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EFFECT OF USING MYCORRHIZAE AND BIOSTIMULANTS ON PRODUCTIVITY OF CANOLA UNDER SALT STRESS

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Abstract

Salinity stress is abiotic stress that has negative impacts on plant growth and productivity. For enhancing productivityunder salinity stress byintegrated use of mycorrhizae and biostimulants. To evaluate the effect of Mycorrhizae (*Glomus macrocarpium*) and Biostimulants including Humic acid at concentration 0.2% and Algal extract at concentration 3% on the growth parameters, yield and the oil components of canola plants (Serw-4), *i.e.* amino and fatty acids contents, as well as microbial activities in soil including total microbial counts, mycorrhizal infection %, number of spores and microbial enzymes (dehydrogenase and nitrogenase) in rhizosphere of canola under salt stress conditions.

A field experiment was conducted at Ras Sudr Research Station, South Sinai, Desert Research Center (DRC), during two successive seasons. The obtained results showed the positive response of canola to mycorrhizal inoculation and biostimulants for most of the studied parameters. Also results indicated that plant inoculation with combined treatment of Mycorrhizae, Algal extract and humic acid showed the highest increases of growth parameters including plant height, fresh and dry weight), both seed yield/plant and oil yield on both seasons, as compared with the un-inoculated plants. Also, combined treatments recorded highest values for NPK concentrations in canola seed, shoots and soil. Also, reduce K/Na ratio even under salt stress. Addition of biofertilizers significantly improved microbial activities in the rhizosphere area of canola plants, represented by dehydrogenase andphosphatase enzymes, Total microbial counts, root mycorrhizal colonization % and number of spores. Mycorrhizae and biostimulants significantly improve amino acid in canola seed and fatty acid composition in canola oil. Combination of Mycorrhizae (*Glomus macrocarpium*) and biostimulants (humic substance and algal extract) might be the mostfavorable cropping strategy for canola production under salt stress conditions.

Key words: Canola- Biostimulants-Mycorrhizae –*Humic substance- Algal extract*

Introduction

Canola which is also known as Rapeseed (*Brassica napus* L.), is one of the world major oilseed crops and the third largest source of vegetable oil in the world. Seeds contain about 40%–45% oil and about 18-25% protein. The oil is edible contains 60% oleic acid and 8.8% linoleic acid. Canola is frequently grown in arid and semiarid regions of the world where salinity is a bigproblem (Hu *et al.*, 2006 and Rajkovic *et al.*,2018).

Abiotic stresses such as drought, salinity and temperature extremes can reduce the yield in most crops and limit agricultural production. Salinity is one of the most limiting factors negatively affecting plant growth and yield production of most plants (Salama and Mona 2016). Salinity causes manyadverse effects on plant developmental stages due to manyfactors such as low water potential of soil solution, nutritionaland hormonal imbalance and ions toxicity which induced saltstress (Miranda, 2011). The development of strategies to alleviate the adverse effects of salinity stress on plants has receive considerable attention.The necessity to use saline water in irrigationcompensating the shortage in the fresh water, the biofertilizers that originated from seaweed are better and moreeconomical than other fertilizers in that cases (Pise and Sabale, 2010).

Agricultural biostimulants include microorganisms and several substances that enhance plant growthwhen applied to plants or the rhizosphere, stimulate natural processes to increase nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality as defined by EBIC (European Biostimulants Industry 2012a). Biostimulants foster plant growth and development throughout different mechanisms including: improving yield and enhanced crop quality; increasing plant tolerance, recovery from abiotic stresses; facilitating nutrient

assimilation, translocation and use; enhancing certain physicochemical properties of the soil and fostering the development of complementary soil micro-organisms". (European Biostimulants Industry 2012b).

Arbuscular mycorrhizal fungi (AMF) establish a symbiotic union with roots of 80% land plants (Smith and Read, 2008). This symbiosis constitutes a distinctive system with more efficiency than roots alone for uptake and transfer of mineral nutrientsfrom soil. AMF can boost several mechanisms in plant to manage salt stress (Ruiz-Lozano *et al.,* 2012). Formation of AMF has been reported to : (i) improve nutrient acquisition and maintain ionic homeostasis; (ii) improve water uptake and maintain osmotic equilibrium in plants; (iii) induce antioxidant system (iv) protectphotosynthetic apparatus and enhance photosynthetic efficiency; and (v) modulate phytohormone profile to minimize salt effects on growth and development (Augé *et al.,* 2014; Khalloufi *et al.,* 2017). Recently, Wang *et al.,* (2018) reported considerable enhancement in fresh and dry weights and N concentration of shoot and root due to mycorrhizal inoculation under moderate saline conditions. The reclamation of salt affected soil requires an improvement of physical, chemical and biological properties.

Seaweeds are multi-cellular, macroscopic organisms found in coastal, marine ecosystems and are a rich source of polysaccharides, polyunsaturated fatty acids (PUFAs), enzymes and bioactive peptides among others (Shukla *et al.,* 2016; Okolie *et al.,* 2018). As such, selected seaweed resources are important sources of plant biostimulants and are widely used to promote agricultural productivity (Du Jardin, 2015; Van Oosten *et al.,* 2017).Effect ofseaweed bio fertilizers have been studied on different cropsand improving their yield. For example, the yield of canola (*Brassica napus*) was improved with foliar spray application ofseaweed extract (Zodape, *et al.,*2010). Exogenous application of micronutrientsand antioxidantssuch as potassium humate and cytokine toplants grown under salt stress conditions have been known tohave stimulatory effect on crop yield and their resistance tosalt stress (Nagesh and Shanwad, 2010 and El-Hak, *et al.,* 2012).

Algae extracts have been used as foliar- and soil-applied treatments in crop production systems due to the presence of a number of plant growth-stimulating compounds and their nitrogen, phosphrous and potash content (Wally *et al.,* 2012). Strik *et al.,* (2004) reported that the algae extracts are effective fertilizers in many crops.

Beneficial anti-stress effects of Algae extracts may be related to cytokine and betaine activites which has a beneficial role in alleviates the effect of water and salt stress in plants by acting as an osmo-protectant and providing partial protection to enzymes against salt inhibition (Khan *et al.,* 2009).

Organicfertilizers are beneficial to soil and plants. Humic acids (HAs) are key components of humic substancesand hence most dynamic constituents of soil and manure (compost organic matter). Humic acids (HA) are the most active components of soil and compost organic matter, stimulate plant growth and consequently yield by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake and enzyme activities thereby increasing the yield of crop plants (Mayi *et al.,* 2014; Arjumend *et al.,* 2015). In particular, optimal concentrations able to affect and stimulate plant growth have been generally found in the range of 50-300 mg/ L, but positive effects have been also exerted by lower concentrations (Chen *et al.,* 2004). A distinction on the effects of humic acids should be made between indirect and direct effects on plants growth. Indirect effects are mainly exerted through properties such as enrichment in soil nutrients, increase of microbial population, higher cation exchange capacity, improvement of soil structure; whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature (Varanini and Penton, 2001; and Chen *et al.,* 2004). Abdelhamid *et al.,* (2011) reported that bio-fertigation of microbial inocula and humic substances could be used as a complementary for mineral fertilizers to improve yield and quality of cowpea under sandy soil conditions which protect the environment chemical pollution and its harmful effect on human and animal health. A foliar application of HA increased the vegetative growth of olive cuttings (Hartwigsen and Evans, 2000 Zandonadi *et al.,* 2010).

Humic substances can also work against environmental stresses,under stress conditions, humic substances lower the uptake of toxic elements and enhancethe uptake of essential nutrients and minerals .Recently, humic acid-rich materials are widely used as soil conditioners and growth regulators. (Mora *et al.,* 2014).

Moreover, several studies have demonstrated that the application of humic acid has many benefits for salt-affected soils.Kulikova *et al.,* (2005) mentioned that, humic substances possibly counteract the negative effects of salinity conditions by reducing the uptake of some toxic elements. Aydin *et al.,* (2012) found that, addition of humic acids to saline soils were reduced the soil electrical conductivity. Khattak *et al.,* (2013) found that, cation

exchange capacity was improved with additions of HA to salinesodic soil. Jiangkuan *et al.,* (2015) added that, presence of humic acid increased the replace of exchangeable Na^+ from soil surface. The current study was carried out to examine the effect Mycorrhizae and biostimulants on the productivity of canola under salt stress.

Materials and Methods

A field experiment was carried out for two successive seasons 2018 and 2019 at Ras Sudr Research Station, South Sinai, Desert Research Center (DRC), to investigate the effect of biofertilization using Mycorrhizae and bio-stimulants using Humic acid and Algal extract on the productivity of canola (serw 4) under salt stress conditions.

Physical and chemical properties of the experimental soil were determined according to Page *et al.,* (1984)as shown in table 1. Chemical properties of irrigation water at Ras Sudr Research Station represented at table 2.

The plot area was 3×3.5 m. The experiment included 5 treatments and was conducted in plot arrangement at a randomized block design with 5 replicates. Phosphate fertilizer as calcium superphosphate (15.5% P_2O_5) was added at rateof 100 kgP_2O_5 /fed. during seed bed preparation, 50 Kg of potassium sulphate (50.0% K_2SO_4) was added at flowering stage, whereas nitrogen fertilizer was applied as ammonium sulfate (20.5% N) at a rate of 48.0 kg N/fed through three equal doses during plant growth.

Biofertilization(Isolation of Mycorrhiza)

Mycorrhizal spores were isolated from rhizosphere by wet sieving and decantation method (Gerdman and Nicolson, 1963).

Identification of vascular arbuscular (VA) mycorrhizal fungi

Table 1: Some physical and chemical properties of the experimental soil.

Depth Cm	pH	EC soil pasteds/m	% OM	$CaCO_3$	Sand	Silt	Clay	CEC Cmole /kg soil	Tex-ture
0-30	7.73	8.56	2.28	26.9	81.20	8.57	10.23	5.81	LS
30-60	7.96	7.35	1.73	27.4	80.08	10.59	9.33	6.65	LS
Cations and anions in soil (meq/L)									
Depth	Na^+	K^+	Ca^{+2}	Mg^{+2}	$CO_3^=$	HCO_3^-	Cl^-	$SO_4^=$	
0-30	47.1	8.9	24.4	5.2	0	8.1	51.3	26.2	
30-60	41.2	12.7	15.8	3.8	0	3.5	46.5	23.5	
Available nutrients in soil (ppm)									
Depth	N	P	K	Fe	Mn	Zn	Cu	B	
0-30	36.8	5.19	48.5	4.26	2.18	1.25	0.57	0.18	
30-60	21.5	3.84	52.3	4.64	2.23	1.31	0.66	0.12	

Table 2: Some chemical properties of irrigation water at Ras Sudr Research Station.

pH	EC	meq/L							
	ds/m	Na^+	K^+	Ca^{+2}	Mg^{+2}	$CO_3^=$	HCO_3^-	Cl^-	$SO_4^=$
7.94	7.85	46.9	2.62	20.5	8.48	0.00	6.3	47.5	24.7

The spores of the isolated mycorrhizae identified microscopically according to the morphological characteristics described by (Schenk and prez, 1987). Schenck & Pérez (1990) manual and descriptions provided by the site of International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http:// invam.caf.wvu.edu) and the original species descriptions.

Preparation of mycorrhizal inoculum: Multiplication of mycorrhizal fungi was carried out by pot culture; mycorrhizal spores were obtained by wet-sieving method (Gerdman and Nicolson, 1963). The AM inocula was mixed with pure sand and kept in the refrigerator to be used in the inoculation.

Collection ofAlgae

Fresh marine algae (green- red- brown) used in the present study were collected from coastal region of Meditreanean sea. They were hand-picked and washed thoroughly with sea-water and hard brush to remove macroscopic epiphytes and sand particles and then washed with tap water to remove adhering salt. Samples were air-dried (26°C) during 2–4 days followed by thermostat dry at 60°C for 12h.

Preparation of Marine Algae Extract (MAE):

Dried marine algae was ground or cut in to pieces. The coarse powder was mixed with distilled water in ratio of 1:20 (w/v)Boiled for 60 minutes and filtered through four folds of white cloth. The filtrate was collected and stored for field study as foliar spray three times during vegetative growth as mentioned bySharma *et al.,* (2014). Nitrogen, phosphorus and potassium were determined as mentioned by (A.O.A.C, 2000). The composition of elements such as copper, manganese, iron, zinc, cobalt, Further, the liquid extract of marine alga was also subjected for estimation of auxin, gibberellinand cytokinine. (Rizzolo *et al.,* 1991).

Humic acid

Potassium humate (12.5% K_2O) was used at concentration of 0.2% as foliar spray three times during vegetative growth.

Determination of growth and yield

Table 3: Chemical analysis of marine algal extract.

Parameters	N	P	K	S	Mg	Fe	Mn	Zn	Cu	Auxin	Gibberellin	Cytokinine
Concentrationppm	19.3	9.6	25.1	3.2	19	14	15.9	4.1	2.5	4.3	10.2	21.1

parameters

Growth parameters: After 85 days from planting shoots of five plantswere taken at random from each plot for determining some growth parameters, *i.e.*, plant height (cm), fresh and dry matter weight (g/plant), Number of branches /plant, number of pods/plant and seed yield/plant.

Yield parameters

At harvest, five plants were taken at random from each plot for determining some yield parameters *i.e.* pods/ plant, seed yield g/p and Oil%.

Chemical determinations

The chosen plants were transferred immediately from the experimental area to laboratory and dried in an electrical air-draft at 70 C forchemical determinations of N, P, K, according to Cottenie *et al.*, (1982). Oil content of seed was determined by solvent extraction method in Soxhlets apparatus with N-hexane as solvent according to A.O.A.C. (2000).

Soil nutrient contents: At the end of the experiment, soil samples were taken from each treatment at major root zone (0–60 cm depth). Nitrogen content was determined in the dry weight using micro-kjeldahl method according to Klute,1986. Phosphorus and Potassium content were determined as described by Page *et al.*, (1984).

Microbial determinations

Rhizosphere soil sample were analyzed for: total microbial counts on Bunt and Rovira medium Nautiyal (1999). Soil samples were analyzed for: Dehydrogenase activity as described by (Friedel *et al.*, 1994). For determination of phosphatase activity disodium phenylphosphate served as enzyme substrate (Õhlinger, 1996).

Assessment of A-mycorrhizal infection: The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic observation. The gridlines intersect method of Giovannetti and Mossa (1980) was used to estimate the A-mycorrhizal infection percentage.

Root colonization (%) =

$$\frac{Total\ number\ of\ segments\ observed}{Number\ of\ AM\ positive\ segments} \times 100$$

Chemical analysis: The best biofertilization treatment (mixed inoculation) were done as follows:

Determination of amino acid as reported by Bailey, (1976). The fatty acids of the oil were determined according to (A.O.A.C. 2000).

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the effects of treatmentson theobtained data.The least significant difference test (LSD) at p = 0.05 level was used to verify the differences between treatments as mentioned by Snedecor and Cochran (1980).

Results and Discussion

Growth and yield parameters of canola plants

Plant height, fresh weight/plant and dry weight/plant of canola plants tended to gradually increase as a result of the studied different treatments (Mycorrhizae, Humic acid, Algal extract and combination of all). All the applied treatments resulted in a significant increase of all the studiedcharacters as compared to the control treatment table 4. The combined treatment of Mycorrhizae, Humic acid and algal extract was the best treatment where it gave the highest values of all thestudied parameters. It exceeds the control treatment by 67% in plant height, 82% in fresh weight, 34% in dry weight and 117% for seed yield at first season. While, 76% in plant height, 70% in fresh weight, 42% in dry weight and 113% for seed yield at second season respectively. For single treatments mycorrhizal inoculation recorded the best value for all studied parameters followed by algae extract

Table 4: Effect of Mycorrhizal inoculation and biostimulants on growth parameters of canola plants grown under saline conditions.

	Plant height cm		FW g/p		DW g/p	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	69	72	51	56	29	31
G. macrocarpium	94	108	82	87	36	42
Humic acid	77	85	73	79	32	37
Algal extract	83	104	80	81	35	40
Mix	115	127	93	95	39	44
L.S.D (5%)	0.174		0.667		0.2309	
Season Bio	0.19		0.1054		0.3651	
Interaction	0.2687		0.1491		0.5164	

Mix:*G. macrocarpium* + Humic acid + Algal extract; FW: Fresh weight, DW: Dry weight.

Table 5: Effect of Mycorrhizal inoculation and biostimulants on yield parameters of canola plants grown under saline conditions.

	Number of branch		Pods/p		Seed yieldgm/p		Oil %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	4.1	4.2	177	198	10.8	11.3	24.3	24.9
G. macrocarpium	4.9	5.1	213	249	19.22	21.7	40.6	41.2
Humic acid	4.7	4.9	188	215	14.7	16.1	37.8	38.2
Algal extract	4.8	4.9	192	221	16.8	17.2	38.5	39.3
Mix	5.6	5.7	239	270	23.4	24.1	42.46	43.9
L.S.D at 5%	0.0115		0.3464		0.104		0.0331	
Season Bio	0.0827		0.8062		0.164		0.0635	
Interaction	0.1169		0.11402		0.0232		0.0424	

and humic acid.

The enhancement effect of the treatments on the growth of canola plants may beattributed to the effect of used biostimulants as growthpromoters. These results are coincided with those obtained by Osman and Salem, 2011. Also it was found that Arbuscular mycorrhizalFungi (AMF) mitigate growth reduction caused by salinity, studies on salt stress tolerance in mycorrhizal plants have suggested that AM plants grow better due to improved mineral nutrition and physiological processes like photosynthesis or water use efficiency (Al-Karaki 2006). AMF are vital endosymbiosis playing an eûective role in plant productivity and the functioning ofthe ecosystem. They are of one importance keys for sustainable cropimprovement (Gianinazzi *et al.,* 2010).

Presented data in table 5 showed that number of branhes /plant, number of pods /plant and seed yield g / plant of canola plants significantly increased as a result of studied treatments. The combined treatment resulted in improvement in all the studied yield parameters as compared to the control. Combined treatment improve yield parameters by (35 and 37 %) for number of branches /plant, (35 and 36%) for number of pods /plant, (113 and 117%) for seed yield and (75 and 76 %) for Oil % at both first and second season respectively. Mycorrhizal inoculation as single treatments recorded the best value for all studied parameters followed by algal extract and humic acid. The same results were reported by (Fatemeh *et al.,*2015). Promoting effect of humic acid on canola yield also reported by Kulikova *et al.,* (2005), they indicated that humic substances might show anti-stress effects under abiotic stress conditions such as unfavourable temperature, salinity, pH, etc.

Effect of Mycorrhizal inoculation biostimulants on macronutrients contents (NPK%) of canola seeds grown under saline conditions

As illustrated in table 6 the applied treatments had a significant effect on the chemical composition of canola seeds. Regarding the nitrogen content, the combined treatment gave the highest percent of nitrogen (1.89 and 1.94%) with almost (16 and 17%) increase as compared to the control treatment at first and second season respectively. While the single treatment of mycorrhizae gave the highest percent of N compared to other single treatments. The highest value of P was 15 and 10% increase for P contents in canola seeds and 29.5 and 46% increase than control for K were recorded with combined treatment at first and second season respectively. These results in agreements with Goel *et al.,* 2000, they reported that, seed inoculation with mycorrhizae significantly increased plant N contents.The second growing season showed higher enhancement effect than the first growth season, these results are in agreement with those reported by (Khalid *et al.,* 2010).

Table 6: Effect of Mycorrhizal inoculation and biostimulants on macronutrients contents (NPK%) of canola seeds grown under saline conditions.

	N		P		K	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.63	1.66	2.27	2.4	1.49	1.49
G. macrocarpium	1.82	1.85	2.53	2.59	1.69	1.75
Humic acid	1.69	1.72	2.32	2.36	1.58	1.61
Algal extract	1.8	1.84	2.48	2.5	1.54	1.57
Mix	1.89	1.94	2.62	2.64	1.93	2.18
L.S.D at 5%	0.0348		0.0231		0.302	
Season Bio	0.0160		0.0208		0.116	
Interaction	0.0227		0.0294		0.0164	

Effect of Mycorrhizal inoculation and biostimulants on macronutrients and sodium (Na) contents of canola leaves grown under saline conditions

Mineral contents of canolaleaves as affected by the applied treatments were represented in table 7. Results showed marked increment for the studied

Table 7: Effect of Mycorrhizal inoculation and biostimulants on macronutrients contents of canola leaves grown under saline conditions.

	N%		P%		K%	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	3.1	3.18	0.39	0.42	2.21	2.29
G. macrocarpium	3.8	3.9	0.63	0.69	2.89	2.96
Humic acid	3.5	3.7	0.48	0.51	2.63	2.81
Algal extract	3.6	3.8	0.54	0.55	2.57	2.75
Mix	4	4.2	0.67	0.7	2.92	3.11
L.S.D at 5%	0.309		0.0375		0.216	
Season Bio	0.0362		0.0167		0.0104	
Interaction	0.0313		0.0236		0.0306	

Table 8: Effect of Mycorrhizal inoculation and biostimulants on macronutrient contents in rhizosphere of canola grown under saline conditions.

	N%		P%		K%	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	0.016	0.0173	0..004	0.0045	0.024	0.028
G. macrocarpium	0.021	0.023	0.006	0.0074	0.031	0.039
Humic acid	0.018	0.021	0.0054	0.0065	0.028	0.035
Algal extract	0.019	0.023	0.0058	0.0069	0.031	0.038
Mix	0.028	0.031	0.0063	0.0081	0.038	0.081
L.S.D at 5%	0.304		0.309		0.008	
Season Bio	0.624		0.151		0.646	
Interaction	0.125		0.061		0.098	

macronutrients due to mycorrhizal inoculation, humic acid and algal extract application, with greatest values when they were combined together. Combined treatment recorded highest values compared to single treatments for all studied parameters at the two growing seasons.

Mycorrhizal inoculation recorded highest values for NPK in canola leaves at first and second season. While it was found that humic acid effectively decrease Na level at first and second season compared to other single treatments.These results in accordance with Al-Karaki 2006 who reported that AM plants grow better due to improved mineral nutrition and physiological processes like photosynthesis or water use efficiency, the production of osmoregulators, higher K^+/Na^+ ratios and compartmentalization of sodium within some plant tissues.

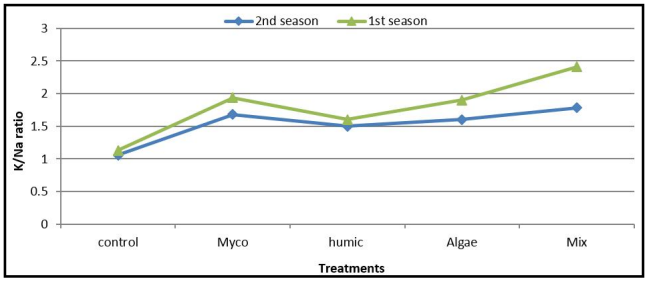

Fig. 1: Effect of Mycorrhizal inoculation and biostimulants on K/Na ratio of canola leaves grown under saline conditions.

Also, humic substances application increases root mass andvolume (Eyheraguibel *et al.,* 2008), which is an importantfactor in nutrient uptake Statistical interaction analyses revealed that valuesof, N, P and K contents of grown wheat plants increasedsignificantly with amino acids combined with humic substances compared to control at both studiedseasons.

K/Na for canola plant

Data presented in Fig. 1 indicated a significantincrease of K/Na ratio in presence each of Mycorrhizae, algae extract andhumic substances along with their combination ascompared to control; such ratio increased by application of mixed treatment.Generally, the positive effect of applied treatmentson K/Na ratio, of both tested seasons may be arranged inthe descending order of combined treatments >mycorrhizae>algal extract >humic> control. These results were explained by Shabala and Cuin (2008) who reported that high level ofproline regulates plasma membrane K ion channels whichare directly detected in the K/Na ratio. Similar results werereported by Abd El-Samad *et al.,* (2010) who found that,amino acids treatments enhanced the uptake of K^+ andhence, K^+/Na^+ ratio to be increased. Disturbances of canola cropsignificantly reduced the Na^+/K^+ ratio in canola leaves under saline soil conditions, indicating amelioration of salt-induced growth, physiological and nutritional Naveed *et al.,* (2020).

Effect of Mycorrhizal inoculation and biostimulants on macronutrient contents, Na, PH and E.C. in rhizosphere of canola grown under saline conditions

Soil macro-nutrients concentrations

Results in table 8 showed that soil total nitrogen, phosphorus and potassium concentrations were higher in the soil of inoculated plants than uninoculated ones. Inoculation with mycorrhiza increases significantly the soil N, P and K by 13.29 & 14.56%; 21.01 & 21.77% and 7.42 & 7.65% for first and second seasons, respectively. Data revealed that the values of available nutrients significantly increased by addition humic acid either as individual or in combinations as compared to control. The combined treatment recorded significantly the highest soil N, P and K concentrations (197.4, 59.2 and 248.3 ppm; 200.1, 60.7 and 253.6 ppm) for the two seasons,

respectively. Tawaraya *et al.,* (2006) reported that mycorrhizae associated with plants rises the availability of phosphate by solubilizing the insoluble fraction of inorganic phosphate, which significantly increase phosphate concentration and uptake in the plant tissue.

The high concentration of humic substances was alsomore effective for increasing P and K availability, possiblydue to the effect of humic substances for increasingphosphorus recovery from calcium phosphate precipitates (Verlinden *et al.,* 2009). Moreover, humic substances, as aresult of their microorganisms activities, decrease soil pHand thus release fixed potassium and produce morechelating ions, leading to an increase in nutrient availableforms of elements in the rhizosphere Zone; these results are agree with those of Khaled and Fawy (2011).

PH and EC

Data illustrated in Figs. 2 and 3 showed the changes ofsome soil chemical properties as affected by the studiedtreatments of mycorrhizal inoculation, Algae extract and Humic substance along withtheir combination, after canola harvesting, under salinityconditions.

Soil (pH): Results revealed in Fig. 2 that, pH values of soil after plantharvesting, in general were slightly affected by the studiedtreatments; The values of pH were slightly decreased forboth tested seasons. The effect of humic substance on decreasing the soilpH, on the other hand, could be explained by the effect ofprotons (H^+)

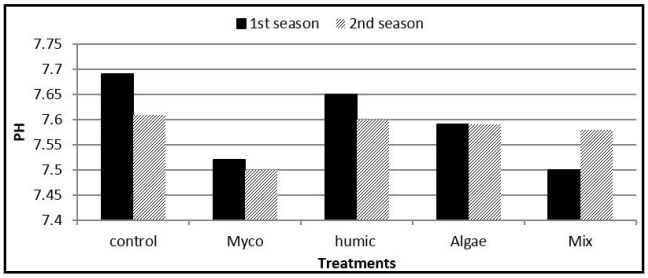

Fig. 2: Effect of Mycorrhizal inoculation and biostimulants on PH in canola rhizosphere soil grown under saline conditions.

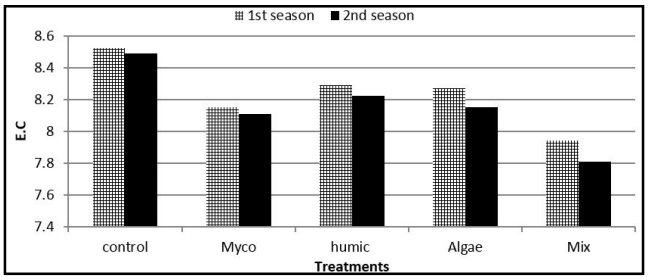

Fig. 3: Effect of Mycorrhizal inoculation and biostimulants on EC in canola rhizosphere soil grown under saline conditions.

release from carboxyl ($COOH^-$) and phenolichydroxyl (OH^-) groups to directly neutralize soil alkalinity,humic substance added to the soil could improve biological activity (Khattak *et al.,* 2013) and thus promote thedecomposition of soil organic matter. The decaying oforganic matter present in humic substance increases theCO_2 concentrations in the soil and releases H+ ions whichreduce the pH values (Wong *et al.,* 2009).

Electric conductivity (EC): Data presented in Fig. 3 indicates that, values of EC were positively affected by humic acidsapplication as compared to control treatment. The lowest EC values wererecorded with the Humic substance (HS) treatment at bothtested seasons, followed by algae extract and mycorrhizal inoculation These results are in agreement with those obtainedby Aydin *et al.,* (2012) who reported that, humic acid addedto saline soil significantly reduced soil electric conductivityand attributed this effect due to the fact that HA absorbedmany times their weight of water, which diluted the salteffect and store it for relatively long time ;such conditionsfacilitated leaching of soluble salts and decreased soilsalinity.

Lakhdar *et al.,* (2009) pointed that organic matter presence in HS decreased soilNa, EC and pH values due to high supplies of Ca, Mg andK; these mineral elements kept the cation-exchange siteson the soil particles to minimize adsorption of Na, soenhancing Na leaching. Moreover, Ouni *et al.,* (2014)added that, reduction of salinity means reduction for themonovalent Na^+ which thus is particularly evident whenreplaced by the monovalent K^+ of the humate. Thus, byelectrostatic repulsion of the high concentration of K^+present in humic complex Na^+ of the adsorption colloiddecreased which finally reduces the soil salinity. Combined treatment recorded the best figure compared to other treatments and control.

Effect of mycorrhizal inoculation and biostimulants on soil microbial activities in rhizosphere of Canola plant

In order to evaluate the effect of mycorrhizal inoculation and biostimulantson the persistence, interaction of soil microbial groups and their activities in rhizosphere of canola plants, the following were determined.

Total microbial counts: Represented data in table 9 showed that the total microbial counts at second season were higher than those of first season and all the treatments exceeded the control. Initial total microbial counts before cultivation were 47×10^5 cfu/g dry soil. Total microbial counts differ with different treatments; highest total microbial counts were recorded with combined

Table 9: Effect of mycorrhizal inoculation and biostimulants on total microbial counts, Mycoorhizal colonization % and number of spore /gm soil in rhizosphere of canola grown under saline conditions.

	TC×10 cfu/g dry soil		My %		No. of Spore /gm soil	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	49	56	0.6	0.8	8.4	8.7
G. macrocarpium	71	79	6.3	8.2	13.3	13.5
Humic acid	54	64	1.2	1.7	8.8	9.1
Algal extract	63	70	2.3	3.4	8.9	9.5
Mix	91	103	9.3	10.2	15.4	16.3
L.S.D at 5%	0.2309		0.0353		0.0267	
Season Bio	0.4082		0.0236		0.0601	
Interaction	0.5774		0.0333		0.0850	

TC: Total microbial counts, M%: Mycorrhizal colonization %.

application of mycorrhizae and biostimulants being 91 and 103×10^5 cfu/g dry soil at first and second seasons respectively. This might be due to the simulative effect of added biofertilizers on microbial community in canola plant rhizosphere which leads to increase total microbial counts. The enhancement effect of microbial activity is a good parameter for many soil improvement indicators (Abd El-Gawad, 2013).

Mycorrhizal root colonization

Data presented in table 9 showed that inoculated plants were significantly increased colonization compared to uninoculated plants. The root colonization of canola plants was affected by mycorrhizal inoculation . The higher percent of root colonization was found in plants inoculated with VAM compared to non-inoculated plants or with both humic acid and algal extract.

Mycorrhizal colonizationvaried from 6.3% at 1st season to 8.2 % at 2nd season in Mycorrhizal treatment, highest values were recorded with combined treatment being 9.3 and 10.2% at first and second season respectively.The positive effect of mixed inoculation on the increase of root colonization % and numbers of VAM spores was recorded by Bahadori *et al.,* (2013).

Number of spores in soil rhizosphere

The number of spores/g soil in the soil rhizosphere was affected by microbial inoculation. Number of mycorrhizal spores in soil increases with inoculation of mycorrhizal as described in table 8. Number of spores increase by 83.33 and 87.4% in inoculated treatments compared to uninoculated treatments at first and second season respectively. While the highest number of spores was recorded with mixed treatment being 15.4 and 16.3 at first and second season respectively. This result is in agreement with Rahman *et al.,* (2017) root colonization (percent) was enhanced significantly by the influence of VAM compared to in absence. Solaiman *et al.,* (2010) also recorded enhanced root colonization in chickpea due to the influence of VAM.

Soil enzymes

Enzymatic activities of soil samples are critical index of soil fertility because enzymes play an important role in nutrient cycles Anwesha *et al.,* (2012), Dehydrogenase and phosphatase enzymes were measured to clarify the effect of the mycorrhizal inoculation and biostimulants on soil enzymatic activity at the harvesting stage.

Dehydrogenase enzyme varied within the different treatments as shown in Fig. 4. Mycorrhiza-linoculation as single treatment gave higher values of soil dehydrogenase than other single treatments, while, combined treatment surpassed all individual treatments. Mycorrhizae inoculation treatments improved the microbial activity in the rhizosphere zone and recorded significant increases, compared to the un-inoculated treatment. Shalaby, (2018) observed an improved dehydrogenase and CO_2evolution in the rhizosphere zone of wheat plants due to biofertilizers application.

Phosphatase enzyme

Presented data in Fig. 5 clearly showed that, phosphatase activity recorded significant increase with

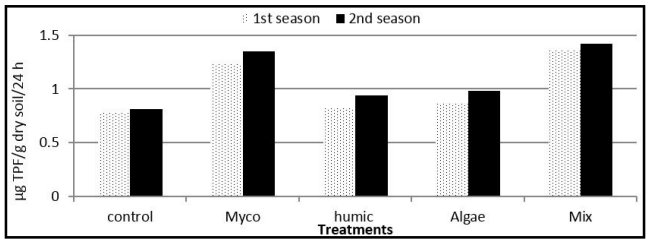

Fig. 4: Effect of of mycorrhizal inoculation and biostimulants on soil dehydrogenase enzyme in canola rhizosphere soil grown under saline conditions.

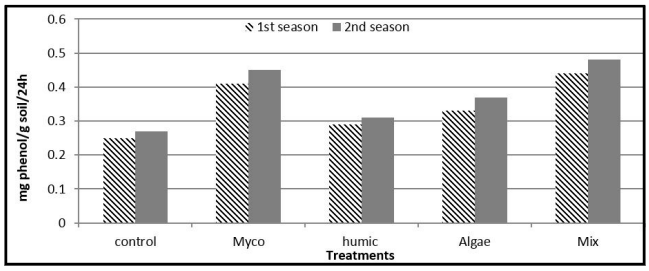

Fig. 5: Effect of of mycorrhizal inoculation and biostimulants on soil phosphatase enzyme in canola rhizosphere soil grown under saline conditions.

mycorrhizae treatments. Mycorrhizae inoculation treatment with biostimulants (combined treatments) recorded the highest phosphatase activity being (0.43 and 0.48 mg phenol/g soil/24h) at first and second seasons, respectively. Mycorrhizal inoculation treatments increase the phosphatase activity to 59.3 and 92% in two seasons compared to uninoculated treatments. George *et al.*, (2002) found that Phosphatase enzyme is able to mineralize organic phosphates into inorganic form of phosphates that available high phosphate for plant.

Effect of mycorrhizal inoculation and biostimulants on amino acids contents in canola seeds

Amino acid content of canola were presented in table 10 showed that, canola seed analysis detected about 3 essential, 7 nonessential, 2 aomatic, 2 sulphur and 3 other amino acids in seeds of canola (control and combined treatments). Canola seeds nutritional value is determined by its protein quality, which depends mainly on its amino acids content. Glutamic acid appeared to be the dominating amino acid in canola seeds. treated with combined treatment recorded the high value of glutamic acid this results indicate enhancement effect of Mycorrhizae with humic acid and algal extract in increasing amino acid content of canola seed. These results are in accordance with Gomaa, (2013), who found that biofertilizer application increased crude protein and mineral elements in canola seeds.

Table 10: Effect of mycorrhizae and biostimulants on amino acids contents (mg/g dry wt.) of canola seed under salt.

Amino acids	1st season		2nd season	
	Control	Mix	Control	Mix
Essential amino acid				
Threonine	9.7	11.65	9.26	11.91
Valine	10.62	12.22	10.29	12.81
Histidine	7.07	9.38	4.09	9.32
Non-essential amino acids				
Arginine	9.23	12.93	9.25	13.28
Aspartic	9.77	11.16	9.8	11.49
Glutamic	21.47	22.63	21.62	24.69
Serine	8.3	9.6	8.61	10.28
Glycine	8.82	9.65	9.16	10.96
Alanine	9.6	11.68	9.52	12.53
Proline	13.77	16.93	13.79	17.22
Aromatic amino acids				
Tyrosine	5.85	8.89	6.19	9.36
Phenyl alanine	10.09	12.86	10.25	13.16
Sulphur amino acids				
Methionine	1.6	3.98	1.23	4.09
other				
Lysine	12.61	14.37	12.9	14.52
Isoleucine	8.35	11.21	8.49	16.15
Leucine	13.4	15.964	13.16	16.23

Effect of mycorrhizae and biostimulants on Fatty acids composition of oil produced from canola

Results for Fatty acids composition of oil produced from canola seeds are presented in table 11. The data revealed that nine fatty acids were detected including saturated, Mono and polyunsaturated fatty acids.

A.Saturated fatty acids contain: caproic,, lauric, myristic, palmitic and stearic. Obtained data indicated that palmitic acid (C 16:0) recorded (13.9 and 13.58 %) is the prominent saturated fatty acids, especially for the control treatments, On the other hand,myristic (C 14:0), stearic (C 18:0) and arachidic (C 20:0) were detected in small amount while,lauric being (11.4 and 12.13%) for combined treatments at first and second season respectively.

(B): Unsaturated fatty acids (UFA): Concerning the mono-unsaturated fatty acids, data in table 10 revealed that oleic acid (C18: 1) was the most prevalent unsaturated fatty. oleic acid represented the highest relative percentage of all identified fatty acids. In this connection, the obtained results were in harmony with that obtained by (Mona, 2015).

On the other hand, the data showed a very low percentage of mono-unsaturated fatty acid, erucic acid (C22: 1) ranging 2.82 to 2.63% at first and second season respectively.

For poly-unsaturated fatty acids, data showed that the essential fatty acid, linoleic (C18: 2) was the most prevalent. So, Serw 4 was characterized with high concentration of the essential fatty acid (linoleic) indicating high nutritional value for mixed treatment compared with control.

Table 11: Effect of biofertilization treatments on Fatty acids composition of oil produced from canola.

Fatty acids	1st season		2nd season	
	Control	Mix	Control	Mix
Saturated				
Palmitic	8.33	13.9	8.29	13.58
Stearic	3.23	5.03	3.41	5.17
Caproic	2.03	8.22	2.37	7.91
Lauric	3.3	11.4	3.09	12.13
Myristic	2.1	2.13	2.15	2.24
Monounsaturated				
Oleic	7.11	23.03	7.29	24.15
Erucic	4.1	2.82	4.19	2.63
Polyunsaturated				
Linoleic	5.7	10.78	6.21	11.29
Linolenic	5.33	10.91	5.42	11.3

Conclusion

Salinity is the main problem limiting agriculture production, mycorrhizal inoculation combined with algal extract and humic acids gave highest growth parameters, maximum macronutrients concentrations in plant, seed and soil, stimulates microbial activity, improve amino acid and fatty acid concentrations. Finally these results suggest that biostimulants application with mycorrhizae inoculation could be used as potential growth regulator to improving plant resistance to salinity stress and therefore enhance plant growth and mineral status.

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