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Response of *Calendula Officinalis* L. which grown in saline soil to Plant Growth Promoters and Some Organic Substances

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Abstract: Two factorial experiments in split plot design were carried out during the two successive growing seasons of 2011/2012 and 2012/2013 at the farm of Soils, Water and Environ. Res. Inst., Agric., Res. Center in Sahl El-Hossynia, EL-Sharkia Governorate; Egypt to investigate effects of some salinity tolerants,[*Azotobacter chroococcum, Azopirillum lipoferum, Bacillus polymyxa, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas fluorescens* and the white rot fungus*(Pleurotus columbines*)] as well as plant growth promoters (PGP) alone or with different organic treatments as Cattle manure (CM) at 20m^3/fed., ascorbic acid (ASC) at 100 ppm, Humic acid (HA) at 100 ppm as foliar spray and their interactions under saline sodic soil conditions on Marigold (*Calendula officinalis* L.) vegetative growth, flowering growth, chemical analysis and chemical properties of soil.

Gradual and significant increases in plant height (cm), number of branches, leaves and inflorescences/plant, leaf area of medium leaf of main stem and fresh and dry weights of shoots, roots and inflorescences per plant (g), diameter of inflorescences (cm) and chemical analysis as, chlorophyll (A&B), total carotenoids in leaves and ray flowers, total carbohydrates, N,P,K percentage in leaves and nitrogenase and dehydrogenase activities of the soil were recorded increasing with the tested treatments especially CM+ ASC+HA with (PGP) followed by that the treatment CM+HA with (PGP) and CM+ASC with (PGP) respectively. While these treatments decrease the proline content in fresh leaves, Ph and EC of the soil, in soil were enhanced by all tested treatments specially in case of organic matter treatments with PGP which reflected on the quality and quantity of marigold plants.

Keywords*:* Marigold, *Calendula officinalis* , PGP, ascorbic acid, Himic acid, saline soil.

Introduction

Soil salinity has become a serious environmental problem which affects on growth and productivity of many crops. High salt content in the soil affects on soil porosity and also decreases the soil water potential that results in a physiological drought. High salt content also affects on the physiology of plants, both at the cellular as well as whole plant levels[1].

Salinity is a common abiotic stress factor seriously affecting on crop production in different regions, particularly in arid and semi-arid regions. It is estimated that over 800 million hectares of land in the world are affected by salinity[2]. In most of the cases, the negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Although both Na^+ and Cl^- are the major ions which produce many physiological disorders in plants, Cl^- is the most dangerous[3]. Salinity at higher levels causes both hyper-ionic and hyper-osmotic stress and can lead to plant demise. The outcome of these effects may cause membrane damage,

nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leads to plant death[4].

Marigold (*Calendula officinalis* L.) is an annual herbaceous plant. It belongs to the family Asteraceae (Compositae). Ancient cultures recognized and used the healing properties of calendula. In some of the earliest medical writings, Calendula was recommended for treating ailments of the digestive tract. It was used to detoxify the liver and gall bladder. The flowers were applied to cuts and wounds to stop bleeding, prevent infection and speed healing. Calendula was also used for various women's ailments, and to treat a number of skin conditions. Calendula flowers were used on the battlefields in open wounds as anti-hemorrhagic and antiseptic, and they were used in dressing wounds to promote healing[5]. [6]mentioned that an oleanane-type triterpene glycosides that isolated from the flowers of marigold were used as anti-inflammatory, anti-tumer-promoting and cytotoxic activities. Furthermore, upon evaluation of the cytotoxic activity against human cancer cell effects against colon cancer, leukemia, and melanoma cells.

Marigold flowers are a rich source of natural carotenoids, especially beta-carotene that used in food processing to retain the orange natural color and as a supplement for vitamin A precursor. So this plant has been added to butter, margarine beverages, ice cream beverage powder, yoghurt and soup as a coloring agent[7].

Plant growth promoters (PGP) are root associated bacteria improve plant growth when artificially introduced onto seeds, seed pieces, roots, or into soil. PGP may improve plant growth and yield by direct and indirect mechanisms[8]. PGPR can also protect plants from the deleterious effects of some environmental stresses including drought and phytopathogens[9]. It can enhance the availability of nutrients to the host plant, lowering of the ethylene level within the plant or by the enhanced production of stimulatory compounds, as plant growth regulators[10]. Different PGPR including associative bacteria such as *Azopirillum, Bacillus, Pseudomonas and Enterobacter* have been used for their beneficial effects on plant growth. Many marketable bio fertilizers are mainly based on plant growth promoting rhizobacteria (PGPR) that exert beneficial effects on plant development often related to the increment of nutrient availability to host plant. Edible macro-fungi like Pleurotus received worldwide attention for their nutritional values such as protein, carbohydrate and antioxidant components such as ascorbic acid, β-carotene and α-tocopherol. Several researches had proven that high amounts of antioxidants may prevent the oxidative stress caused by the presence of free radicals which lead to disorder in physiological effectiveness[11].

Humic acid *(HA)* could be used as one of the main organic fertilizers, which is an important component of humic substances. Humic acid are the most significant constituents of organic matter in both soils and municipal waste compost, and have a relevant role in the cycling of many elements in the environment and in soil ecological functions. According to previous investigations, humates seem to have a particular favorable effect on the nutrient supply. Therefore, application of humates was tested as an approach to improve both the nutrient balance and plant vitality, also promote growth and increase the yield and quality of plant[12]. Therefore, HA are useful for reclaimed, saline soils because they help plants to resist salinity and drought, help to establish a desirable environment for the development of microorganisms and stimulate plant growth[13].

Ascorbic acid (vitamin C) is one of the most important water soluble antioxidants in plants, acting as a modulator of plant development through hormone signaling and as coenzyme in reactions by which carbohydrates, fats and proteins are metabolized. It has some role in aspects of plant growth, such as cell division and enlargement, development of cellular wall, and other physiological procedures[14]. Application of ascorbic acid can reduce the harmful effects of salt stress and may have stimulatory effects on plants; ascorbic acid is synthesized in the higher plants and improves plant growth. It is a product of D-glucose metabolism which affects some nutritional cycle activities in higher plants and plays an important role in the electron transport system. Ascorbic acid is an essential cofactor for α-ketoglutarate-dependent dioxygenases (e.g. prolyl hydroxylases) important for formation of covalent adducts with electrophilic secondary metabolites in plants[15].

Organic farming is one of the practices to make the production system more sustainable without adverse effects on the natural resources and the environment[16]. It not only maintains soil fertility but also conserves soil moisture[17]. Organic fertilizers and their extracts enhance soil fertility via improved nutrient retention and cycling and also plays an essential role in growth and yield. The use of organic matter plays an essential role in the growth and development of Marigold plants. It positively affects on vegetative growth

parameters including plant height, stem diameter, fresh and dry shoot weight, as well as flower parameters, including flower number per plant, flower height, and flower size of the plant[18].

Considering that salinity is a major problem in the Egypt there is a need to determine the effects of salinity. In this context, the objective of the current study was to determine the relations between the treatment by ascorbic acid, humic substances, cattle manure with or without Plant growth promoters (PGP) and the growth, yield and nutrient uptake of Marigold (*Calendula officinalis* L.), under saline soil stress.

Materials and Methods

Two Field experiments were conducted in clay soil at Sahl El-Hossynia Agric. Res. Station Farm in EL-Sharkia Governorate; Egypt to study the effect of Plant growth promoters (PGP) with different organic treatments on marigold cultivated in saline soil. Some physical and chemical characteristics of the studied soil and irrigation water are presented in Table (A&B) respectively, according to[19].

The experiments were carried out during two successive seasons 2011/2012 and 2012/2013, on Marigold (*Calendula officinalis* L.). The seeds of marigold were sown on September 18th and 20th 2011 and 2012, respectively. The seedlings were transplanted after 40 days in both seasons and planted in plots 2.0 ×2.5 m with 3 rows/ plot in hills at 30 cm apart within the same row. Each plot contained 21 plants. The experimental design was a split plot design with three replicates. Plant growth promoters (PGP) was set at the main plots while, organic treatments were set at the sub plots included; cattle manure *(CM)* at 20 m^3/fed., Ascorbic acid (*ASC*), Humic acid *(HA)* at 100 ppm, for each one and their interactions. So, the experiment implicated 16 interaction treatments.

The cattle manure *(CM)* was obtained from the same farm was added in one dose, which was incorporated into the soil to a depth of 15-20 cm, two weeks before transplanting date (on 13–th and 19th Novamber 2011 and 2012, in the first and second seasons, respectively).The physical and chemical characteristics of the cattle manure are presented in Table (C) described by[20].

ASC and *HA* (Al drich Chemical Co., Ltd., Egypt) were dissolved in tap water at the previously mentioned concentrations, and they were applied as foliar sprayer. Plants were sprayed till run off using hand sprayer and Nestapon as wetting agent at a rate of 1 ml/ L was used. Treated plants were received three sprays throughout the growing season. The first spray was done after transplanting directly. The 2nd and 3rd sprays were conducted to enhance plant growth monthly.

Table (A) The main physical and chemical properties analyses of experimental soil

Course sand (%)	Fine sand (%)	Silt(%)	Clay(%)	Soil Texture	OM(%)	$CaCO_3$(%)
2.85	38.37	12.14	46.64	Clay	0.59	10.33

pH (1:2.5)	EC*(dSm^{-1})	Cations (meq/l)				Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SO^{-}_4
8.34	15.38	10.85	14.63	175	1.30	10.29	172	22.33

Available Macronutrients (mg/kg)			Available Micronutrients (mg/kg)			
N	P	K	Fe	Mn	Zn	Cu
30	3.55	196	1.73	2.33	0.72	0.080

Table (B). Chemical analysis of irrigation water.

pH (1:2.5)	**EC (dSm^{-1})**	**Sodium Adsorption Ratio(SAR)**
8.25	1.21	4.55

Macro-micronutrients (mg/L)						
NO_3 -N	NH_4 -N	P	K	Fe	Mn	Zn
20.04	10.52	2.88	6.83	1.90	2.20	0.77

Table (C): Physical and chemical characteristics of the used cattle manure

The character	1st season	2ed season
Weight of 1 m^3 (kg)	480	475
Moisture content (%)	9.12	9.05
Organic Matter (%)	62.72	62.21
Organic Carbon (%)	30.88	32.26
Total N (%)	1.55	1.53
C:N ratio	12.3:1	14.5:1
Total P (%)	0.27	0.60
Total K (%)	1.06	1.12
Fe (ppm)	1880	2051
Mn (ppm)	188	180
Zn (ppm)	89.9	160

Plant growth promoters (PGP) :

Plant growth promoters (PGP) are root associated bacteria included *Azotobacter chroococcum*, *Azopirillum lipoferum, Bacillus polymyxa, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas fluorescens* and the white rot fungus *Pleurotus columbinus* were kindly supplied from Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Agriculture Research Center (ARC), Ministry of Agriculture, Giza, Egypt. These PGPR were grown on maximum density in Difico nutrient broth medium[21] for 24 hrs. Marigold seedlings were soaked for 30 minute before planting in the PGP culture. The plants after transport, were sprayed with these PGP until flower stage.

-Isolation of rhizospheric bacteria:

Ten grams of rhizosphere soil were suspended in 90ml of sterilized water in conical flask (250ml), thoroughly shaked for 10 minutes, and dilution series up to(10^7) were prepared. Dilutions from each soil sample were plated on King' s Media[22]**.** Plates were incubated at 28^0C ± 2 for 1-3 days then individual colonies were picked up, purified and microscopically examined for morphological characterestics. Isolates were maintained on nutrient agar at 4^0C. Subculture of the purifird isolates was done monthly.

Pleurotus columbinus source and growth condition:

The white rot fungus (*Pleurotus columbinus*) was obtained from the Unit of Mushroom Production , Faculty of Agriculture , Ain Shams University .*Pleurotus columbinus* was grown on potato dextrose medium, in a rotary shaker at 200 rpm for 7 days[23]. After propagation , the growth and its medium was put in an electric mixer , then filtered and kept at 4 ^{o}C till field application.

Quantitative determination of plant growth promoting substances in culture media:

Azotobacter chroococcum, *Azopirillum lipoferum, Bacillus polymyxa, Bacillus subtilis, Klebsiella pneumonia* and *Pseudomonas fluorescens* isolates were tested for their quantitative capabilities to produce plant growth promoting substances i.e. indole acetic acid (IAA) and gibberellins (GA) according to[24] which shown in Table (D).

Table (D): Some characteristics of plant growth promoting in the study

Strain	N_2-ase µmole C_2H_4/ml/hr	Phytohormons (µg/L culture)		
		Indole acetic acid	Gibberellins	Cytokinins
Azotobacter chroococcum	286.5	69.3	100.2	14
Azopirillum lipoferum	60.23	4.4	80.1	29
Bacillus polymyxa	56.18	33.5	98.2	97
Klebsiella pneumonia	54.49	19.6	96.5	20
Bacillus subtilis	----	192.0	85.1	19
Pseudomonas fluorescens	-----	163.0	509	21

Recorded Data:

Marigold (*Calendula officinalis* L.) plants were harvested in six picks in the two experimental seasons, the inflorescences were taken every 13 days started at (3ed March,16th March, 29th March,11th April, 24th April and 7th May) when 80% of the flowers were opened. The recorded data were implicated:

Vegetative parameters: plant height (cm), Number of branches/plant, Number of leaves/plant, leaf area of median leaf of main stem and fresh and dry weights of shoots and roots / plant (g).

Flowering parameters: Number of inflorescences/plant, diameter of inflorescences (cm) and fresh and dry weights of inflorescences(g).

Chemical analysis: Chlorophyll (a& b) and total carotenoids contents (mg/gm fresh matter) were determined in leaf samples according to[25]**.** Total carotenoids in air-dried ray flowers was determined using the method described by[26]**.** Leaves samples were taken for both tested seasons and were dried at 70 ^{o}C for 72 hours and finely grounded, then determine for total carbohydrates percentage as described by[27]**.** Total nitrogen was determined using the modified micro Kjeildahl as described by[28]**.** Phosphorus was determined according to[29]**.** Potassium was determined using the atomic absorption spectroscopy[30]**.** Free proline was determined according to[31]**.**

Marigold rhizosphere soil was sampled after harvest plants in each season to determine the soil biological activity in terms of Dehydroengenase enzyme (DHA) activity[32], Nitrogenase enzyme (N_2-ase) activity of rhizosphere according to[33] and determination of pH, EC and available N,P, and K[34]**.**

Statistical Analysis

The collected data were subjected to statistical analysis according to[35]**.** Mean separation was done using least significant difference test at 5% level (LSD 0.05).

Results and Discussion

I-Vegetative growth characteristics:

Results represented effects of organic applications, Plant growth promoters (PGP) and their interactions during the two tested seasons are recorded in Tables (1-4).

Respecting that the application of different organic treatments had a considerable effect on the different vegetative growth characteristics of Marigold (*Calendula officinalis* L.) plant expressed as: plant height (cm), branches No./ plant, leaves No./ plant, leaf area of medium leaf of main stem and fresh and dry weights of shoots and roots per plant (g). In most cases, application of different organic treatments promoted vegetative growth and resulted in significant increases in the values of these characteristics, compared to the untreated control plants. Gradual increases in the above mentioned traits were noticed were determined with the plants which received CM+ ASC+HA followed by that the treatment by CM+HA and CM+ASC respectively. Such results were confirmed during the two tested seasons. Similar enhancing effects on plant growth after organic applications for ASC were noticed by [36]on *Canola Okapi* and [37]on *Puccinellia distans*. While these results for HA are in accordance with those reported by [38]on bean and [39]on corn. However, these results for CM are in harmony with results by [40]on fennel,[41]on *Anethum graveolens* and [42]on *Matricaria chamomilla*.

For Plant growth promoters (PGP), there were significant, in general, enhancement effects on vegetative growth characteristics under the effect of PGP applications comparing to the plants untreated with PGP (Table 1-4). As applied with PGP these growth parameters were significantly increased and the highest values in this respect were recorded during the two tested seasons. Such result was obtained by [43]on tomato and [10]on strawberry.

Concerning, Plant growth promoters (PGP) interacted with organic treatments, significant effects on vegetative growth traits were recorded during the two tested seasons (Table 1-4). Generally, as the interacted applied organic treatments were increased under tested PGP, values of these characteristics were significantly increased. The interaction treatments of CM+ ASC+HA with PGP followed by that the treatment CM+HA with PGP and CM+ASC with PGP respectively, were the more effective in increasing the recorded marigold plant

growth parameters in (Table 1-4) comparing to the control and all other interaction treatments. This was true during the two seasons.

Table 1: Response plant height (cm) and number of branches/plant of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/ 2012 and 2012/2013 seasons

Organic Treatments (OT)	Plant height (cm)						Number of branches/plant					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)
control	22.15	25.50	23.83	20.70	24.11	22.41	2.25	3.05	2.65	2.03	2.67	2.35
CM 20m^3/fed	27.00	29.55	28.28	26.02	28.03	27.03	3.75	4.37	4.06	3.09	3.52	3.31
ASC 100ppm	30.76	32.06	31.41	29.40	30.45	29.93	4.64	5.80	5.22	4.07	5.00	4.54
HA 100ppm	35.13	38.00	36.57	33.07	35.45	34.26	5.30	6.75	6.03	5.02	5.80	5.41
CM+ASC	43.20	48.64	45.92	41.50	46.19	43.85	6.33	7.70	7.02	6.00	7.00	6.50
CM+HA	50.16	56.13	53.15	49.20	52.38	50.79	7.15	8.64	7.81	6.55	7.16	6.86
ASC+HA	40.67	45.67	43.17	38.80	43.33	41.07	6.00	7.08	6.54	5.45	6.15	5.80
CM+ASC+HA	53.19	58.67	55.93	51.00	55.33	53.17	8.33	9.70	9.02	7.33	8.10	7.72
Mean of PGP	37.78	41.78	-----	36.21	39.41	----	5.47	6.61	---	4.94	5.68	----
L.S.D. at 0.05 PGP	3.02			2.43			0.85			0.062		
OT	3.75			2.86			1.04			0.92		
Interaction PGP X OT	4.28			3.87			1.48			1.20		

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP)= Plant growth promoters

Table 2: Response number of leaves/plant and leaf area medium leaf (cm^2)of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/ 2012 and 2012/2013 seasons

Organic Treatments (OT)	Number of leaves/plant						Leaf area medium leaf (cm^2)					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)
control	20.76	25.33	23.05	17.88	21.15	19.52	70.2	86.3	78.25	64.7	72.1	68.4
CM 20m^3/fed	28.45	39.30	33.88	24.19	32.70	28.45	74.3	91.2	82.75	70.2	79.6	74.9
ASC 100ppm	37.12	46.45	41.79	31.45	40.92	36.19	86.6	95.7	91.15	78.3	87.5	82.9
HA 100ppm	45.08	56.70	50.89	40.50	51.30	45.90	100.8	105.4	103.1	86.4	92.4	89.4
CM+ASC	66.30	73.67	69.99	54.15	67.55	60.85	115.7	120.2	117.95	95.3	112.5	103.9
CM+HA	71.16	80.29	75.73	65.46	76.34	70.90	119.6	125.5	122.55	104.5	118.4	111.45
ASC+HA	53.43	65.80	59.62	47.75	60.67	54.21	108.3	112.8	110.55	87.3	102.6	94.95
CM+ASC+HA	80.45	88.30	84.38	77.06	82.75	79.91	124.3	132.5	128.4	118.6	125.3	121.95
Mean of PGP	50.34	59.48	-----	44.81	54.17	----	99.98	108.7	----	88.16	98.8	----
L.S.D. at0.05 PGP	5.61			6.35			6.43			8.15		
OT	6.43			7.21			3.52			5.48		
Interaction PGP X OT	7.07			6.01			3.85			3.52		

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters

Table 3: Response fresh and dry weights of shoots/plant (g) of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/ 2013 seasons

Organic Treatments (OT)	Fresh weight of shoots/plant (g)						Dry weight of shoots/plant (g)					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of(OT)
control	252.64	267.83	260.24	248.80	261.44	255.12	59.70	67.61	63.66	57.92	66.25	62.09
CM 20m^3/fed	263.03	284.05	273.54	258.24	280.19	269.22	62.63	70.52	66.58	60.82	71.33	66.08
ASC 100ppm	271.07	295.10	283.09	265.11	292.45	278.78	65.12	73.82	69.47	63.41	72.11	67.76
HA 100ppm	285.50	303.07	294.29	280.10	302.64	291.37	96.36	76.19	86.28	67.60	74.03	70.82
CM+ASC	305.70	320.55	313.13	300.64	317.45	309.05	74.43	80.15	77.29	73.20	79.12	76.16
CM+HA	317.72	333.64	325.68	313.12	329.28	321.20	78.40	82.45	80.43	76.83	80.37	78.60
ASC+HA	292.13	315.25	303.69	287.43	310.75	299.09	72.14	78.43	75.29	71.64	77.42	74.53
CM+ASC+HA	328.45	340.92	334.69	321.15	337.43	329.29	86.45	90.88	88.67	85.15	89.12	87.14
Mean of PGP	289.53	307.55	----	284.32	303.95	----	74.40	77.51	----	69.57	76.22	----
L.S.D. at0.05 PGP	10.57			13.65			2.14			4.45		
OT	11.24			12.06			1.75			2.53		
Interaction PGP X OT	9.07			8.74			2.04			1.81		

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters

Table 4: Response fresh and dry weights of roots/plant (g) of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/ 2013 seasons

Organic Treatments (OT)	Fresh weight of roots/plant (g)						Dry weight of roots/plant (g)					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of (OT)
control	12.33	14.82	13.58	11.50	13.76	12.63	6.55	7.81	7.18	5.68	7.55	6.62
CM 20m^3/fed	14.57	16.70	15.64	13.64	15.14	14.39	7.33	8.56	7.95	6.90	8.34	7.62
ASC 100ppm	16.22	18.45	17.34	15.86	17.40	16.63	8.37	9.72	9.05	7.68	9.60	8.64
HA 100ppm	20.12	21.06	20.59	18.43	20.00	19.22	9.06	10.15	9.61	8.40	10.34	9.37
CM+ASC	22.31	24.50	23.41	21.03	23.70	22.37	10.00	11.45	10.73	10.02	11.80	10.91
CM+HA	23.54	26.24	24.89	22.72	25.03	23.88	10.73	12.70	11.72	10.70	12.83	11.77
ASC+HA	21.82	22.66	22.24	20.25	21.10	20.68	9.55	10.80	10.18	9.06	10.63	9.85
CM+ASC+HA	25.43	28.75	27.09	23.90	26.43	25.17	12.25	14.80	13.53	11.75	13.30	12.53
Mean of PGP	19.54	21.65	----	18.42	20.32	----	9.23	10.75	----	8.77	10.55	----
L.S.D.at 0.05 PGP	2.00			1.72			0.73			1.34		
OT	1.74			1.12			0.42			0.88		
Interaction PGP X OT	1.81			1.94			0.30			0.67		

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters

The application of organic treatments and plant growth-promoters can play an important role in organic forage production. In the presence of PGP, the plant height and the number of branches were superior by 11.9 and 20.84% for cattle manure and 10.3 and 16.4% for the mixture of all treatments compared to the absence of PGP, respectively. Also, number of leaves exceeded by 9.7% while surpassed in the case of leaf area of medium by 6.7%. While, the fresh weight of shoot/plant rise from 252.64 g in the control into 328.45 g in the mixture of all treatments without PGP while the weight of fresh shoot/plant rise from 267.83 g in the control to 340.92 g in the mixture of all treatments with PGP. The increasing ration in the two cases was almost 1.3 fold. The same trend was occurred for dry weight of shoot/plant where the increased rat in the mixture of all treatments averaged between 1.4 fold in the case of without PGP and 1.3 fold in the case of with PGP.

II-Flowering growth characteristics:

The effects of organic applications, Plant growth promoters (PGP) as well as the combinations of these two types of fertilizer, during the two tested seasons are obtained in Tables (5-7). Data in Table 5 present that the highest Value of weight inflorescences/plant was obtained in the fourth pick at 11th April followed by fifth pick at 24th April.

Regarding the effect of different organic treatments the data in Tables (5-7) show that, in the both seasons, the different organic treatments increased number of inflorescences/plant, diameter of inflorescences (cm) and fresh and dry weights of inflorescences (g), compared to the control. These treatments gave a significant increase in these parameters. The highest values were obtained in plants fertilized with CM+ ASC+HA followed by that the treatment by CM+HA and CM+ASC respectively. The positive effect of ASC in accordance with the results obtained by [37]on *Puccinellia distans* and [36]on *Canola Okapi*. While the results of HA are in agreement with [39]on corn and [38]on bean. The results for CM are in harmony with results by [40]on fennel, [42]on *Matricaria chamomilla* and [44]on Indian spinach.

The data in Tables (5-7) also show that in the both seasons, plants receiving the Plant growth promoters (PGP) treatment gave significantly effect on number of inflorescences/plant, diameter of inflorescences (cm) and fresh and dry weights of inflorescences (g), than the control. Application of PGP caused a steady increase in these parameters. These values were insignificantly higher than those recorded without PGP These conclusions are in agreement with the findings of Similar works on growth enhancement under saline stress was reported by [9]on *Cicer arietinum* L., [45]on (*Ociumum basilicum L.*) and [46]on tomato

Concerning the interaction between the effects of Plant growth promoters (PGP) and organic treatments, the data in the Tables (5-7) show that in the both seasons, this interaction was significant. The plants which receiving treatments without PGP treatments had the lowest effect on the investigated parameters .The combination CM+ ASC+HA with PGP resulted in the highest significantly effect, followed by that the treatment CM+HA with PGP and CM+ASC with PGP respectively. Among plants receiving treatments of both PGP and another organic treatments, had the lowest effect on these parameters. In the case of diameter of inflorescences, the mixture of all organic treatments without PGP reached to 2.5 fold of control, while with PGP the increase reached to 3.3 fold of control. The mixture of all organic with PGP matter was superior to without PGP by 1.4 fold. In addition that the increase rate caused by that treatment without PGP compared to control in the fresh weight was 2.0 folds while with PGP was two folds in the first season.The mixture of all organic matter with PGP was superior to without PGP by 6.25%. These results are in harmony with those reported by [47]on *Sorghum almum* and [48]on *Lactuca sativa L.*

Table 5: Response weight of inflorescences/plant (g) in each pick of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/2013 seasons

Organic Treatments (OT)	weight of inflorescences/plant (g) in each pick													
	Without PGP							With PGP						
	3ed March	16th March	29th March	11th April	24th April	7th May	Total (OT)	3ed March	16th March	29th March	11th April	24th April	7th May	Total (OT)
	First - season													
control	2.30	3.07	4.18	5.10	4.72	3.67	23.04	3.09	3.52	4.36	5.46	5.02	3.95	25.40
CM20m3/fed	3.25	4.17	6.20	7.25	6.68	5.33	32.88	3.64	4.44	6.75	7.64	7.12	5.86	35.45
ASC 100ppm	3.78	5.07	6.54	7.88	7.12	5.71	36.10	3.98	5.38	7.08	8.17	7.56	6.19	38.36
HA100ppm	3.96	5.35	6.82	8.05	7.67	6.08	37.93	4.35	5.62	7.26	8.64	8.02	6.65	40.54
CM+ASC	4.47	6.22	7.35	8.96	8.15	6.86	42.01	4.92	6.48	7.95	9.37	8.44	7.15	44.31
CM+HA	4.78	6.65	7.94	9.06	8.58	7.28	44.29	5.11	7.18	8.34	9.97	8.95	7.87	47.42
ASC+HA	4.12	5.92	7.05	8.53	7.95	6.25	39.82	4.61	6.08	7.53	9.07	8.27	7.02	42.58
CM+ASC+HA	5.18	7.00	8.27	9.31	8.94	7.83	46.53	5.64	7.30	8.85	10.07	9.46	8.12	49.44
Total of PGP	31.84	43.45	54.35	64.14	59.81	49.01	-----	35.34	46.00	58.12	68.39	62.84	52.81	------
	Second-season													
control	2.00	2.36	3.22	4.46	3.81	2.95	18.80	2.57	3.02	4.10	5.03	4.43	3.65	22.80
CM20m3/fed	2.63	3.07	4.15	5.33	4.75	3.72	23.65	3.12	3.68	4.87	6.14	5.65	4.24	27.70
ASC 100ppm	3.23	3.75	5.03	6.34	5.60	4.25	28.20	3.77	4.35	5.92	7.16	6.30	5.05	32.55
HA100ppm	3.71	4.32	5.44	6.97	6.18	4.87	31.49	4.11	4.82	6.23	7.90	7.08	5.44	35.58
CM+ASC	4.27	4.93	6.14	8.13	7.02	5.25	35.74	4.55	5.23	6.84	8.62	7.73	6.07	39.04
CM+HA	4.62	5.24	7.09	8.48	7.90	6.06	39.39	4.87	5.81	7.38	9.20	8.15	6.92	42.33
ASC+HA	4.00	4.58	5.84	7.26	6.46	5.03	33.17	4.35	5.16	6.52	8.00	7.27	5.71	37.01
CM+ASC+HA	5.06	5.77	7.68	8.93	8.32	6.34	42.10	5.30	6.14	8.03	9.86	8.85	7.07	45.25
Total of PGP	29.52	34.02	44.59	55.90	50.04	38.47	-----	32.64	38.21	49.89	61.91	55.46	44.15	-----

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters

Table 6: Response number of inflorescences/plant and diameter of inflorescences (cm) of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/2013 seasons

Organic Treatments (OT)	Number of inflorescences/plant						Diameter of inflorescences (cm)					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of (OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)
control	18.84	22.15	20.50	17.14	20.66	18.90	2.20	2.60	2.40	2.00	2.24	2.12
CM20m3/fed	20.48	26.20	23.34	18.37	27.76	23.07	2.45	3.06	2.76	2.15	2.84	2.50
ASC 100ppm	23.68	30.50	27.09	20.72	27.37	24.05	2.88	3.42	3.15	2.35	3.12	2.74
HA100ppm	25.37	33.92	29.65	23.64	31.00	27.32	3.25	4.00	3.63	2.60	3.65	3.13
CM+ASC	32.75	40.45	36.60	30.55	38.00	34.28	4.15	5.10	4.63	3.25	4.48	3.87
CM+HA	36.24	43.72	39.98	34.12	41.75	37.94	4.85	6.40	5.63	3.65	5.88	4.75
ASC+HA	28.28	37.16	32.72	26.07	35.47	30.77	3.70	4.30	4.00	3.00	4.06	3.53
CM+ASC+HA	40.88	45.30	43.09	38.44	43.80	41.12	5.50	7.82	6.66	4.70	6.75	5.73
Mean of PGP	28.32	34.93	----	26.13	33.23	----	3.62	4.59	---	2.96	4.13	-----
L.S.D. at 0.05 PGP	4.53			5.08			0.43			1.01		
OT	1.65			2.95			0.12			0.19		
Interaction PGP X OT	1.20			0.94			0.10			0.07		

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters

Table 7: Impact of plant growth promoters (PGP) and some organic substances on fresh and dry weights of inflorescences / plant (g) of *(Calendula officinalis* L.) plant under salinity stress during, 2011/ 2012 and 2012/ 2013 seasons

Organic Treatments (OT)	Fresh weight of inflorescences/plant(g).						Dry weight of inflorescences/plant(g).					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)
control	23.04	25.40	24.22	18.80	22.80	20.80	11.20	12.82	12.01	10.61	11.43	11.02
CM20m3/fed	32.88	35.45	34.17	23.65	27.70	25.68	13.78	14.10	13.94	12.60	13.49	13.05
ASC 100ppm	36.10	38.36	37.23	28.20	32.55	30.83	14.15	15.45	14.80	13.02	14.36	13.69
HA 100ppm	37.93	40.54	39.24	31.49	35.58	33.54	14.47	15.70	15.09	13.53	14.51	14.02
CM+ASC	42.01	44.31	43.16	35.74	39.04	37.39	15.06	16.32	15.69	14.45	15.58	15.02
CM+HA	44.29	47.42	45.86	39.39	42.33	40.86	15.30	16.75	16.03	15.04	16.04	15.54
ASC+HA	39.82	42.58	41.20	33.17	37.01	35.09	14.78	16.08	15.43	13.86	14.89	14.38
CM+ASC+HA	46.53	49.44	47.99	42.10	45.25	43.68	16.90	17.10	17.00	15.77	16.43	16.10
Mean of PGP	37.83	40.44	-----	31.57	35.28	----	14.46	15.54	-----	13.61	14.59	---
L.S.D. at 0.05												
PGP	4.43			3.54			0.18			0.14		
OT	3.57			2.15			0.22			0.20		
Interaction PGP X OT	3.12			1.24			0.41			0.30		

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters

III- Chemical analysis:

1- Plant contents:

The results in Fig. (1-3) obtained that the effects of organic applications, Plant growth promoters (PGP) as well as treatment combinations of these two fertilizers on chemical analysis of marigold plant .

Respecting organic applications effects on chemical analysis of marigold plant, data in (Fig. 1-3) clear that all organic treatments at the two tested levels, increased chemical analysis of marigold plant expressed as: chlorophyll A, and B, total carotenoids in leaves, total carotenoids in flowers, total carbohydrates, nitrogen, Phosphorus and Potassium The highest values were recorded increases in plants received the CM+ ASC+HA followed by that the treatment by CM+HA and CM+ASC respectively. On the other hand, the same treatments gave the lowest content of free proline..Such results were confirmed during the two tested seasons. Similar enhancing effects on chemical analysis after organic applications were noticed by[49]on onion, [50]on *Hibiscus sabdariffa* and [44]on Indian spinach**.**

For Plant growth promoters (PGP), there were, in general, enhancement effects on chlorophyll A, and B, total carotenoids in leaves, total carotenoids in flowers, total carbohydrates, nitrogen, Phosphorus, Potassium and free proline. in Marigold plant under the effect of PGP applications comparing to the plants un fertilized with PGP (Fig. 1-3). As the applied PGP these parameters were increased and the highest values in this respect were recorded during the two tested seasons. But decrease the free proline content in fresh matter. These results are in line with [51]on mung bean and [52]on maize.

Concerning, Plant growth promoters (PGP) interacted with organic treatments, had effects on chemical analysis of marigold plant were recorded during the two tested seasons (Fig. 1-3). Generally, as the interacted applied organic treatments were increased under tested PGP. The interaction treatments of CM+ ASC+HA with PGP were the more effective in increasing the recorded fennel chemical analysis parameters in (Fig. 1-3) comparing to the control and all other interaction treatments followed by that the treatment CM+HA with PGP and CM+ASC with PGP respectively. This was true during the two seasons. So, the mixture of organic matters with PGP increased the chlorophyll A by 6.2 % than treatments without PGP while increased chlorophyll B by 8.9%. Also, the mixture of treatments with PGP was superior to without PGP by 10.6% for total carbohydrates. While, The first season was the best specially with PGP which increased by 8.3 %, 12.9 % and 4.7 %in percentage of nitrogen, phosphorous and potassium in leaves than without PGP, respectively. On the other hand, It was noticed that decrease of proline was due to the vital action of PGP in all treatments. The mixture of

all treatments with PGP had great effect on decreasing proline excretion. The decrease of proline content was nearly by 49 % of control in the two seasons without PGP, while the decrease was almost by 60 % of control with PGP in the two seasons. These results are in harmony with those reported by[53]on barley and [54] on (*Lycopersicum esculentum* L.) and (*Cucumis sativus* L.).

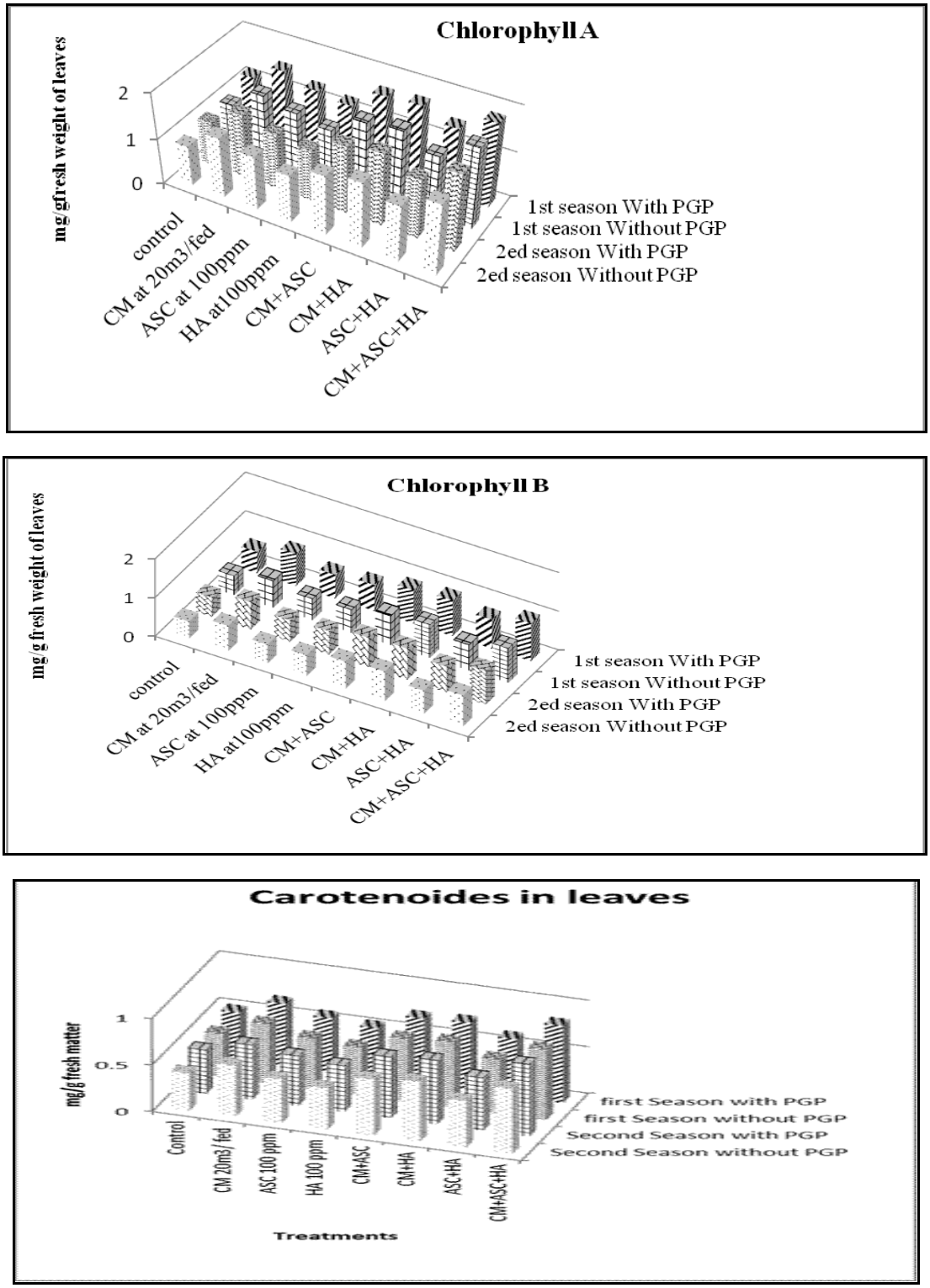
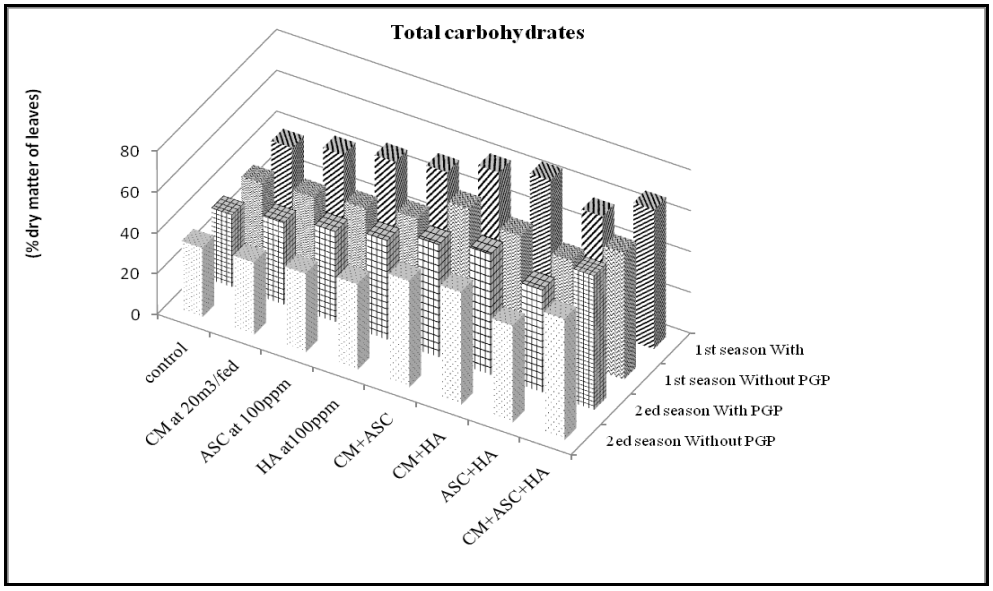
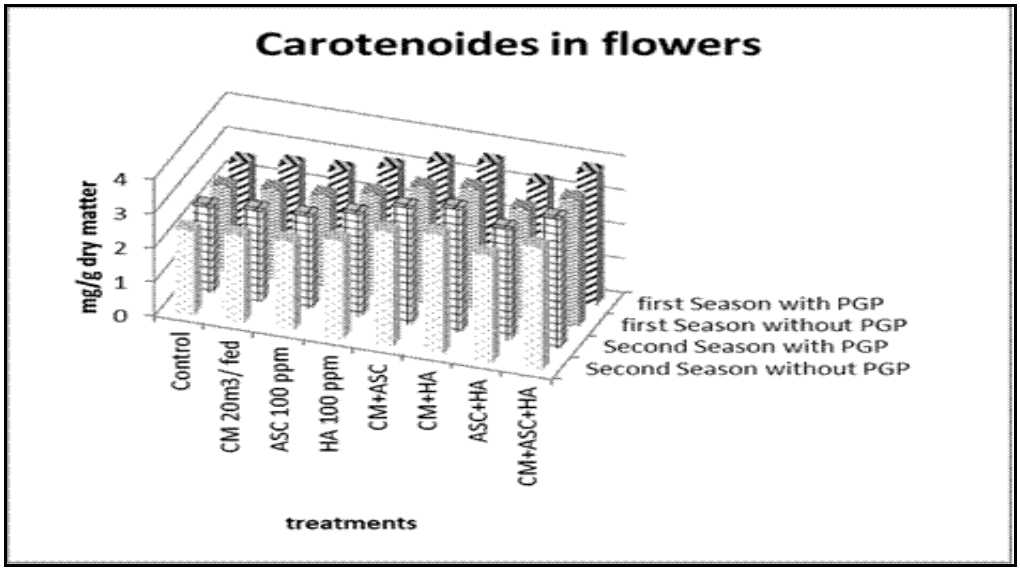


Fig. (1): Response chlorophyll A & B and carotenoids content in leaves of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/ 2012 and 2012/2013 seasons





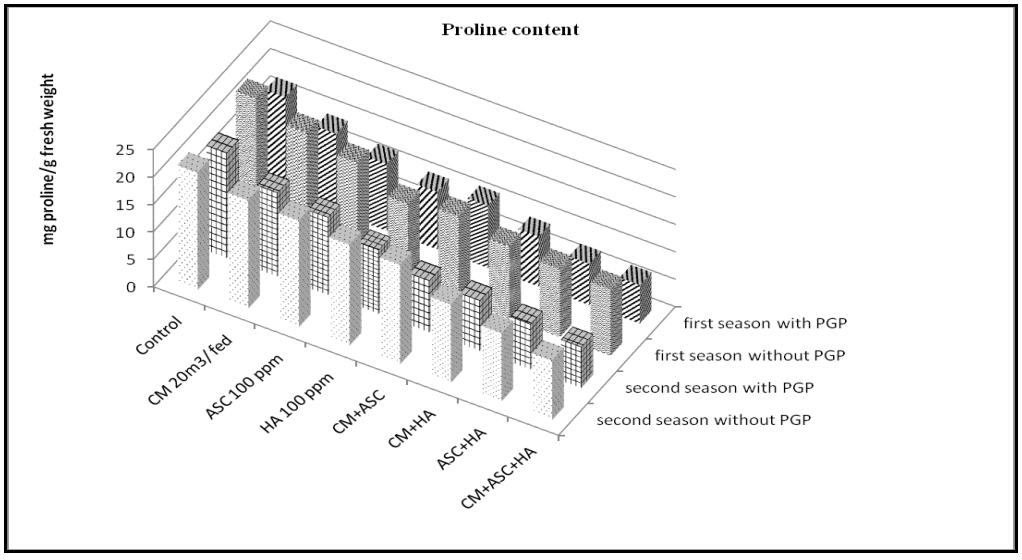
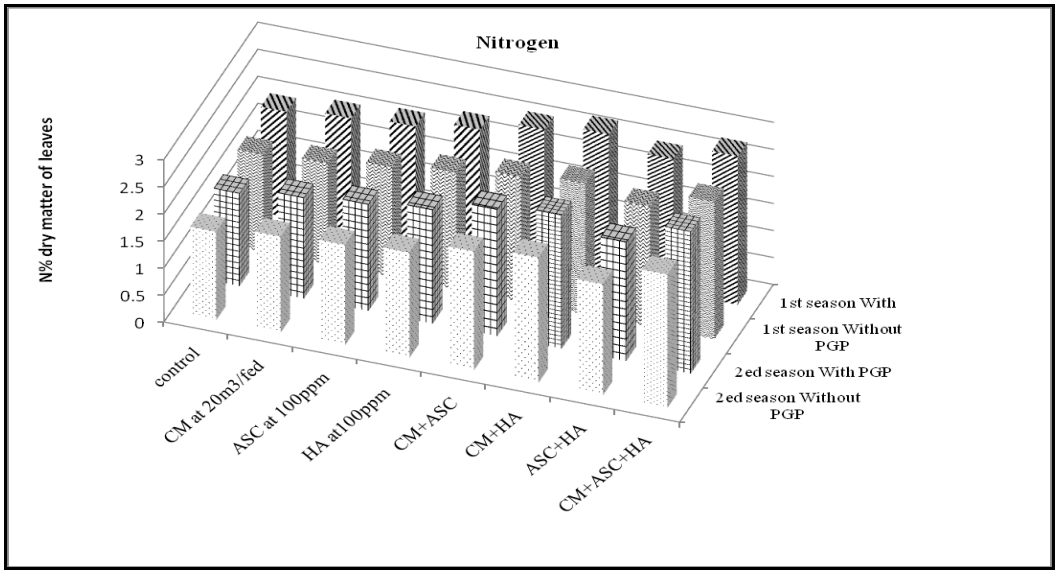
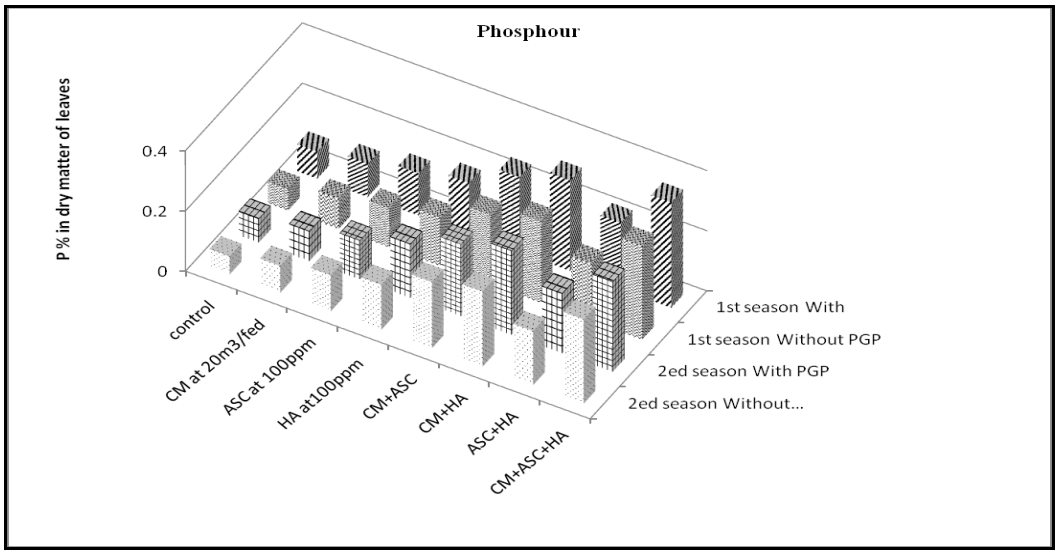


Fig. (2): Response total carbohydrates (%), proline content in leaves and total carotenoids in flowers of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/2013 seasons





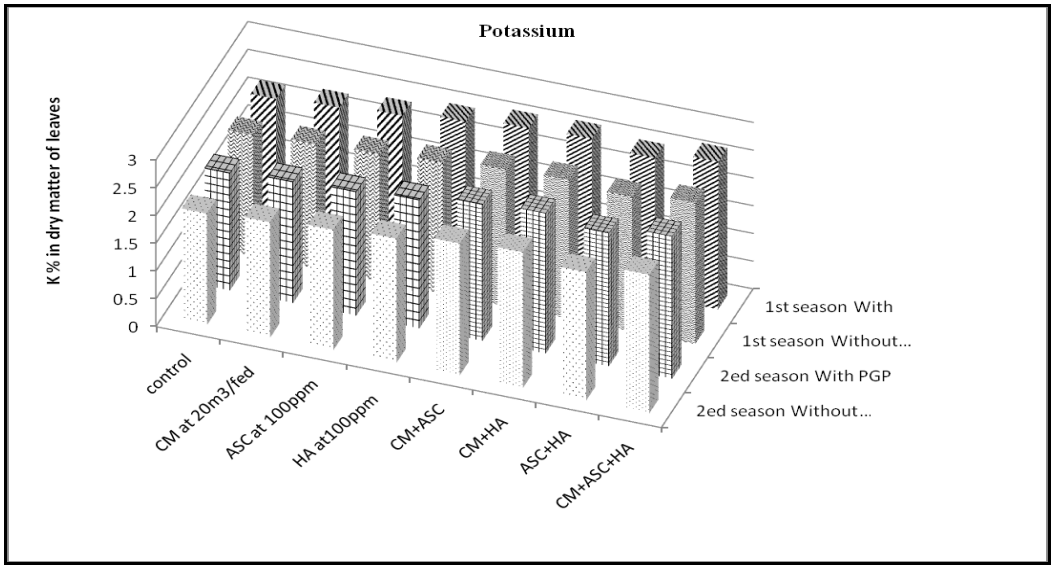


Fig. (3): Response percentage of nitrogen, phosphorous and potassium in leaves of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/2013 seasons

2-Soil properties:

The effects of organic applications, Plant growth promoting (PGP) as well as the combinations of these two types of fertilizer, during the two tested seasons are obtained in Table (8) and fig.(5-6).

Regarding the effect of different organic treatments the data in Table (8) and fig.(5-6).show that, in the both seasons, the different organic treatments had more effect on decreasing pH and EC of soil. Particularly, CM+ ASC+HA followed by that the treatment by CM+HA and CM+ASC respectively. On the other hand, the same treatments increased the soil properties as Dehydroengenase enzyme (DHA), Nitrogenase enzyme (N_2-ase) activities of rhizosphere and available N,P, and K, The positive is accordance with the results obtained by[50]on *Hibiscus sabdariffa* and [44]on Indian spinach.

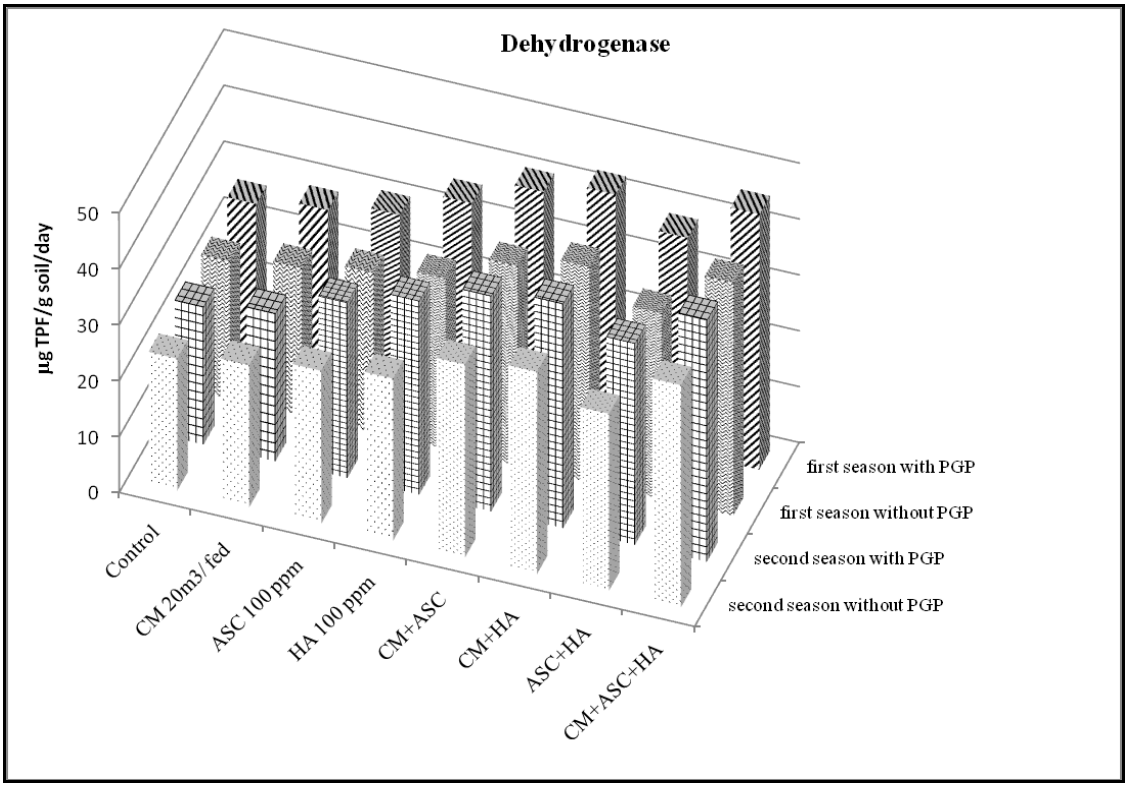
The data in Table (8) and fig.(5-6).also show that in the both seasons, plants receiving the Plant growth promoters (PGP) treatment gave more effect on Dehydroengenase enzyme (DHA), Nitrogenase enzyme (N_2-ase) activities of rhizosphere and available N,P, and K, than the control. While the same treatments caused a steady decrease in pH and EC of soil. These values were lower than those recorded withoue PGP, Table (8).These conclusions are in agreement with the findings of Similar works on growth enhancement under saline stress was reported by [51]on mung bean and [52]on maize.

Concerning the interaction between the effects of Plant growth promoters (PGP) and organic treatments, the data in the Table (8) and fig.(5-6).show that in the both seasons. The plants which receiving treatments without PGP treatments had the lowest effect on the pH and EC of soil. While the mixture of all organic treatment with PGP had positive effect on decreasing pH and EC of soil. On the other hand, Dehydroengenase enzyme (DHA), Nitrogenase enzyme (N_2-ase) activities of rhizosphere and available N,P, and K. The combination CM+ ASC+HA with PGP resulted in the highest effect, followed by that the treatment CM+HA with PGP and CM+ASC with PGP respectively. Addition PGP to the mixture of all organic treatment increased the dehydrogenase activity in the first season by 9.9 % than without PGP. Also, it was superior in nitrogenase activity than without PGP almost by 3 folds in the two seasons. On the other hand, The first season was the best specially with PGP which increased by 4.9%, 2.8% and 4.9% of nitrogen, phosphorous and potassium content in the soil than without PGP, respectively. These results are in harmony with those reported by [54] on (*Lycopersicum esculentum* L.) and (*Cucumis sativus* L.), [53]on barley, and [55]on corn.

Table 8: Response pH (1:2.5) and EC (dS m^{-1}) of the soil that *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/ 2013 seasons

Organic Treatments (OT)	PH (1: 2.5)						EC (dS m-1)					
	1st season			2ed season			1st season			2ed season		
	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of(OT)	Without PGP	With PGP	Mean of (OT)
control	7.98	7.53	7.76	8.34	8.05	8.20	15.12	13.80	14.46	15.38	14.06	14.72
CM 20m3/fed	7.30	7.21	7.26	8.07	8.02	8.05	13.64	11.53	12.59	13.88	11.90	12.89
ASC 100ppm	7.10	7.00	7.05	8.03	8.00	8.02	14.85	12.10	13.48	15.20	13.07	14.14
HA 100ppm	7.02	6.94	6.98	8.05	7.92	8.00	14.11	11.96	13.04	14.47	12.35	13.41
CM+ASC	6.60	6.44	6.52	7.82	7.75	7.79	12.70	10.34	11.52	13.08	10.92	12.00
CM+HA	6.47	6.25	6.36	7.65	7.54	7.60	12.05	10.10	11.08	12.84	10.26	11.55
ASC+HA	6.75	6.60	6.68	7.88	7.81	7.85	13.17	11.00	12.09	13.52	11.58	12.55
CM+ASC+HA	6.27	6.15	6.21	7.00	7.24	7.12	12.25	9.55	10.90	12.77	9.70	11.24
Mean of PGP	7.03	6.90	----	7.99	7.91	---	13.49	11.30	----	13.89	11.73	----

(CM) = Cattle Manure (ASC) = Ascorbic acid (HA) = Humic acid (PGP) = Plant growth promoters



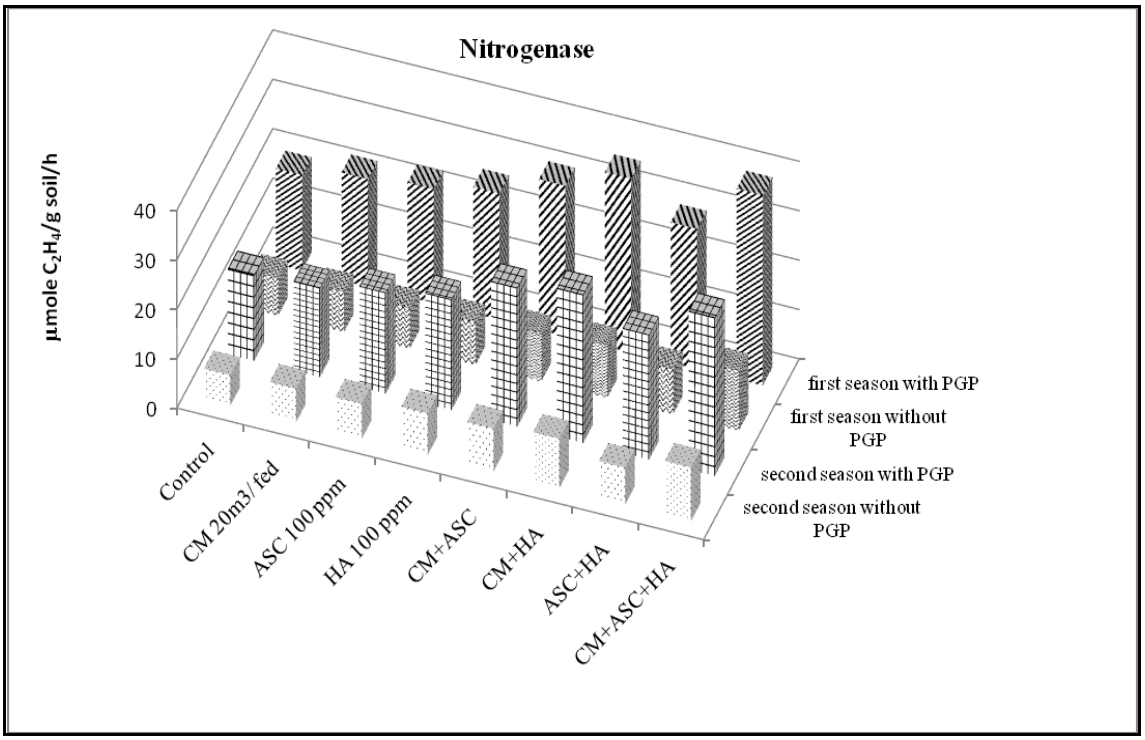


Fig (5): Response Dehydroengenase enzyme (DHA) and Nitrogenase enzyme (N_2-ase) activities of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/2013 seasons

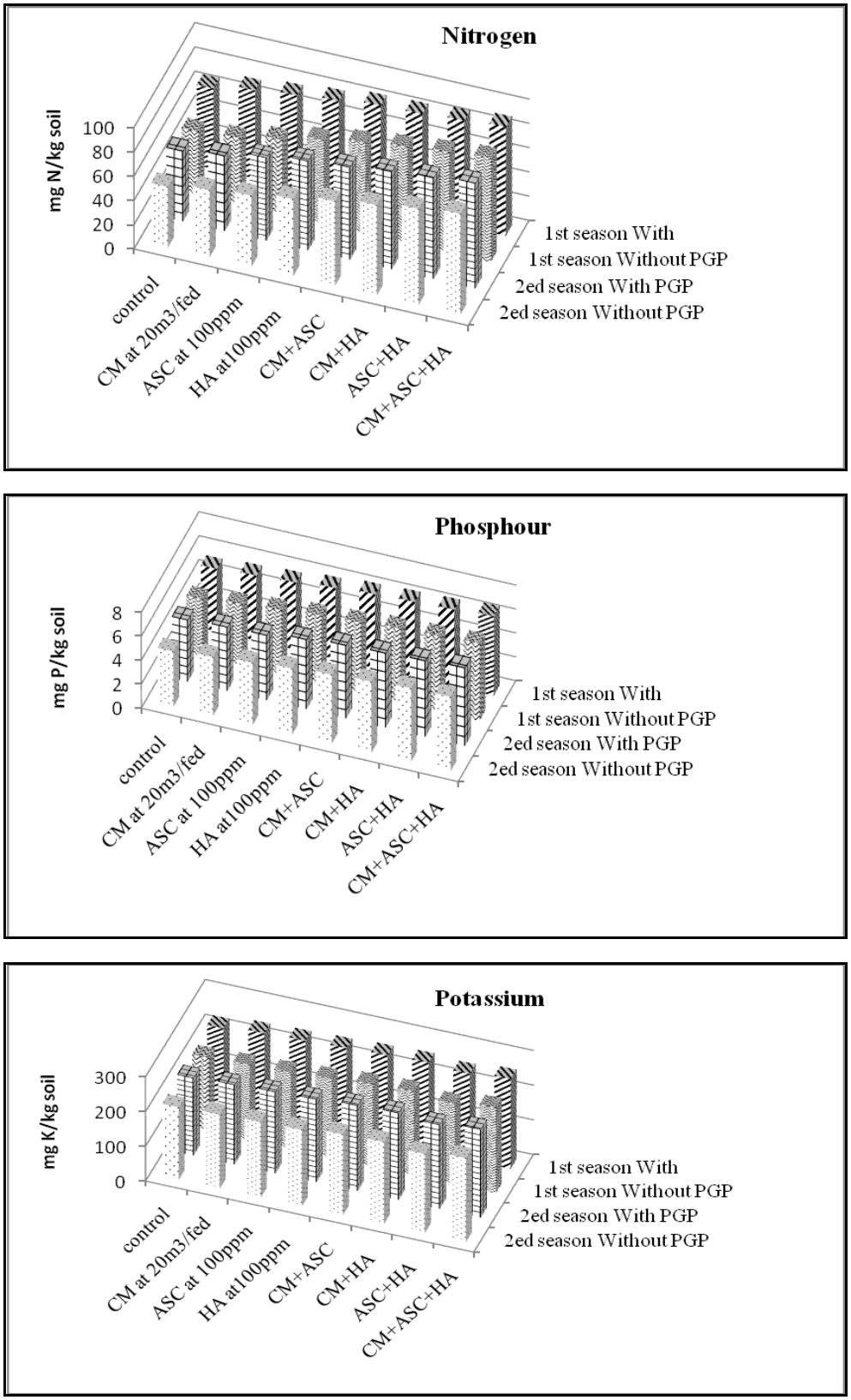


Fig. (6): Response nitrogen, phosphorous and potassium content in soil of *Calendula Officinalis* L. which grown in saline soil to plant growth promoters and some organic substances during, 2011/2012 and 2012/2013 seasons

Discussion

The main problem at Sahl El-Hossynia soil is related to high salinity conditions. Salt affected soil is a major environmental issue, as it limits plant growth and development, causing productivity losses [56]**.** Salt affected soils are characterized by excessively high levels of water- soluble salts, including sodium chloride (NaCl), The NaCl is a major salt contaminant in the soil. It has a small molecule size and when oxidized by water, producing sodium ions (Na^+) and chloride ions (Cl^-), which are easily absorbed by the root cells of higher plants and transferred to the whole plant using xylem uploading channels, also cause ionic and osmotic stresses at the cellular level of higher plants, especially in susceptible species [57]. There are many procedures that can be used to improve salt affected soils, such as: the applications which studied in this experiments.

There is no doubt that the used plant growth promoters (PGP) led to an increase in growth parameters of marigold and soil properties. However, such increases in these parameters and under PGP treatments effect might be the PGP are usually defined as microorganisms that can grow in, on, or around plant tissues, stimulating plant growth by a variety of mechanisms[58]. Plant defense against ROS (reactive oxygen species) is related to antioxidant defense systems including catalase, peroxidase, superoxide dismutase, glutathione reductase, ascorbate peroxidase and nonenzymitic compounds includes ascorbate, α-tocopherol and carotenoides, ascorbate [59]. Under stress, most of the rhizobacteria produce osmo-protectants (K+, glutamate, trehalose, glycine, beatine, proline and ectoine etc.) to modulate their cytoplasmic osmolarity and some others produce exopolysaccharides. Exopolysaccharides produced by pseudomonads can bind to cations including Na+ thus making it unavailable for plants under saline conditions [60]. *Pleurotus* plays an important role as growth promoter and as an anti-oxidant because it excretes high amounts of exo-polysaccharides, flavonoids, carotenoids and it has high contents of protein, amino acids, vitamins and essential minerals including Ca, K, Mn, P, Mg, F, Cu and Se[61].

For Humic acid (HA**)** application effect, results showed that treatment with (HA**)** led to an increase in tested parameters. Humic acid might benefit plant growth by chelating unavailable nutrients and buffering pH and stimulate microbial activity, soil enzymatic activities thereby improves physicochemical and biological environment of soil[62]. The increase of Na^+ may be related to humic acid causing greater root permeability by increasing lateral root development and total root bio-mass. [63]conclude that, treatment with humus enhanced the uptake of nutrients in plant under saline conditions. Therefore, the humic substances may interact with the phospholipid structures of the cell membranes and react as carriers of nutrients through them.

For ascorbic acid (ASC**)** effect found that, Application of ascorbic acid can reduce the harmful effects of salt stress and may have stimulatory effects on plants; ascorbic acid is synthesized in the higher plants and improves plant growth. It is a product of D-glucose metabolism which affects some nutritional cycle activities in higher plants and plays an important role in the electron transport system[64]. [65]reported that exogenous ascorbic acid enhanced the productivity of wheat plants under salinity stress conditions. These effects may be attributed to the protective role of ascorbic acid in plant cells from the oxidative stress induced by salinity. Moreover, the increase in yield and its compounds might be due to the effect of the antioxidant role on enhancing protein synthesis and delaying senescence. Foliar spray of ascorbic acid increased photosynthetic metabolites, which leads to the accumulation of different fractions of soluble sugars and nitrogen content in plant tissues under saline conditions[66] .

For Cattle manure application effect, results showed that applying CM with any tested application treatments led to an increase in tested parameters. However, the Cattle manure may be attributed to enhancing soil aggregation, soil aeration, increasing water holding capacity and offers good environmental conditions for root system[67]. [44]found that the increased growth and nutrient content of plant suggest the positive effects of organic manures in amelioration of saline soils by enhancing soil fertility through the release of essential macro and micro elements.

Conclusively, although all the applied treatments improved the growth parameters of marigold and soil properties under saline stress, the combination of Plant growth promoters (PGP) with organic treatments especially CM+ ASC+HA proved to be the best treatment for increasing, vegetative and flowering parameters and soil properties, followed by that the treatment by CM+HA and CM+ASC respectively.

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