالريزوبكيتريا بتركيز ١٠ %.