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Original article

Evaluation of the effects of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Enterobacter cloacae* on growth, yield and active compound compositions of *Moringa oleifera* under salinity stress

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ABSTRACT

Application of *Chlorella vulgaris*, *Nannochloropsis salina* and *Enterobacter cloacae* has been reported to improve the growth of multiple plant species. *Moringa oleifera* is a medicinal plant found in Saudi Arabia. Its leaves, flowers and fruit have been used as food. *Moringa oleifera* is rich in rutin and gallic acid and many other bioactive compounds, which collectively contribute to its demonstrated range of pharmacological activities. In Saudi Arabia, the semi-arid and arid weather presents a significant challenge to agriculture. High salinity in cultivated land is a particular threat. We applied *Chlorella vulgaris*, *Nannochloropsis salina*, and *Enterobacter cloacae* at multiple salinities to *Moringa oleifera* to investigate their effects on the growth, yield, and photosynthetic pigment content. We also examined possible changes in the phytochemical composition. The application of *Chlorella vulgaris*, *Nannochloropsis salina* and *Enterobacter cloacae* enhanced plant growth and yield, while inhibition was observed at high (6000 ppm) salinity. The presence of *Chlorella vulgaris* and *Nannochloropsis salina* altered plant growth and yield and rutin and gallic acid content of *Moringa oleifera* plants grown in saline conditions. Microalgae species were recommended for use as a bio-fertiliser alternative to mainstream synthetic fertilisers.

1. Introduction

Biotic and abiotic stresses continue to affect crop production and productivity adversely. Damage from these stresses is responsible for massive economic losses worldwide. Salinity is one of the main abiotic environmental stresses (Safarnejad, 2004; Schwabe et al., 2006). The rise in arable land salinisation is likely to have negative global consequences (Hasegawa et al., b, 2000a; Zhu, 2000; FAO, 2005). Salinity places plants under two kinds of stress. The first is the nutritional imbalance created by saline ions and low soil water capacity both in uptake and translocation. The second is toxicity due to high ion accumulation in the cytoplasm (Kafkafi and Bernstein, 1996). In Saudi Arabia, the semi-arid and arid climate presents great challenges to agriculture. Increased salinity on cultivated land is becoming a major threat. Different approaches such as the adoption of salt-tolerant cultivars and different agricultural practices to mitigate the negative effects of salinity on plant growth and yield have been investigated.

Moringa oleifera Lam, a widely cultivated plant of the Moringaceae family, is commonly known as the drumstick or ben oil tree (Verdcourt 1985). It is a fast-growing soft-wooded tropical perennial tree with a long history of traditional medical and culinary uses. It is widely grown in India, the Philippines, Sudan, South Africa, tropical Asia, the Caribbean and the Pacific Islands. It is ideal for cultivation in Saudi Arabia, as it is extremely drought tolerant and is widely cultivated in arid and semi-arid regions (Stephenson and Fahey, 2004; Galvez Tan and Galvez Tan, 2008; Mridha, 2015).

The *Moringa* tree produces nutrients and antipyretic, anti-inflammatory, antispasmodic, diuretic, anti-hypertensive, anticholesterol, antioxidant and antidiabetic compounds (Guevara et al., 1999; Bennett et al., 2003; Khanuja et al., 2005; Anwar et al., 2007; Shanker et al., 2007; Kasolo, 2010; Mbikay, 2012; Jung and Pandey, 2014; Anwar et al., 2007). Various phenolic acids

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and flavonoid compounds such as rutin and gallic acid have also been found in *Moringa* (Alam et al., 2020). Rutin has shown a range of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective (Ganeshpurkar and Saluja, 2017; Javed et al., 2012; Richetti et al., 2011). Gallic acid has potential preventive and therapeutic effects in many conditions in which oxidative stress has been implicated, including cardiovascular diseases, cancer, neurodegenerative disorders and in ageing (Kaur et al., 2005; Nikolic, 2006; Singh et al., 2018). The moringa tree is also salt tolerant, but the salinity level of this plant affects growth and yield up to 8.0 dS/m (Radovich, 2010; Nouman et al., 2012; Hussein and Abou-Baker, 2013; Fatima et al., 2018).

Excessive use of synthetic chemical fertilisers may have adverse environmental effects. Thus alternative sources, namely biofertilisers, have been proposed to replace chemical fertilisers partially or fully. These biofertilisers are cost-effective and environmentally friendly. *Enterobacter* spp. and microalgae such as *Chlorella vulgaris* and *Nannochloropsis salina* have been identified as plant growth enhancers, as they have many growth promoters and have played a pivotal role in building and sustaining soil fertility, thereby raising the growth and yield of several agricultural crops (Deepa et al., 2010; Ramesh et al., 2014, Khalifa, et al., 2016; Pemmaraju et al., 2018; Ortiz-Moreno et al., 2019; Dineshkumar et al., 2020). The goal of this study was to investigate the effect of *Chlorella vulgaris* and *Nannochloropsis salina* alga and *Enterobacter cloacae* bacteria on the growth, yield and rutin and gallic acid of diluted seawater irrigated *Moringa oleifera*.

2. Material and methods

2.1. Sources of plant, algae and bacteria

Moringa oleifera Lam seeds were collected from the Department of Medicinal and Aromatic Plants, Institute for Horticultural Science, Agricultural Research Center (ARC), Giza, Egypt. Fresh *C. vulgaris* and *N. salina* alga were obtained from the Fisheries Research Centre, King Faisal University, Kingdom of Saudi Arabia. *Enterobacter cloacae* was obtained from the Microbiology Laboratory, Department of Biological Sciences, Faculty of Science, King Faisal University.

2.2. GC/MS analysis

The GC/MS analyses of *C. vulgaris* and *N. salina* were performed at the Department of Clinical Studies, College of Veterinary Medicine, King Faisal University. Both air-dried algae samples were extracted with methanol, according to McKennedy et al. (2016). The methanol extracts were analysed by gas chromatography coupled with mass spectrometry (GC/MS-QP 2010 Plus), equipped with an auto-sampler AOC-20i, (Shimadzu, Kyoto, Japan). Separation was performed with a 30 m × 0.25 mm × 0.1 μm RTX®-5SilMS capillary column (Restek, Bellefonte, PA, USA). The stationary phase was composed of 5% diphenyl and 95% dimethylpolysiloxane and high purity helium gas (99.9999%) used as a carrier gas. The helium gas flow rate, sample volume, and temperature program setting were as described by El Sherif et al. (2020). The computation of composition was according to Lee et al. (2018) with slight modification.

2.3. Moringa plant cultivation and algal/bacterial treatment

The experiment was conducted under greenhouse conditions at the King Faisal University Agriculture and Veterinary Research and Training Centre. The temperature was maintained between 32 and 36 °C, the relative humidity was 47–56%, and the average photoperiod was 14 h. Seeds were sown on 1 March 2019 in germination trays (depth of 1.0–2.0 cm.) filled with a moist mixture of (1:1 v: v) of sand and peat moss. After one month, the seedlings were transplanted into 20 cm diameter plastic pots with a depth of 15 cm, containing 4.5 kg of a moist mixture of (1:1 v: v) of sand and peat moss per pot. In a split-plot pattern, pots were arranged with two factors: *C. vulgaris*, *N. salina* and *E. cloacae* strain MSR1 OD (500) as a sub-factor and saline water as a major factor (0, 3000 and 6000 ppm) with 20 pots per treatment (one plant/pot). The cell suspensions of *C. vulgaris* and *N. salina* were adjusted to 1.5×10^7 cells/ml. Suspensions were mixed with either fresh or saline water at 3000 or 6000 ppm salinity to achieve a concentration of 0.4% (v/v) and applied to the pots at the beginning of the experiment. Fresh water (864 ppm) (Table 1) was used as a control. Seawater (45,000 ppm) from the Arabian Gulf, Dammam, Saudi Arabia, was diluted with fresh tap water (Table 1) to prepare saline solutions of 3000 and 6000 ppm salinity. An electrical conductivity meter (EcoSense ® EC300) was used to measure the salinity of all solutions. Throughout the experimental period, plants were irrigated with corresponding salinity treatments to raise the soil water holding capacity. A pH meter (CRISON Simple 20) was used to measure the pH. Various agricultural activities, such as weeding, were performed as recommended.

2.4. Vegetative growth

The plants were harvested after eight months from the date of their planting. Tree growth parameters such as plant height (cm), stem diameter (mm), number of leaves/plant (n), and leaves, roots and stem dry weight (g) of 10 random plants selected from each treatment group were recorded.

2.5. Chlorophyll pigments determinations

The plant pigments [chlorophyll *a* (Chl-a), chlorophyll *b* (Chl-b), and carotenoid] were extracted with 80% acetone from the 3rd bottom fresh leaf of 4 randomly selected 8-month-old moringa trees. Based on methods in A.O.A.C (1984), these pigments were measured spectrophotometrically and then estimated on a fresh weight basis as mg/100 g.

2.6. Determination of sodium and potassium concentrations in leaves

Plant leaves sampled 8 months after replanting were dried at room temperature and the air-dried matter was ground and digested, according to Piper (1947). Sodium and potassium were determined using atomic absorption flame photometry (3300), according to Wilde et al. (1985).

2.7. HPLC method for determination of rutin and chlorogenic acid

2.7.1. Instrumentation

The contents of rutin and gallic acid were determined from the air-dried samples of leaves and root per each treatment using the Waters 2690 Alliance HPLC system (USA) equipped with a Waters 996 photodiode array detector.

2.7.2. Materials and reagents

Authentic standards of rutin and gallic acid were obtained from Sigma-Aldrich. A rutin stock solution of 2 mg/ml in methanol was prepared and diluted to obtain standard solutions of 900 μg/ml, 750 μg/ml, 600 μg/ml, 450 μg/ml and 300 μg/ml. A gallic acid stock solution of 2 mg/ml in methanol was prepared, and 5 serial dilutions were prepared in concentrations of 1000 μg/ml, 800 μg/ml,

Table 1
Chemical content of the irrigation water.

Salinity Level (ppm)	Cations (meq L^{-1})				Anions (meq L^{-1})				SAR
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}	Cl^{-}	
864	5.72	2.02	7.27	0.38	0.28	2.68	4.03	8.4	3.43

Table 2
Phytochemical composition of methanol extracts from *Chlorelle vulgaris* and *Nanochloropsis salina* by GC MS.

Algae species	RT	Area	Area%	MF	MW	Compound Name
Chlorella vulgaris	7.694	6426	3.75	57	C2H3NO	Isocyanic acid, methyl ester
	17.391	10062	5.87	102	C5H10O2	Capric acid methyl ester
	18.764	1751	1.02	194	C8H6N2O4	p,.beta.-Dinitrostyrene
	19.402	30187	17.61	268	C17H32O2	9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate
	19.628	110359	64.36	270	C17H34O2	Palmitic acid, methyl ester
	21.495	2446	1.43	98	C5H6O2	Vinyl acrylate
	21.825	509	0.3	207	C11H17N3O	p-Mentha-6,8-dien-2-one, semicarbazone
	22.008	505	0.29	207	C7H4F3NO3	p-Cresol, 2-nitro-.alpha.,.alpha.,.alpha.-trifluor
	22.326	9199	5.37	277	C14H9Cl2NO	2H-Indol-2-one, 1-(2,6-dichlorophenyl)-1,3-dihydro
Nannochloropsis salina	7.701	735	0.31	57	C2H3NO	Isocyanic acid, methyl ester
	17.39	14795	6.19	186	C11H22O2	Capric acid methyl ester
	18.767	1789	0.75	194	C8H6N2O4	p,.beta.-Dinitrostyrene
	19.401	50496	21.13	268	C17H32O2	9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate
	19.628	148332	62.08	270	C17H34O2	Palmitic acid, methyl ester $
	21.496	15416	6.45	111	C7H13N	5-Methylhexanenitrile
	22.324	6881	2.88	242	C14H7CLO2	beta.-Chloroanthraquinone
	34.425	504	0.21	207	C11H17N3O	Imidazole, 2-bromo-4-methyl-5-nitro

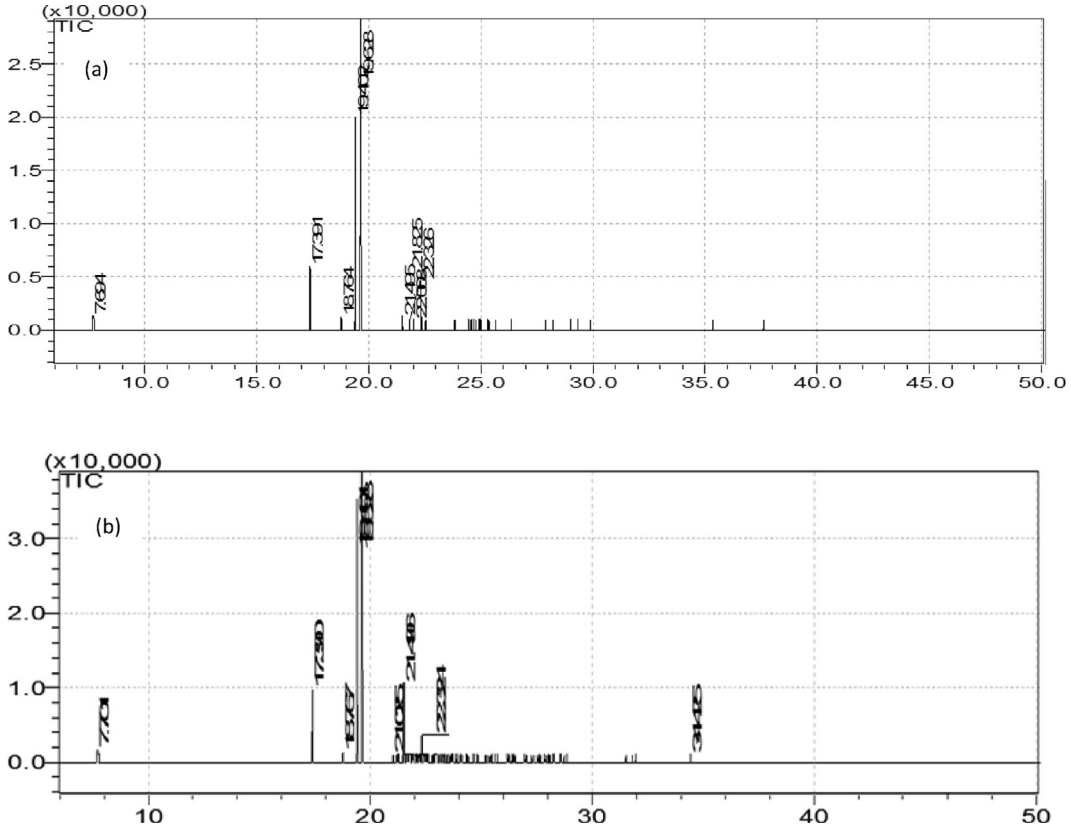

Fig. 1. Components identified in the methanol extracts from (a) *Chlorella vulgaris* and (b) *Nannochloropsis salina* by GC/MS analysis.

600 μg/ml, 400 μg/ml and 200 μg/ml. Each of the dilutions was filtered using a 0.22 μm syringe filter, and 10 μL were injected.

2.7.3. Sample preparation

The extracts were prepared from the dried samples by ultrasonic-aided extraction with methanol. Different weights of

Table 3
Effect of sea water concentrations, *Chlorelle vulgaris, Nanochloropsis salina* and *Enterobacter cloacae* on steam diameter, leaves number and plant height of *Moringa oleifera* plants.

Algae and bacteria	Salinity (ppm)	Stem diameter (mm)	Leaves number (n)	Plant height (cm)
(a) Effect of alga and bacteria				
Control		11.09 b	18.71 bc	129.43 c
Chlorelle vulgaris		17.04 a	25.57 ab	191.64 a
Nannochloropsis salina		16.01 a	26.46 a	195.23 a
Enterobacter cloacae		12.13 b	13.50 c	149.43 b
(b) Effect of sea water concentrations				
	Control	18.99 a	24.40 a	231.15 a
	3000	16.66 b	26.18 a	196.74 b
	6000	6.27 c	11.10 b	68.00 c
(c) The interaction between sea water concentration , alga and bacteria				
Control	Control	19.26 ab	21.00 b	227.80 ab
Chlorelle vulgaris	Control	19.45 ab	22.75 b	237.00 a
Nannochloropsis salina	Control	17.94 ab	46.20 a	219.80 ab
Enterobacter cloacae	Control	19.48 a	19.33 b	237.20 a
Control	3000	14.75 cde	39.25 a	168.25 c
Chlorelle vulgaris	3000	17.73 abc	22.25 b	211.75 ab
Nannochloropsis salina	3000	16.88 bcd	25.40 b	206.60 b
Enterobacter cloacae	3000	16.92 bcd	21.17 b	211.00 b
Control	6000	0.00 f	0.00 c	0.00 e
Chlorelle vulgaris	6000	14.44 de	20.40 b	132.6 d
Nannochloropsis salina	6000	13.25 e	15.50 b	157.75 cd
Enterobacter cloacae	6000	0.00 f	0.00 c	0.00 e

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test.

Table 4
Effect of sea water concentrations , *Chlorelle vulgaris, Nanochloropsis salina and Enterobacter cloacae* on dry weight of leaves, stem and root of *Moringa oleifera* plants.

Algae and bacteria	Salinity (ppm)	Dry weight of leaves (g)	Dry weight of stem(g)	Dry weight of root (g)
(a) Effect of alga and bacteria				
Control		3.22 b	36.09 c	5.66 b
Chlorelle vulgaris		5.51 a	70.58 ab	10.57 a
Nannochloropsis salina		5.26 a	79.29 a	10.28 a
Enterobacter cloacae		3.71 b	59.17 b	11.96 a
(b) Effect of sea water concentrations				
	Control	5.12 a	94.75 a	12.98 a
	3000	5.92 a	71.37 b	12.00 ab
	6000	2.04 b	16.91 c	4.43 b
(c) The interaction between sea water concentration, alga and bacteria				
Control	Control	4.6 cd	61.70 cd	8.50 bcd
Chlorelle vulgaris	Control	6.18 abc	104.70 ab	13.53 abc
Nannochloropsis salina	Control	5.36 abcd	107.00 a	10.22 abc
Enterobacter cloacae	Control	4.84 bcd	104.17 ab	17.7 ab
Control	3000	5.07 bcd	49.18 de	7.05 cd
Chlorelle vulgaris	3000	6.95 a	83.45 abc	12.58 abc
Nannochloropsis salina	3000	6.38 ab	82.24 bc	18.17 a
Enterobacter cloacae	3000	6.13 abc	73.35 c	8.40 bcd
Control	6000	0.00 e	0.00f	0.00 d
Chlorelle vulgaris	6000	4.22 d	32.04 e	7.44 cd
Nannochloropsis salina	6000	4.15 d	39.98 de	13.53 abc
Enterobacter cloacae	6000	0.00 e	0.00 f	0.00 d

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test.

each sample were combined with 50 ml methanol in conical flasks and sonicated for 30 min. The solvent was collected and replaced with 50 ml of fresh methanol every day for three consecutive days to ensure complete extraction before evaporating the methanol using a rotary evaporator at 40 °C to obtain dry residue for each sample. Complete extraction was confirmed by thin-layer chromatography and high-performance liquid chromatography. For the HPLC analysis, a known weight of the residue was dissolved in 5 ml of the mobile phase in a volumetric flask. The contents of each flask were shaken vigorously for 10 min, then sonicated for 15 min before filtrated through a 0.45 μm disposable filters. Before injection, the sample was filtered with a 0.22 μm syringe filter. A sample of 10 μL was then injected, and the concentrations of rutin and gallic acid were calculated.

2.7.4. HPLC analysis conditions

The HPLC separation and quantitation were performed with a Column C18 Kromasil: 4.6 × 150 mm, 5 μm ODS column (Waters, USA). The mobile phase was prepared by mixing 0.1% phosphoric acid in water and acetonitrile in a ratio 5:95 v/v. The flow rate was 1 ml/min. All determinations were performed at ambient temperature (25 °C), Wavelength: 280 nm. The mobile phase was filtered using 0.45 μm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use.

2.8. Statistical analysis

The data from all measurements were analysed using the Statistica 6 program ANOVA/MANOVA (StatSoft, 2001). The mean differ-

Table 5
Effect of sea water concentrations, *Chlorelle vulgaris, Nanochloropsis salina and Enterobacter cloacae* on chlorophyll *a*,b and carotenoid of *Moringa oleifera* plants.

Algae and bacteria	Salinity (ppm)	Chl b (mg/100 g F.W.)	Chl a (mg/100 g F.W.)	Carotenoids (mg/100 g F.W.)
(a) Effect of alga and bacteria				
Control		82.06 b	25. 76 a	100.68 b
Chlorelle vulgaris		124.19 a	25.36 a	126.38 ab
Nannochloropsis salina		132.14 a	27.98 a	150.76 a
Enterobacter cloacae		103.30 ab	25.35 a	121.45 ab
(b) Effect of sea water concentrations				
	Control	138.71 a	35.75 a	156.16 a
	3000	120.17 ab	29.56 ab	139.29 ab
	6000	72.39 b	13.02 b	79.00 b
(c) The interaction between sea water concentration, alga and bacteria				
Control	Control	111.98 bc	14.58 cd	117.40 bc
Chlorelle vulgaris	Control	125.57 abc	23.54 bc	133.97 abc
Nannochloropsis salina	Control	178.29 a	44.83 a	202.00 a
Enterobacter cloacae	Control	138.99 abc	35.29 ab	171.27 ab
Control	3000	131.61 abc	32.45 abc	162.35 ab
Chlorelle vulgaris	3000	86.99 c	32.12 abc	87.43 c
Nannochloropsis salina	3000	154.86 ab	37.68 ab	176.62 ab
Enterobacter cloacae	3000	107.19 bc	40.75 ab	130.77 bc
Control	6000	0.00 d	0.00 d	0.00 d
Chlorelle vulgaris	6000	160.02 ab	29.37 abc	174.32 ab
Nannochloropsis salina	6000	129.57 abc	22.73 bc	141.68 abc
Enterobacter cloacae	6000	0.00 d	0.00 d	0.00 d

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table 6
Effect of sea water concentrations , *Chlorella vulgaris, Nannochloropsis salina and Enterobacter cloacae* on (K% and Na%) contents of *Moringa oleifera* plants.

Algae and bacteria	Salinity (ppm)	Na%	k%
(a) Effect of alga and bacteria			
Control		1.2306 a	0.0236 b
Chlorelle vulgaris		1.0197 b	0.0453 a
Nannochloropsis salina		0.6995 c	0.0498 a
Enterobacter cloacae		0.7110 c	0.0249 b
(b) Effect of sea water concentrations			
	Control	0.5110 b	0.0353 ab
	3000	1.0825 a	0.0456 a
	6000	1.1071 a	0.0242 b
(c) The interaction between sea water concentration, alga and bacteria			
Control	Control	1.2709 a	0.0277 c
Chlorelle vulgaris	Control	1.0394 bcd	0.03890 abc
Nannochloropsis salina	Control	0.9655 d	0.0429 abc
Enterobacter cloacae	Control	1.0542 bcd	0.0317 bc
Control	3000	1.2019 ab	0.0389 abc
Chlorelle vulgaris	3000	1.0591 bcd	0.0469 abc
Nannochloropsis salina	3000	1.000 cd	0.04874 ab
Enterobacter cloacae	3000	1.1675 abc	0.0514 ab
Control	6000	0.0000 e	0.0000 d
Chlorelle vulgaris	6000	1.2151 ab	0.0483 ab
Nannochloropsis salina	6000	1.0049 cd	0.0566 a
Enterobacter cloacae	6000	0.0000 e	0.0000 d

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test.

ence between the treatment groups was evaluated at a probability level of p = 0.05.

3. Results

This study is the first to assess the effects of *Chlorella vulgaris*, *Nannochloropsis salina* and *Enterobacter cloacae* on the growth, yield and phytochemical composition of *Moringa oleifera*.

3.1. GC/MS analysis of C. vulgaris and N. salina

The phytochemical composition of methanol extracts from *C. vulgaris* and *N. salina* are shown in Table 2 and Fig. 1. Fatty acid methyl esters (FAME) such as capric acid and palmitic acid were found in the methanol extract of both alga species. The results confirm the prerogative of polyunsaturated fatty acids in their cellular content is rich and diverse.

3.2. Growth components

The survival rate of explants was 100% in the control group and 3000 ppm seawater treatments. However, at 6000 ppm, the survival rate was 0.0% (data not shown). These data suggest that the *M. oleifera* under study could tolerate salinity up to 3000 ppm.

The stem diameter, leaf number and plant height under salt stress and *C. vulgaris*, *N. salina* and *E. cloacae* exposure and both treatments together are presented in Table 3. The results indicate that increased seawater concentration significantly decreased stem diameter and plant height, except for plants treated with 3000 ppm in terms of leaf number (Tables 3b, c).

Stem diameter and plant height increased in the plants treated with *E. cloacae*, but this increase was not significant (Table 3c). The leaf number significantly increased in the plants treated with *N. salina* (Table 3c).

In the 3000-ppm salinity treatment group, the stem diameter and height of plants exposed to *C. vulgaris* treatments were greater than the control (3000 ppm) treatment (Table 3c). Treatment of *Moringa* seedlings with 6000 ppm salinity led to the death of plants in the control group and *E. cloacae* treatments. In contrast, 100% of the plants treated with *C. vulgaris* and *N. salina* survived at 6000 ppm salinity. The highest stem diameter and leaf number were observed in plants treated with *C. vulgaris* (Table 3c). The presence of *N. salina* enhanced the plant height under 6000 ppm salinity (Table 3c).

3.3. Yield of M. oleifera

The dry weight of leaves, stems and roots of *M. oleifera* under salt stress, with or without *C. vulgaris*, *N. salina* and *E. cloacae* is given in Table 4. The data showed that treatment supplemented with *C. vulgaris*, *N. salina* and *E. cloacae* resulted in the highest dry weight of leaves, stems and roots compared to the control

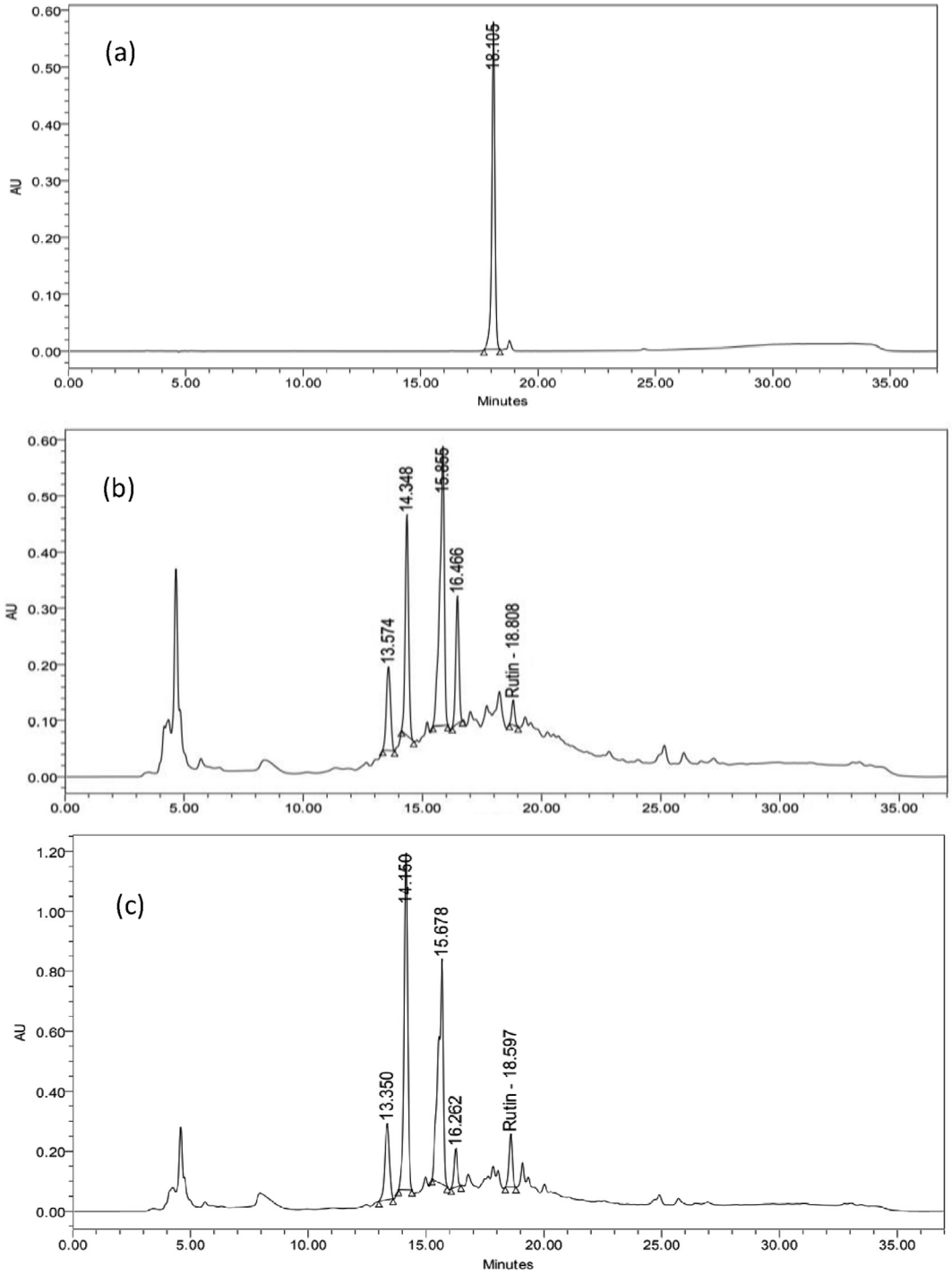

Fig. 2. HPLC chromatogram of the methanolic extract of *Moringa oleifera* leaves (a) rutin authentic compounds, (b) the control plants and (c) plants exposed to *Chlorella vulgaris* and 6000 ppm salinity.

treatment (Tables 4a, c). In contrast, the presence of salinity stress (except that of 3000 ppm) significantly decreased the dry weight of stems and roots and increased the dry weight of leaves (Table 4b, c). *Chlorella vulgaris*, *N. salina* and *E. cloacae* had stimulation effects on the dry weight of leaves, stems and roots in plants grown under 3000 ppm salinity stress (Table 4c). Under high salinity stress (6000 ppm), the dry weight of leaves increased in plants treated with *C. vulgaris*. A higher dry weight of stems and roots was observed in plants treated with *N. salina* (Table 4c).

3.4. Chemical analyses

3.4.1. Sodium and potassium contents

The presence of all three species enhanced the chlorophyll *a*, b and carotenoid contents relative to the control (Table 5c). *N. salina* treatment conferred greater enhancement effects on these parameters than the control, *C vulgaris* and *E. cloacae* treatments (Table 5a,c). The control treatment had inhibitory effects as demonstrated in the lower chlorophyll *b* and carotenoid contents (Table 5a, c). In contrast, these variables decreased in response to

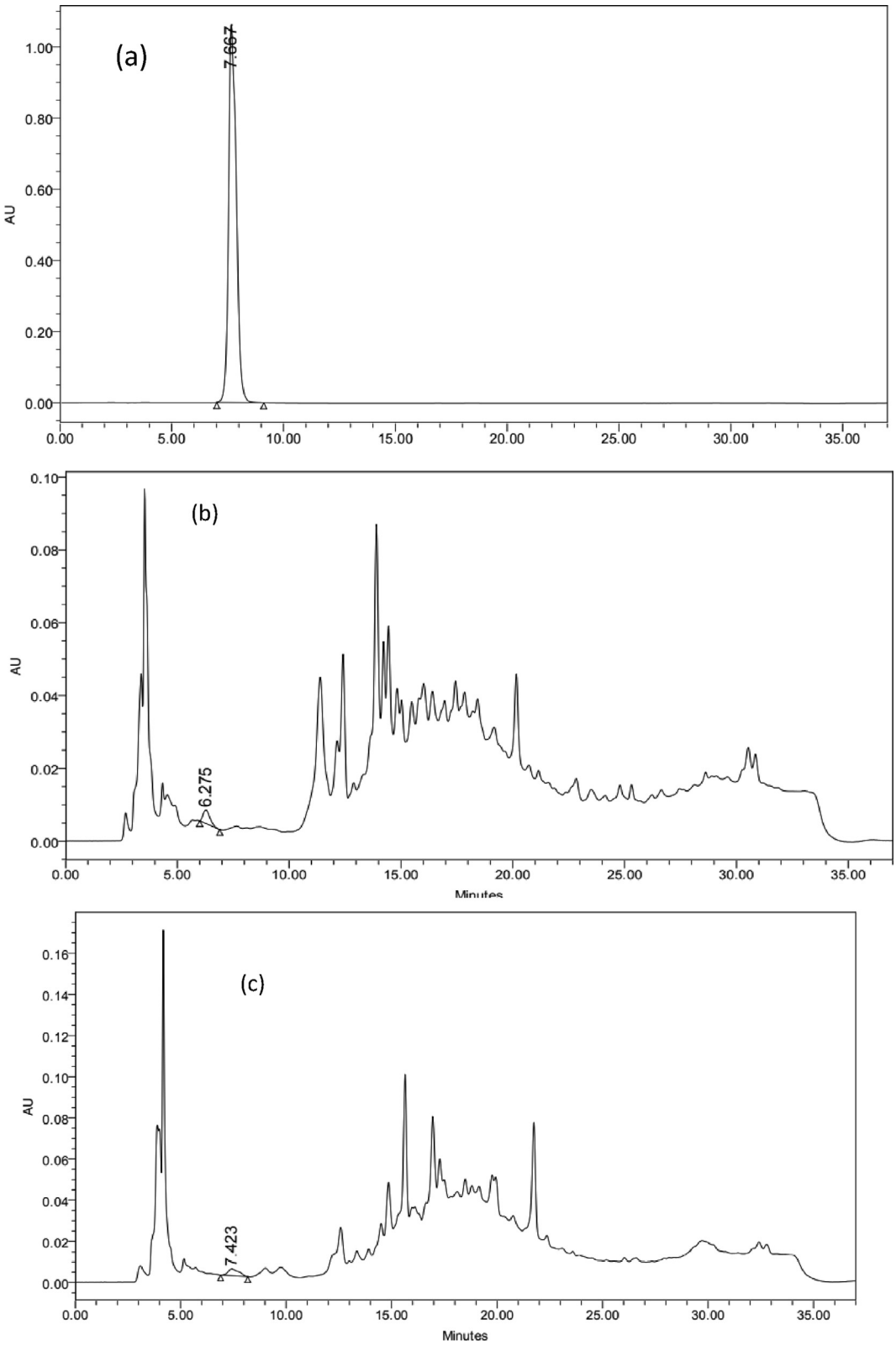

Fig. 3. HPLC chromatogram of methanolic extract of *Moringa oleifera* (a) galic acid authentic compounds, (b) root from plant exposed to *Nannochloropsis salina* + 6000 ppm salinity levels and (c) root of plant exposed to *Enterobacter cloacae* treatment.

salt stress (Tables 5b, c). *N. salina* treatment under 3000 ppm salinity resulted in the highest chlorophyll *a*, b and carotenoid contents relative to the control, *C. vulgaris* and *E. cloacae* treatments (Table 5c). However, *C. vulgaris* treatment led to a smaller increase in the above parameters compared to *N. salina* treatment under 6000 ppm salinity.

Table 7
Effect of sea water concentrations , *Chlorelle vulgaris, Nanochloropsis salina and Enterobacter cloacae* on rutin and galic acid contents of *Moringa oleifera* plants.

Algae and bacteria	Salinity (ppm)	Rutin mg/g extract in plant leave	Galic acid mg/g extract in plant leave	Galic acid mg/g extract in plant root
(a) Effect of alga and bacteria				
Control		0.27810 c	0.12786 b	0.09594 a
Chlorelle vulgaris		0.66961 a	0.33943 a	0.021732 a
Nannochloropsis salina		0.49467 b	0.17938 ab	0.035162 a
Enterobacter cloacae		0.30724 c	0.09207 b	0.014837 a
(b) Effect of sea water concentrations				
	Control	0.50708 a	0.240399 a	0.08969 a
	3000	0.424073 a	0.190564 a	0.016644 a
	6000	0.381042 a	0.12309 a	0.019419 a
(c) The interaction between sea water concentration, alga and bacteria				
Control	Control	0.44858 bc	0.28021 ab	0.274176 a
Chlorelle vulgaris	Control	0.57607 b	0.40585 a	0.0104592 b
Nannochloropsis salina	Control	0.52910 bc	0.10452 ab	0.040883 b
Enterobacter cloacae	Control	0.47458 bc	0.171016 ab	0.033255 b
Control	3000	0.25814 cd	0.103363 ab	0.013653 b
Chlorelle vulgaris	3000	0.46102 bc	0.33540 ab	0.022305 b
Nannochloropsis salina	3000	0.52997 bc	0.21829 ab	0.018623 b
Enterobacter cloacae	3000	0.44717 bc	0.105194 ab	0.011256 b
Control	6000	0.00000 d	0.00000 b	0.00000 b
Chlorelle vulgaris	6000	1.10871 a	0.27705 ab	0.04598 b
Nannochloropsis salina	6000	0.50545 bc	0.215334 ab	0.03169 b
Enterobacter cloacae	6000	0.00000 d	0.00000 b	0.00000 b

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test.

The data in Tables 6a and 6c show that Na^+ content increased in plants under the control and 3000 ppm salinity treatments relative to that of *C. vulgaris*, *N. salina* and *E. cloacae* treatments, smaller Na^+ content was observed in plants exposed to *N. salina* treatment (Tables 6a, c). An increase of salinity levels also increased the Na^+ content in leaves (Table 6b). Under 6000 ppm salinity, the *C. vulgaris* treatment contained higher Na^+ percentage than the *N. salina* treatment (Table 6c).

The *N. salina* treatment produced the highest K^+ percentage in leaves, and the other doses caused an increase in K^+ compared to the control (Table 6a, c). Increased salinity treatments up to 3000 ppm resulted in a decrease in K^+ in the plants in the control and *C. vulgaris* treatments compared to *N. salina* treatment (Table 6a, c).

3.4.2. Results for the HPLC method

A new single, isocratic, selective reverse phase-liquid chromatographic method has been developed for quantification of the rutin and gallic acid in the extracts of different treatments (Table 6 and Figs. 2 and 3). The method allowed good separation and quantification of the rutin and gallic acid within 18.808 and 7.667 min, respectively. The HPLC method was selective for rutin and gallic acid components. It was able to detect rutin and gallic acid components in the complex natural extract with minimal interference from other compounds in the extract.

The effect of salt stress, with or without *C. vulgaris*, *N. salina* and *E. cloacae* on rutin and gallic acid accumulation in *M. oleifera* have not been previously reported.

Rutin was found in the leaf extract of *M. oleifera* plant only, and the absence of this compound in the root extract is consistent with a previous study (Alam et al., 2020). Data (Table 7a and c) showed that the presence of *C. vulgaris*, *N. salina* and *E. cloacae* enhanced the rutin content compared to the control treatment. *C. vulgaris* treatment conferred a higher enhancement effect on rutin content than the control, *N. salina* and *E. cloacae* treatments (Table 7a and c). However, plants irrigated with 3000 and 6000 ppm saltwater showed a decrease in rutin compared to the control (Table 7b). The highest rutin production (1.10871 mg/g extract) was obtained in plants treated with *C. vulgaris* and irrigated with 6000 ppm salinity. The total amount of gallic acid (mg/g extract) in the leaves and roots of *M. oleifera* are presented in Table 7 and Fig. 3a and 3b. Results indicated that the leaf sample accumulated a higher amount of gallic acid compared to the root sample (Table 7). The amount of gallic acid increased in the leaf samples of plants exposed to *C. vulgaris* and *N. salina* treatment, respectively (Table 7c). Under 3000 and 6000 ppm salinity levels, the gallic acid accumulated at higher amounts in the leaf and root samples of *M. oleifera* treated with *C. vulgaris* (Table 7c). Increased salinity levels led to a decrease in gallic acid in both leaf and root samples of *M. oleifera* (Table 7a).

4. Discussion

Our study indicated that *C. vulgaris*, *N. salina* and *E. cloacae* as microorganisms were good elicitors in enhancing growth and phytochemicals accumulation in *M. oleifera* plant grown under different salinity levels. In terms of stem diameter and plant height, *E. cloacae* was a better elicitor than *C. vulgaris* or *N. salina*. However, *N. salina* resulted in higher leaf number and photosynthetic pigments. The presence of *C. vulgaris*, *N. salina* and *E. cloacae* enhanced the leaf, stem and root dry weight of the plants. The plant growth-stimulating effect of *E. cloacae* is due to its ability to make organic nitrogen sources available to plants (Santoyo et al., 2016; White et al., 2018; Macedo-Raygoza et al., 2019). Microalga and *E. cloacae* as biofertilisers have been previously shown to increase plant growth and yield of some crops (Özdemir et al., 2016; Borham et al., 2017; White et al., 2018; Satheeswaran and Jun, 2020).

Salinity level of 3000 and 6000 ppm showed some toxic effects, as seen in the inhibition of plant growth, yield, and contents of chlorophyll *b* and carotenoids. A negative relationship was previously demonstrated between the degree of salt stress and *M. oleifera* plant growth characters, i.e. the dry weight of roots, stems and leaves, which decreased as the salt concentration increased in the diluted seawater (Hussein and Abou-Baker, 2013; Soliman et al., 2015). This reduction could be resulted by the toxicity associated with excessive uptake of Na^+ and nutrient imbalance (El Sherif et al., 2013; El-Garhy et al., 2016; Khattab and El-Garhy, 2016; Fatima, et al., 2018). Uptake of K^+ mineral is reduced during saline conditions. This could be due to the blockage or reduced activity of the transporters caused by the high level of Na^+. As the result, there

is a K^+ and Na^+ imbalance in the plant (Soliman et al., 2015; El-Garhy et al., 2016). A decreased K^+ content is a response commonly observed in plants under salt stress because K^+ directly competes with Na^+ for binding sites that are charged dependent (Chen and Yu, 2007). The results herein showed that the application of micro-alga limited toxic ion accumulation, thus increased K^+ contents (Kuşvuran and Can, 2020). Increased salinity levels decreased rutin and gallic acid content. A decrease in the bioactive compounds content of *M. oleifera* resulting from increased salinity levels has been reported by Anwa et al. (2006). The presence of *C. vulgaris* and *N. salina* promoted plant growth and yield as well as rutin and gallic acid content in *M. oleifera* plants grown under different salinity levels. *Nannochloropsis* spp. and *C. vulgaris* are beneficial microscopic species that can increase nutrient uptake, growth and abiotic stress tolerance in plants (Agwa et al., 2017; Faheed and Fattah, 2008; Kang et al., 2015; Oancea et al., 2013; Ördög et al., 2004; Rajasekaran et al., 2015; Zayadan et al., 2014; Han et al., 2018). It offers substantial levels of macro and micronutrients, metabolites such as carbohydrates, proteins, fatty acid methyl esters and growth-promoting factors, such as cytokinins, which affect plant growth and yield (Elarroussia et al., 2016; Kholssi et al., 2018; Kuşvuran and Can, 2020).

5. Conclusions

Our study has indicated that the application of *C. vulgaris*, *N. salina* and *E. cloacae* have enhanced the rutin and gallic acid compositions while promoting growth and yield *M. oleifera* plants grown under different salinity levels**.** The presence of *C. vulgaris* incresed plant growth and yield as well as rutin and gallic acid content in *M. oleifera* plants grown under different salinity levels. compared to the *N. salina* and *E. cloacae*. The study opens the possibility of utilising *C. vulgaris*, *N. salina* and *E. cloacae* live cells as potential sources of biofertiliser without causing environmental pollution.

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References

AOAC, 1984. Official Methods of Analysis of the Association of Official Chemists. In: Howitz (Ed.); Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC.

Agwa, O.K., Ogugbue, C.J., Williams, E.E., 2017. Field evidence of *Chlorella vulgaris* potentials as a biofertiliser for *Hibiscus esculentus*. Int. J. Agric. Res. 12 (4), 181–189.

Alam, P., Alam, P., Sharaf-Eldin, M.A., Algarni, M.H., 2020. Simultaneous identification of rutin, chlorogenic acid and gallic acid in Moringa oleifera by densitometric high-performance thin-layer chromatography method. JPC – J. Planar Chromatogr. – Modern TLC 33, 27–32. https://doi.org/10.1007/s00764-019-00002-2.

Anwar, F, Ashraf, M, ., Gilani H, A, 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytotherapy Research 21 (1), 17–25.

Anwar, F., Latif, S., Ashraf, M., Gilani, A.H., 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother. Res. 21 (1), 17–25. https://doi.org/10.1002/ptr.2023.

Bennett, R.N., Mellon, F.A., Foidl, N., Pratt, J.H., Dupont, S.M., Perkins, L., Kroon, P.A., 2003. Profiling Glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *M. oleifera* L. (Horseradish Tree) and *Moringa stenopetala* L. J. Agric. Food. Chem. 51, 3546–3553.

Borham, A., Belal, E., Metwaly, M., El-gremy, S., 2017. Phosphate solubilisation by enterobacter cloacae and its impact on growth and yield of wheat plants. J. Sustain. Agric. Sci. 43 (2), 89–103. https://doi.org/10.21608/jsas.2017.1035.1004.

Chen Q, X, Yu J, B, 2007. Ionic effects of Na+ and Cl on photosynthesis in Glycine max seedlings under iso osmotic salt stress. J. Plant Physiol. Mol. Biol 33, 294–300.

Deepa, C.K., Dastager, S.G., Pande, A., 2010. Isolation and characterisation of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea *Vigna unguiculata* L. Walp. seedling growth. World J. Microbiol. Biotechnol. 26, 1233–1240.

Dineshkumar, R., Subramanian, J., Sampathkumar, P., 2020. Prospective of chlorella vulgaris to augment growth and yield parameters along with superior seed qualities in black gram, *Vigna mungo* (L.). Waste Biomass Valorisation 11, 1279–1287. https://doi.org/10.1007/s12649-018-0465-9.

Elarroussia, H., Elmernissia, N., Benhimaa, R., Isam, M.E.K., Najib, B., Abedelaziz, S., Imane, W., 2016. Microalgae polysaccharides a promising plant growth biostimulant. J. Algal Biomass Utilization 7, 55–63.

El Sherif, F., Albotnoor, N., Yap, Y.-K., Meligy, A., Khattab, S., 2020. Enhanced bioactive compounds composition of *Lavandula officinalis* in-vitro plantlets using NaCl and *Moringa oleifera*, *Aloe vera* and *Spirulina platensis* extracts. Ind. Crops Prod. 157, 112890.

El Sherif, Fadia, Khattab, Salah, Ibrahim, Amany K., Ahmed, Safwat A., 2013. Improved silymarin content in elicited multiple shoot cultures of *Silybum marianum* L. Physiol. Mol. Biol. Plants 19 (1), 127–136. https://doi.org/10.1007/s12298-012-0141-7.

El-Garhy, H., Khattab, S, MahmoudMoustafa, M.A., Rania Abou Ali, Azeiz, AZ, Elhalwagi, A, El Sherif, F., 2016. Eliciting the expression of chalcone synthase genes (CHSs) enhanced the silymarin yield and induced salinity tolerance of Silybum marianum L. Plant Physiology and Biochemistry 108, 191–202.

Faheed, F.A., Fattah, Z.A., 2008. Effect of *Chlorella vulgaris* as bio-fertiliser on growth parameters and metabolic aspects of lettuce plant. J. Agric. Soc. Sci. 4, 165–169.

FAO, 2005. Global Network on Integrated Soil Management for Sustainable Use of Salt-affected Soils. FAO Land and Plant Nutrition Management Service, Rome, Italy. http://www.fao.org/ag/agl/agll/spush.

Fatima, N., Akram, M., Shahid, M., Abbas, G., Hussain, M., Nafees, M., Wasaya, A., Tahir, M., Amjad, M., 2018. Germination, growth and ions uptake of moringa (*Moringa oleifera* L.) grown under saline condition. J. Plant Nutr. 41 (12), 1555–1565. https://doi.org/10.1080/01904167.2018.1459690.

Galvez Tan, J.Z., Galvez Tan, M.A., 2008. Medicinal Fruits and Vegetables. Health Future Foundation, Quezon City, Philippines.

Ganeshpurkar, Aditya, Saluja, Ajay K., 2017. The Pharmacological Potential of Rutin. Saudi Pharm. J. 25 (2), 149–164. https://doi.org/10.1016/j.jsps.2016.04.025.

Guevara, A.P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuka, M., Ito, Y., Tokuda, H., Nishino, H., 1999. An antitumor promoter from M. Oleifera Lam. Mutatation Res. 440, 181–188.

Han, X., Zeng, H., Bartocci, P., Fantozzi, F., Yan, Y., 2018. Phytohormones and Effects on Growth and Metabolites of Microalgae: A Review. Fermentation 2018, 4–25.

Hasegawa, P.M., Bressan, R.A., Pardo, J.M., 2000a. The dawn of plant salt to tolerance genetics. Trends Plant Sci. 5, 317–319.

Hasegawa, P.M., Bressan, R.A., Zhu, J.-K., Bohnert, H.J., 2000b. Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology 51, 463–499.

Hussein, M.M., Abou-Baker, M., 2013. Growth and mineral status of Moringa plants as affected by silicate and salicylic acid under salt stress. Int. J. Plant Soil Sci. 3 (2), 163–177. https://doi.org/10.9734/IJPSS/2014/6105.

Javed, H., Khan, M.M., Ahmad, A., Vaibhav, K., Ahmad, M.E., Khan, A., Ashafaq, M., Islam, F., Siddiqui, M.S., Safhi, M.M., Islam, F., 2012. Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience 210, 340–352. https://doi.org/10.1016/j.neuroscience:2012.02.046.

Jung, I.L., 2014. Soluble extract from Moringa oleifera leaves with a new anticancer activity. PloS One 9(4), e95492. doi: 10.1371/journal.pone.0095492.

Kafkafi, U., Bernstein, N., 1996. Root Growth under Salinity Stress. In: Plant Roots – the Hidden Half. Marcel Dekker, New York, pp. 463–499.

Kang, N.K., Jeon, S., Kwon, S., Koh, H.G., Shin, S.-E., Lee, B., Choi, G.-G., Yang, J.-W., Jeong, B.-R., Chang, Y.K., 2015. Effects of overexpression of a bHLH transcription factor on biomass and lipid production in *Nannochloropsis salina*. Biotechnol. Biofuels 8, 200. https://doi.org/10.1186/s13068-015-0386-9.

Kasolo, J., 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J. Med. Plant Res. 4 (9), 753.

Kaur, Swayamjot, Michael, Husheem, Arora, Saroj, Härkönen, Pirkko L., Kumar, Subodh, 2005. The in vitro cytotoxic and apoptotic activity of Triphala—an Indian herbal drug. J. Ethnopharmacol. 97 (1), 15–20. https://doi.org/10.1016/j.jep.2004.09.050.

Khalifa, A.Y.Z., Alsyeeh, A.-M., Almalki, M.A., Saleh, F.A., 2016. Characterisation of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi J. Biol. Sci. 23 (1), 79–86. https://doi.org/10.1016/j.sjbs.2015.06.008.

Khanuja, S.P.S., Arya, J.S., Tiruppadiripuliyur, R.S.K., Saikia, D., Kaur, H., Singh, M., Gupta, S.C., Shasany, A.J., Darokar, M.P., Srivastava, S.K., Gupta, M.M., Verma, S. C., Pal, A., 2005. Nitrile glycoside is useful as a bioenhancer of drugs and nutrients, process of its isolation from Moringa oleifera. (United States Patent 6,858,588).

Kholssi, R., Marks, E.A.N., Miñón, J., Montero, O., Debdoubi, A., Rad, C., 2018. Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth. J. Plant Growth Regul. 1–6. https://doi.org/10.1007/s00344-018-9879-7.

Khattab, S., El-Garhy, H.A., 2016. In vitro assessment of salt tolerance in male poplar trees and associated molecular changes. J. Hortic. Sci. Biotechnol. 91 (6), 2–11.

Kuşvuran, A., Can, A.G., 2020. Effects of Microalga (Chlorella vulgaris Beijerinck) on Seconder Metabolites and Antioxidative Defense System Improve Plant Growth and Salt Tolerance in Guar [Cyamopsis tetragonoloba (L.) Taub.]. Legume Res. - An Int. J. 43, 56–60.

Lee, Kyoung Bok, Choi, Jaehyuk, Ahn, Soon Kil, Na, Jong-Kuk, Shrestha, Krishna Kumar, Nguon, Samnang, Park, Sang Un, Choi, Sangho, Kim, Jae Kwang, 2018.

Quantification of arbutin in plant extracts by stable isotope dilution gas chromatography–mass spectrometry. Chromatographia 81 (3), 533–538. https://doi.org/10.1007/s10337-017-3461-5.

Macedo-Raygoza, G.M., Valdez-Salas, B., Prado, F.M., Prieto, K.R., Yamaguchi, L.F., Kato, M.J., Canto-Canché, B.B., Carrillo-Beltrán, M., Di Mascio, P., White, J.F., Beltrán-García, M.J., 2019. *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black Sigatoka pathogen. Front. Microbiol. 10, 804.

Mbikay, M., 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. Front. Pharmacol. 3, 24.

McKennedy, Janet, Önenç, Sermin, Pala, Mehmet, Maguire, Julie, 2016. Supercritical carbon dioxide treatment of the microalgae *Nannochloropsis oculata* for the production of fatty acid methyl esters. J. Supercrit. Fluids 116, 264–270. https://doi.org/10.1016/j.supflu.2016.06.003.

Mridha, M.A.U., 2015. Prospects of Moringa Cultivation in Saudi Arabia Journal of Applied Environmental and Biological Sciences 5(3), 39–46.

Nikolic, Katarina M., 2006. Theoretical study of phenolic antioxidants properties in reaction with oxygen-centered radicals. J. Mol. Struct. (Thoechem) 774 (1-3), 95–105. https://doi.org/10.1016/j.theochem.2006.07.017.

Nouman, W., Siddiqui, M.T., Basra, S.M.A., Khan, R.A., Gull, T., Olson, M.E., Munir, H., 2012. Response of *Moringa oleifera* to saline conditions. Int. J. Agric. Biol. 14 (5), 557–562.

Oancea, F., Velea, S., Fătu, V., Mincea, C., Ilie, L., 2013. Micro-algae based plant biostimulant and its effect on water stressed tomato plants. Romanian J. Plant Prot. 6, 104–117.

Ördög, V., Stirk, W.A., Lenobel, R., Bancířová, M., Strnad, M., van Staden, J., Szigeti, J., Németh, L., 2004. Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. J. Appl. Phycol. 16 (4), 309–314. https://doi.org/10.1023/B:JAPH.0000047789.34883.aa.

Ortiz-Moreno, M.L., Sandoval-Parra, K.X., Solarte-Murillo, L.V., 2019. Chlorella, ¿un potencial biofertilizante? Orinoquia 23 (2), 71–78. https://doi.org/10.22579/20112629.582.

Özdemir, S., Sukatar, A., Oztekin, G.B., 2016. Production of *Chlorella vulgaris* and its effect on plant growth, yield and fruit quality of organic tomato grown in greenhouse as biofertiliser. Tarim Bilimeri Dergisi 22, 596–605.

Pemmaraju, Deepak, Appidi, Tejaswini, Minhas, Gillipsie, Singh, Surya Prakash, Khan, Nooruddin, Pal, Mahadeb, Srivastava, Rohit, Rengan, Aravind Kumar, 2018. Chlorophyll rich biomolecular fraction of A. cadamba loaded into polymeric nanosystem coupled with Photothermal Therapy: a synergistic approach for cancer theranostics. Int. J. Biol. Macromol. 110, 383–391. https://doi.org/10.1016/j.ijbiomac.2017.09.084.

Piper, O.S., 1947. Soil and plant analysis. University of Adelaide, Adelaide, pp. 258–275.

Radovich, T., 2010. Farm and Forestry Production and Marketing profile for Moringa (*Moringa oleifera*). In: Elevitch, C.R. (Ed.), Specialty Crops for Pacific Island Agroforestry. Holualoa, Hawai'i.

Rajasekaran, S., Sundaramoorthy, P., Sankar, G.K., 2015. Effect of FYM, N, P fertilisers and biofertilisers on germination and growth of paddy (Oryza sativa L.). Int. Lett. Nat. Sci. 35, 59–65.

Ramesh, Aketi, Sharma, Sushil K., Sharma, Mahaveer P., Yadav, Namrata, Joshi, Om P., 2014. Plant growth-promoting traits in *Enterobacter cloacae* subsp. *dissolvens* MDSR9 isolated from Soybean Rhizosphere and its impact on growth and nutrition of soybean and wheat upon inoculation. Agric. Res. 3 (1), 53–66. https://doi.org/10.1007/s40003-014-0100-3.

Richetti, S.K., Blank, M., Capiotti, K.M., Piato, A.L., Bogo, M.R., Vianna, M.R., Bonan, C. D., 2011. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. Behav. Brain Res. 217 (1), 10–15. https://doi.org/10.1016/j.bbr.2010.09.027.

Safarnejad, A., 2004. Characterization of somaclones of alfalfa (*Medicago sativa* L.) for drought tolerance. J. Agric. Sci. Technol. 6, 121–127.

Santoyo, Gustavo, Moreno-Hagelsieb, Gabriel, del Carmen Orozco-Mosqueda, Ma., Glick, Bernard R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008.

Satheeswaran, T., Jun, S., 2020. The biotechnological potential of the marine diatom *Skeletonema dohrnii* to the elevated temperature and pCO_2. Mar. Drugs 18 (5), 259. https://doi.org/10.3390/md18050259.

Schwabe, Kurt A., Kan, Iddo, Knapp, Keith C., 2006. Drainwater management for salinity mitigation in irrigated agriculture. Am. J. Agric. Econ. 88 (1), 133–149. https://doi.org/10.1111/j.1467-8276.2006.00843.x.

Shanker, K., Gupta, M.M., Santosh, K., Srivastava, S.K., Bawankule, D.U., Pal, A., Khanuja, S.P., 2007. Determination of bioactive nitrile glycoside(s) in drumstick (*Moringa oleifera*) by reverse phase HPLC. Food Chem. 105 (1), 376–382.

Singh, M.P., Avneet, G., Sisodia, S.S., 2018. Gallic acid: pharmacogical promising lead molecule: a review. Int. J. Pharm. Phytochem. Res. 10 (4), 132–138.

StatSoft. 2001. STATISTICA Fur Windows (software-system fur Datenanalyse).

Soliman, A, El-feky, S, Darwish, E, 2015. Alleviation of salt stress on *Moringa peregrina* using foliar application of nanofertilizers. Journal of Horticulture and Forestry 7 (2), 36–47.

Stephenson, Katherine K., Fahey, Jed W., 2004. Development of tissue culture methods for the rescue and propagation of endangered *Moringa* Spp. Germplasm. Econ. Bot. 58 (sp1), S116–S124. https://doi.org/10.1663/0013-0001(2004)58[S116:DOTCMF]2.0.CO;2.

Verdcourt, B.A., 1985. Synopsis of the moringaceae. Kew Bull. 40, 1–24.

White, J.F., Kingsley, K.L., Verma, S.K., Kowalski, K.P., 2018. Rhizophagy cycle: an oxidative process in plants for nutrient extraction from symbiotic microbes. Microorganisms 6, 1–20. https://doi.org/10.3390/microorganisms6030095.

Wilde, S.A., Corey, R.B., Lyer, J.G., Voight, G.K., 1985. Soil and Plant Analysis for Tree Culture. Oxford and IBM publishing, New Dehli, pp. 93–106.

Zayadan, B.K., Matorin, D.N., Baimakhanova, G.B., Bolathan, K., Oraz, G.D., Sadanov, A.K., 2014. Promising microbial consortia for producing biofertilisers for rice fields. Microbiology 83, 391–397.

Zhu, J.-K., 2000. Genetic analysis of plant salt tolerance using Arabidopsis. Plant Physiol. 124, 941–948.