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Allelopathic potential of Egyptian halophytes *Arthrocnemum macrostachyum* and *Halocnemum strobilaceum* from two coastal areas.

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ABSTRACT

We studied the allelopathic effects of two halophytes (*Arthrocnemum macrostachyum* and *Halocnemum strobilaceum*), growing in coastal areas of Burj Alarab and Manzala, Egypt, on the seed germination, chlorophyll content, leaf area, shoot length, and esterase and superoxide dismutase activities of *Eruca sativa*. The shoot aqueous extracts (0, 10, 20, 30 %) of both species had similar phytotoxicity. The *H. strobilaceum* was more allelopathic than *A. macrostachyum*. The samples from the Burj Alarab area were more phytotoxic than from the Manzala area. GC-MS analysis detected 27 and 67 compounds in *Arthrocnemum* and 25 and 31 in *Halocnemum* extracts, respectively. The dominant components in the Burg Alarab *Arthrocnemum* extract were: bicyclo[3.3.1]nonane-2,4-dione,3-(2,2-dimethyl propylidene) and 6,8-nonadien-2-one, 6-methyl-5-(1-methy lethylidene). In Manzala *Arthrocnemum* extracts, the antioxidant 3,4-dihydro-2h-1,5-(3"-t-butyl) benzodioxepine, nonadien and ethanonaphthalene, were most abundant. In the Burg Alarab *Halocnemum* extract dominant allelochemicals were : hexadecanoic acid and octadecenoic acid. While, in the Manzala extract the allelochemicals were : thieno[3,4-c] pyridine, 1,3,4,7-tetraphenyl, benzothieno[2,3-c]quinolin-6(5h)-one, 2-methoxy (11.43 %). These results suggested the influence of habitat on the diversity, allelochemicals contents and allelopathy.

Keywords: Allelopathy, *Arthrocnemum macrostachyum*, coastal areas, Egypt, *Eruca sativa*, esterase, GC-MS, *halocnemum strobilaceum*, halophytes, SOD, phytotoxicity, seed germination, seedling growth.

INTRODUCTION

Worldwide the coastal wetlands suffers from salinization due to rise in sea level, decreased fresh water flow and storm surges (33), and only halophytes (salt-tolerant plants) survive and reproduce in these ecosystems (37). Halophyte plants over produce the secondary metabolites, as an adaptive response to saline conditions and these compounds play vital roles as osmolytes and in scavenging of reactive oxygen species (ROS) under saline conditions (32,55,76). Additionally, secondary metabolites play crucial role in allelopathic effects and are present in different plant organs. Allelochemicals are released from plants into the environment via leaching from the shoots, volatilization, exudation from the roots and decomposition of plant residues (8,35,65,71). Allelochemicals directly inhibits the seed germination and growth of neighbouring species due to their negative effects on cell division, photosynthesis and respiration (29). Additionally, allelochemical stress causes oxidative damage in target plants through overproduction of ROS such as hydrogen peroxide,

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superoxide anion and hydroxyl radical (13,75). Although plants contain non-enzymatic compounds and antioxidant enzyme systems to control ROS levels under stress conditions (3), high levels of ROS inhibits the enzymes such as superoxide dismutase and peroxidase (44). Esterases have major functions in breaking down lipids and detoxifying xenobiotics, and used as biomarkers for allelochemical stress in target organisms (18,66).

The structure and composition of plant cover in dry and semi-arid habitats are significantly affected by the biotic interactions between the plants (6,59,67). The release of secondary metabolites is associated with tolerance against stresses (43). Allelopathy change the vegetation properties viz., growth of single species, inhibition zones and root segregation. The phytotoxicity increases under abiotic stress [drought and extreme temperature (5,6,19,27,34,53)], however, the toxicity of allelopathic plants may vary among habitats (16,39).

Arthrocnemum macrostachyum and *Halocnemum strobilaceum* (family Amaranthaceae) are succulent, perennial halophytic shrubs with ecological, nutritional and medicinal value thriving in saline habitats along the coastal zone of Mediterranean Sea (2,24,50,60,69,77); however, human activities changes the plant communities (15,30,60). *Eruca sativa* is medicinal plant native to Mediterranean region with antifungal, anticarcinogenic and antibacterial activities (28).

This study aimed to evaluate the allelopathic potential of shoot extracts of these two halophytes collected from two habitats in Egypt on the seed germination, growth, chlorophyll content, and superoxide dismutase and esterase activities of *Eruca sativa* and to identify potential allelochemicals in these halophytes.

MATERIALS AND METHODS

2.1 Samples collection

The shoots of two halophytes (*A. macrostachyum* and *H. strobilaceum*) were collected in October 2016 from two coastal areas of Egypt: the Manzala shore (31˚ 12' 22" N, 32˚ 15' 38" E) and Burj Alarab (30˚ 55' 31" N, 29˚ 31' 27" E). In Burj Alarab, both species grow in sandy soil with *Zygophyllum coccenium*. The concentrations of total dissolved solids and nitrates in the Burj Alarab soil were 530 and 2 ppm, respectively. The Manzala coastal area has silty soil and both halophytes grow with *Z. coccenium*, *Zygophyllum album* and *Suaeda fruticosa*. The total dissolved solids and NO_3 content in Manzala soil were 43,800 and 153 ppm, respectively.

2.2. Water extracts

The shoots of two species were separately washed with distilled water and air dried at room temperature (25 ℃) for 7 days. The dried material was chopped into 1-cm pieces and powdered in blender. To prepare stock solution, 75 g powdered materials were added to 500 ml distilled water in flask and incubated with shaking for 24 h. The extracts were filtered through muslin cloth followed by filter paper, as described by Sarkar *et al.* (58), then stored at 4 °C. Three dilutions (10 %, 20 % and 30 %) of this stock extract were prepared for this study. For GC-MS, 5 ml of each extract was air dried and then dissolved in 15 ml methanol.

2.3. Germination bioassay

(i). **Petriplate Bioassay:** *Eruca sativa* was selected in this study as target plant due to its fast growth and sensitivity to allelochemicals (4,26,36). Thirty seeds of *E. sativa* were sown in Petri dishes (11 cm dia) on filter paper (Whatman No. 1) wetted with 8 ml of

each extract; distilled water was used as control. The treatments were replicated thrice in complete randomnised design. All Petri dishes were kept in dark at 25 ℃ for 15 days. Seeds were considered germinated when the radicle length was about 1-2 mm (21).

(ii). **Pot culture :** To examine the effects of allelochemicals on the growth of *E. sativa*, its 15- seeds were sown per pot (13 cm height × 10 cm dia) filled with 350 g of sieved clay garden soil + sand (33 % clay and 67 % sand). The soil was irrigated with 20 % MS for 2-weeks (field capacity). After that, the seedlings were irrigated with different extract concentrations (30 ml every 3- days) for 4-weeks; the control was treated only with 20 % MS. The treatments were replicated thrice in complete randomnised design. All pots were kept in a growth chamber [25 ℃ and 200 µmol m^{-2} s^{-1}] for 6-weeks. Afterwards, the seedlings were harvested and used for measuring different parameters.

2.4. Leaf area

The leaf area was determined using the chart paper method (74). Shoot length was measured (centimetres) using a ruler (20).

2.5. Chlorophyll measurement

One hundred mg of fresh leaf tissue was ground in 10 ml of 80 % acetone and centrifuged (1000 rpm) for 10 min. The absorbance of the supernatant was measured at 663, 645 and 452 nm using a spectrophotometer (Jenway, UK). Chl a, Chl b, and total carotenoid contents were determined as per Lichtenthaler (42).

2.6. Enzyme extraction and detection

Leaves tissues (0.2 g) were homogenized at 4 °C in 0.5 ml 20 mM Tris HCl buffer (pH 7.8) containing 1 mM DDT, 1 mM EDTA and 20 mg PVP. The homogenates were centrifuged for 10 min at 14,000×*g*, and the supernatant was collected for esterase and superoxide dismutase analyses. The protein concentration was measured spectrophotometrically (Jenway, UK) as per method of Bradford (11) with BSA as standard. A non-denaturing polyacrylamide gel was prepared as per Laemmli (41) with sodium dodecyl sulphate. Electrophoretic separation was done at 100 V for 120 min at 4 °C. Equal amounts of protein samples were loaded onto each gel lane.

(i). **SOD activity:** For SOD separation, gels were soaked in 200 mM K-phosphate buffer (pH 7.8) containing 1 mM EDTA, 0.24 mM nitro blue tetrazolium and 0.1 mM riboflavin for 30 min. After that, the gels were exposed to fluorescence light. Isozymes appear as colourless bands and the negative band is presented as positive for clarification (47).

(ii). **Esterase activity :** It was assessed as per the following method of Smith *et al.* (64). The gel was soaked in 200 ml of 0.1 M sodium phosphate buffer (pH 7) containing 0.2 g fast blue RR and 30 mg α-naphthyl acetate (dissolved in 1 ml acetone) for 30 minutes at 37 °C in dark. The gel was then transferred to and stored in 7 % acetic acid.

2.7. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the extracts was analysed using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-5MS capillary column (30 m x 0.25 mm inner diameter x 0.25 µm film thickness). The temperature programme was initially at 60 °C followed by an increase to 250 °C at a rate of 5 °C /min and maintained for 2 min. After that, the temperature was increased to 300 °C at 15 °C/min. The injector and MS transfer line temperatures were maintained at 270 °C. Helium was used as the carrier gas in this system at a flow rate of 1 ml/min. The sample (1

µl) was injected using an Autosampler AS3000 coupled to the GC in split mode. The MS conditions were characterized by an EI ion source and transfer line temperature at 200 °C and 280 °C, respectively, an ionization energy of 70 eV, and a mass scan range of m/z 40–650 in full-scan mode. The compounds were identified by comparison based on their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral databases.

2.8. Statistical analysis

The germination (%), leaf area, shoot length, and Chl a, Chl b and carotenoid contents were expressed as means of relative percentage of control with the standard error. Data were subjected to one-way analysis of variance (ANOVA) and Tukey's test using SPSS 16.0 statistical software. The mean comparison was set at $P < 0.05$. Values denoted by the same letter are not significantly different.

RESULTS AND DISCUSSION

3.1. Seed germination

Explorations of the allelopathic potential of two species that commonly grow together in different habitats, provide unique information about the factors affecting their allelopathy. To examine the allelopathic activity of *A. macrostachyum* and *H. strobilaceum*, their shoot extracts (0, 10 %, 20 %, 30 %) effects were studied on *E. sativa* seed germination and seedlings germination growth for 15 days and 30 days, respectively (Fig. 1). Using the maximum germination percentage (100 %) detected with distilled water, the degree of seed germination inhibition appeared as a function of the concentration of *A. macrostachyum* and *H. strobilaceum* extract (Fig. 1).

Based on habitat differences, the phytotoxicity of the two species exhibited the same trend with various degrees of seed germination inhibition (Fig. 1). Regarding *Arthrocnemum*, the extract of shoots sampled from the Burj Alarab area appeared to be more toxic than that of samples collected from the Manzala area, where germination of *Eruca* seeds was completely inhibited with treatment of 30 % water extract. Regardless of habitat differences, *Halocnemum* was more toxic to seed germination than *Arthrocnemum*, a difference that was significant ($P < 0.05$). In the Burj Alarab coastal area, non-significant differences in the percent germination of *Eruca* seeds were recorded when treated with 20 % extracts of both species. These results suggested that the shoot extracts of both species collected from the two habitat types contains allelochemicals that act as barriers to water uptake during the germination process (32). The differential phytotoxic potential of *Arthrocnemum* reflects the role of habitat in its allelopathy.

3.2. Shoot growth

The effect of the shoot extracts of both species on the length of *E. sativa* shoots also appeared to be habitat and concentration dependent. The shoot length of *E. sativa* was slightly decreased at 10 % concentration of *Arthrocnemum* and *Halocnemum* from Manzala but was significantly decreased at higher concentrations. The extract of the two species from the Burj Alarab area exhibited toxicity on *Eruca*, as determined by shoot length, even at the low concentration of 10 % (Fig. 2).

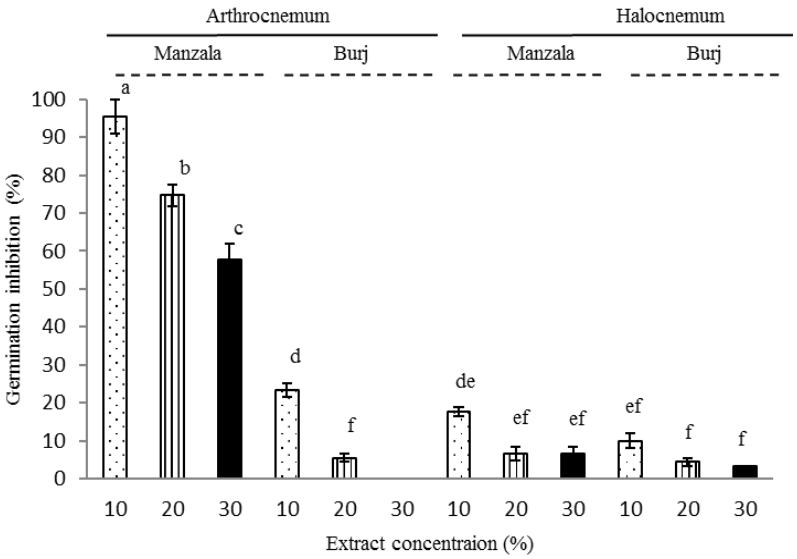

Figure 1. Inhibitory effects of *A. macrostachyum* and *H. strobilaceum* on seed germination of *Eruca sativa*.

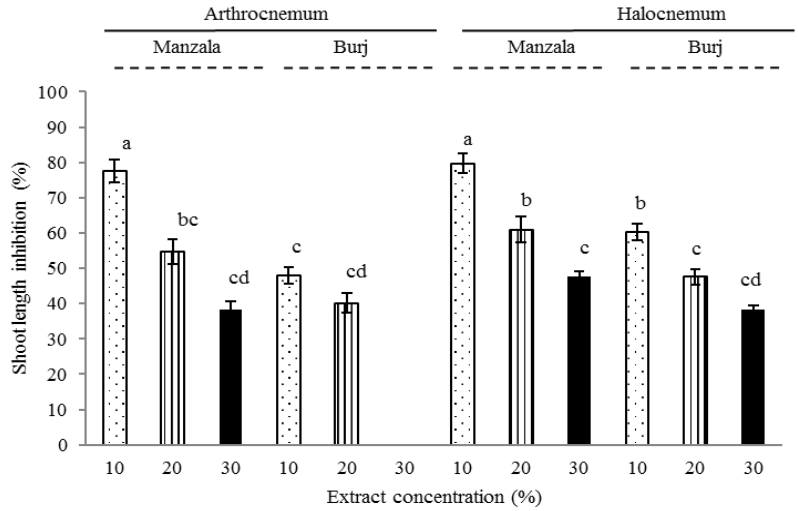

Figure 2. Inhibitory effects of *A. macrostachyum* and *H. strobilaceum* on shoot length of *Eruca sativa*.

The results also showed that the leaf area of *E. sativa* was significantly inhibited by the concentrations applied. The effect of *Arthrocnemum* from Manzala had a lower inhibitory effect when compared to plants from Burj Alarab. Conversely, no significant difference was detected for the effect of *Halocnemum* from either habitat at 20 % extract (Fig. 3).

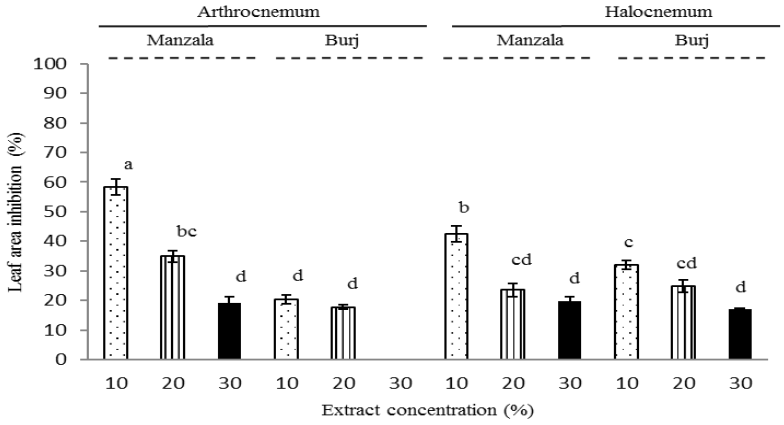

Figure 3. Inhibitory effects of *A. macrostachyum* and *H. strobilaceum* on leaf area of *Eruca sativa*.

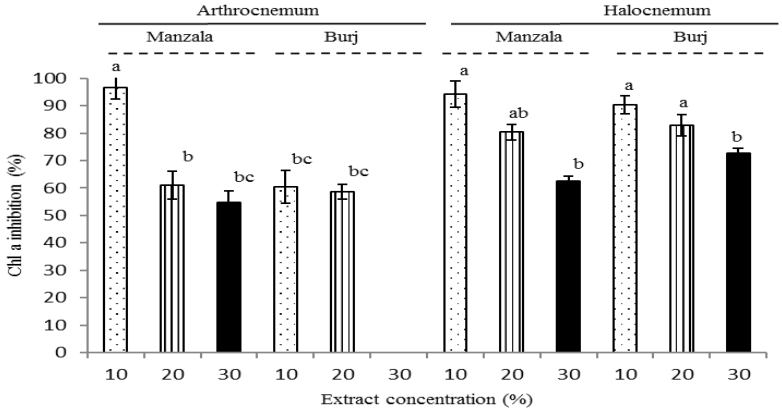

Figure 4. Inhibitory effects of *A. macrostachyum* and *H. strobilaceum* on chlorophyll a content of *Eruca sativa*.

Our results suggested that the *Arthrocnemum* extract from Burj Alarab had stronger inhibitory effects on leaf area and shoot length than from Manzala, which might be due to the difference in allelochemicals in the extracts of these species. Kong *et al.* (38) reported that *Ageratum conyzoides* produces different amounts of allelochemicals in various habitats and the degree of phytotoxicity was a function of the extract concentration. El-khatib *et al.* (22) also found that the degree of *Chenopodium murale* extract inhibition on the growth parameters of *Melilotus indicus, Trifolium alexandrinum, Triticum pyramidal, Lycopersicon esculentum* and *Cucumis sativus* was proportional to the concentration of extract applied. Overall, the reduction in leaf area and shoot length of *E. sativa* might be due to the suppressive effects of allelochemicals on cell division and elongation, nutrients` uptake, membrane permeability and growth regulation systems (12).

3.3. Photosynthetic pigments

The relative percentage of Chl a and Chl b contents of *E. sativa* treated with the 10 % extract of both species collected from the two habitats were insignificantly different except for *Arthrocnemum* from Burj Alarab. Whereas there was no significant inhibition in Chl a and Chl b content at 20 % extract of *Halocnemum* from Burj Alarab, the 20 % and 30 % concentrations of all other specimens displayed significant inhibition in relation to those of the control (Figs. 4 & 5). In contrast, the carotenoid content of the treated *Eruca* plants showed a non-significant decrease from that of the control at all concentrations (Fig. 6). In this regard, many authors (51,62,63) have reported inhibition of Chl a and Chl b by allelochemical stress. In addition, the reduction in chlorophylls might be attributed to inhibition of chlorophyll synthesis and promotion of chlorophyll degradation (79). However, the applied extracts only slightly reduced the carotenoid content of *E. sativa*, suggesting the vital role of carotenoids in this plant as antioxidant compounds to scavenge free radicals under stress (7).

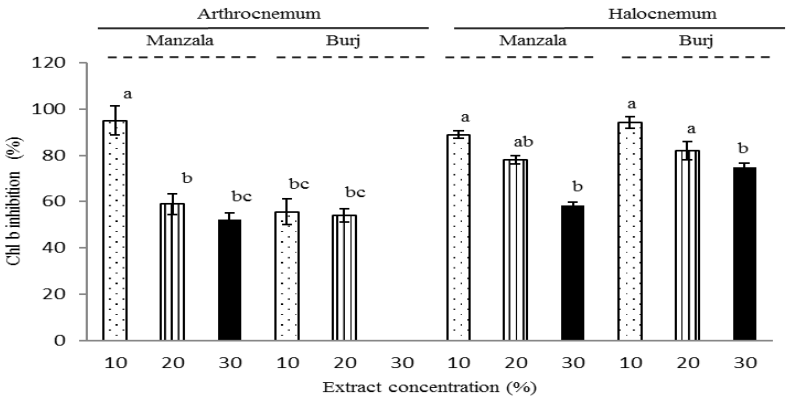

Figure 5. Inhibitory effects of *A. macrostachyum* and *H. strobilaceum* on chlorophyll b content of *Eruca sativa*.

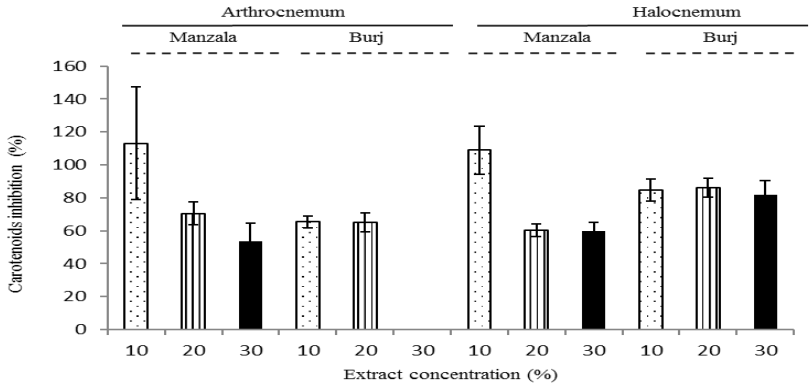

Figure 6. Inhibitory effects of *A. macrostachyum* and *H. strobilaceum* on carotenoid content of *Eruca sativa*.

3.4. Esterase and superoxide dismutase

Esterases play vital role in the metabolism of xenobiotics, pesticides and pollutants by catalysing the hydrolysis of carboxyl ester bonds (14,57). Three esterases were detected in the control and treated *E. sativa* plants: EST1, EST2 and EST3. Both EST1 and EST2 exhibited the same activity in the control and treated plants. Compared with control plants, the activity of EST3 was lower in *E. sativa* plants treated with the extracts of *Arthrocnemum* shoots from Burj Alarab (10 % and 20 %) and *Halocnemum* from Manzala (30 %) (Fig. 7). According to Mukherjee *et al.* (49), esterase activities in *Lemna minor* were increased under heavy metal stress and three new esterase isozymes were induced. Additionally, an increase in esterase activity in germinated seeds of *Pancratium maritimum* under salinity was reported by Mohamed *et al.* (45). Esterases also hydrolyse many plant allelochemicals, such as lactones, aliphatic, aromatic and polycyclic esters (2). Our data revealed inhibition of esterase activity in *E. sativa* in response to allelopathy by shoot extracts of *Arthrocnemum* from Burj Alarab (10 % and 20 %) and *Halocnemum* from Burj Alarab (30 %). These results suggests that the allelochemicals in these extracts produce inhibitory effects on the esterase activity of *E. sativa*.

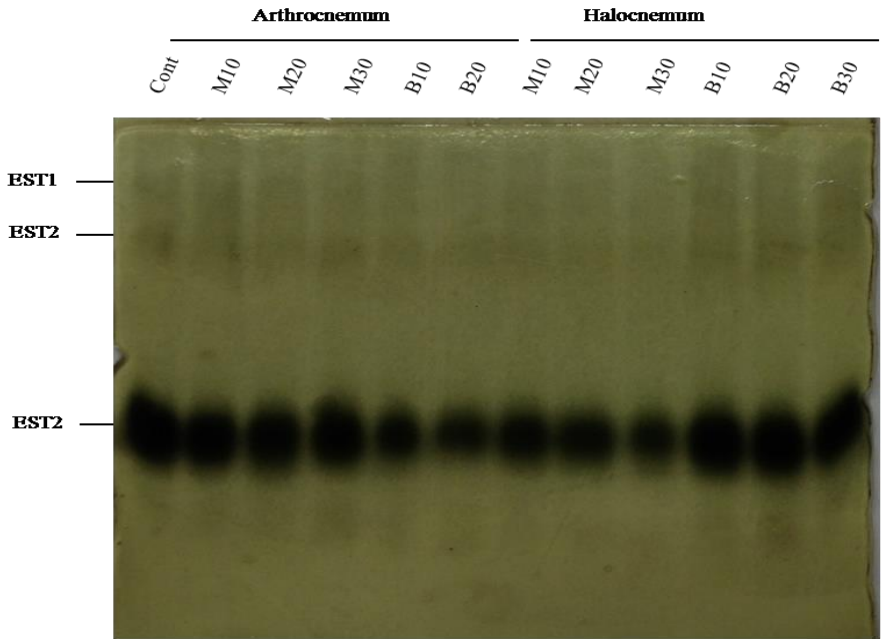

Figure 7. Effects of *Arthrocnemum macrostachyum* and *Halocnemum strobilaceum* shoot extracts concentration on the esterase activity of *Eruca sativa* seedlings after 28 days.

ROS are generated under allelochemical stress and their accumulation produces oxidative damage in target species (31,63). Superoxide dismutase catalyses the conversion of superoxide anions to hydrogen peroxide and molecular oxygen (9); therefore, SOD plays a pivotal role in alleviating the oxidative damage produced by stress, such as salinity and

allelochemicals (31,46). To explore the response of superoxide dismutase isozymes to allelopathy, cell-free extracts of the control and extract-treated *Eruca* plants were subjected to an in-gel SOD assay. Two SOD bands were observed in the leaves of both the treated and control plants. SOD1 activity was decreased in plants treated with the shoot extract of *Arthrocnemum* from Burj Alarab (10 % and 20 %) and *Halocnemum* from both habitats (10 % and 20 %) compared with the control. In contrast, SOD2 activity was similar in the control and treated plants (Fig. 8). Inhibition of SOD activity suggests that the allelochemicals in these extracts might cause excessive ROS production, which results in SOD inhibition, rendering the target tissue susceptible to oxidative stress (44,54).

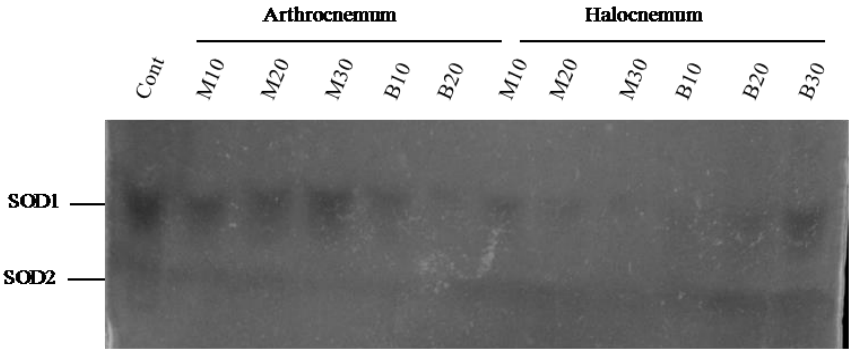

Figure 8. Effects of *Arthrocnemum macrostachyum* and *Halocnemum strobilaceum* shoot extracts concentration on the Superoxide dismutase activity of *Eruca sativa* seedlings after 28 days.

3.5. GC-MS analysis

The chemical composition of the shoot extracts of both species was analysed by GC-MS. Twenty-seven allelochemicals were identified in *Arthrocnemum* from Burj Alarab, and the most abundant constituents were bicyclo[3.3.1]nonane-2,4-dione,3-(2,2-dimethyl propylidene), with a relative value of 40.31 %, and 6,8-nonadien-2-one, 6-methyl-5-(1-methylethylidene), with a relative value of 14.55 %. *Arthrocnemum* from Manzala yielded 67 compounds, and the major constituents were the antioxidant 3,4-dihydro-2h-1,5-(3"-t-butyl) benzodioxepine (9.86 %), 6,8-nonadien-2-one, 6-methyl-5-(1-methylethylethylidene), with a relative value of 8.41 %, and 1,4-ethanonaphthalene-2,3-dicarbonitrile, 1,4-dihydro-9,9-dimethyl, with a relative value of 6.53 % (Table 1). For *Halocnemum*, 25 and 31 allelochemicals were detected in the extracts of shoots from Burj Alarab and Manzala, respectively (Table 2). The main constituents in the extract of the specimen from Burj Alarab were hexadecanoic acid, methyl ester (27.91 %), 17-octadecenoic acid, methyl ester (24.62 %) and 2-butanene, 4-(2,6,6-trimethyl-1,3-yclohexadien-1-y (8.66 %); the major compounds in specimen from Manzala were thieno[3,4-c]pyridine, 1,3,4,7-tetraphenyl (19.9 %), benzothieno[2,3-c]quinolin-6(5h)-one, 2-methoxy (11.43 %) and 15-methyltricyclo [6.5.2(13,14).0(7,15)] pentadeca-1,3,5,7,9,11,13-heptene (10.79 %).

From the foregoing results, numerous allelochemicals were found in shoot extracts of *Arthrocnemum* from Manzala and Burj Alarab, with higher diversity with the specimen from Manzala than that from Burj Alarab. However, the Burj Alarab specimen displayed

Table 1. Chemical constituents of shoot extracts of *Arthrocnemum* from Manzala and Burj Alarab.

Compounds type		Compound Name	AR-M area (%)#	AR-B area (%)*
Alicyclic	1	Bicyclo[3.3.1]nonane-2,4-dione,3-(2,2-dimethylpropylidene)	2.96	40.31
	2	2,6-Dihydroxyacetophenone, 2TMS derivative	--	3.31
	3	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl	--	2.14
	4	9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol,(3á,5Z,7E)	0.23	1.24
	5	Calcitriol	0.90	0.80
	6	7-Epi-cis-sesquisabinene hydrate	0.79	0.33
	7	Bufa-20,22-dienolide	4.93	--
	8	25-Norisopropyl-9,19-cyclolanostan-22-en-24-one, 3-acetoxy-24-phenyl-4,4,14-trimethyl	3.3	--
	9	Santalol	2.67	--
	10	D-1-,(5à,7à,13à,14á,15á,17aà)	1.68	--
	11	4-Hydroxy-á-ionone	1.17	--
	12	4-(3,3-dimethyl-1-butynyl)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one	1.14	--
	13	Retinol, acetate	1.13	--
	14	4-(2,5-Dihydro-3-methoxyphenyl)butylamine	0.94	--
	15	Cyclopropanebutanoic acid	0.78	--
	16	2-Cyclohexene-1-carboxylic acid	0.37	--
	17	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	0.34	--
	18	3a,6,6,12a-tetramethyl-1-[1-methyl-4-oxo-4-phenyl-2-butenyl]tetradecahydro-1h-cyclopenta[a]cyclopropa[e]phenanthren-7-yl acetate	0.31	--
	19	Aromadendrenoxid	0.27	--
	20	Cyclopropanebutanoic acid,	0.25	--
	21	Cyclopropanebutanoic acid,	0.25	--
	22	4,4-Dimethylcholesta-5,7-dien-3-ol	0.25	--
	23	2-(2,6,6-Trimethylcyclohex-1-enyl)cyclopropanecarboxylic acid, methyl ester	0.22	--
Aliphatic	24	6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)	8.41	14.55
	25	Methyl 5,11,14-eicosatrienoate	1.23	1.93
	26	Dotriacontane	2.06	--
	27	7-Heptadecene, 1-chloro	0.75	--
Aromatic	28	3,4-dihydro-2h-1,5-(3"-t-butyl)benzodioxepiNe	9.86	7.84
	29	1,4-methanonaphthalene-2,3-dicarbonitrile, 1,4-dihydro-9,9-dimethyl	6.53	0.47
	30	à-Curcumene, dihydro	2.74	4.69
Esters	31	Hexadecanoic acid, methyl ester	4.98	4.06
	32	16-Octadecenoic acid, methyl ester	3.33	3.49
	33	6,9,12,15-Docosatetraenoic acid, methyl ester	0.63	0.64
	34	i-Propyl 5,8,11,14,17-eicosapentaenoate	0.97	--
	35	10,13-Octadecadiynoic acid, methyl ester	0.60	--
	36	Cis-5,8,11,14,17-Eicosapentaenoic acid	0.59	--
	37	10,12-Tricosadiynoic acid, methyl ester	0.56	--
	38	Docosanoic acid, 1,2,3-propanetriyl ester	0.44	--
	39	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	0.30	--
Organosilicon	40	cyclotrisiloxane, hexaphenyl	3.66	--
	41	Cyclotetrasiloxane, octamethyl	2.61	0.95

	42	Cycloheptasiloxane, tetradecamethyl	2.60	2.29
	43	Cyclooctasiloxane, hexadecamethyl	1.34	--
	44	Cyclohexasiloxane, dodecamethyl-	1.12	--
Heterocyclic	45	Benzenamine, 2-(3-pyridinylmethyl)-,n-oxide	1.53	--
	46	Thieno[3,4-c]pyridine, 1,3,4,7-tetraphenyl	1.02	--
	47	pyrazolo[1,5-a]pyrimidine, 2,5-bis (methylthio)-3-phenyl-7-(6-phenyl-1,3,5-hexatrienyl)-	0.53	--
	48	Ethanone,1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta[b]furan-5-yl)-	0.47	--
	49	8H-Azecino[5,4-b]indol-8-one, 5-ethylidene-1,2,3,4,5,6,7,9-octahydro-6-(2-hydroxyethyl)-3-methyl-, [S-(E)]-	0.40	--
	50	2H-pyran, 2-(7-heptadecynyloxy)tetrahydro	0.38	--
	51	2H-1-benzopyran,7-ethoxy-8-methoxy-2,2-dimethyl	--	1.07
Terpenes	52	Furoscrobiculin b	--	1.91
	53	1-Heptatriacotanol	0.25	1.14
	54	Isochiapin b	0.93	--
	55	19-d-torulosol	0.50	--
Carotenoid	56	Astaxanthin	0.59	0.72
	57	psi.,.psi.-carotene,7,7',8,8',11,11',12,12',15,15'-decahydro	2.49	--
Aldehyde	58	2-Butenal, 2-chloro	0.65	--
	59	11-Octadecenal	1.50	--
Spirocompound	60	1-Oxaspiro[2.5]octane,5,5-dimethyl-4-(3-methyl-1,3-butadienyl)	0.40	--
	61	Gleenol	0.71	--
Fatty acid	62	Doconexent	0.39	0.25
Alkene	63	Z,Z,Z-4,6,9-Nonadecatriene	0.67	1.36
Alkaloids	64	Spirosolan-3-ol, 28-acetyl-, acetate (ester),(3á,5à,22á,25s)	0.25	1.07
Steroid	65	8,9-Seco-3,19-epoxyandrostane-8,9-dione	1.16	0.74
Acetylic	66	Falcarinol	0.92	--
Organometallic	67	Hahnfett	1.56	2.03
Organic alcohol	68	Hexadecanol	1.24	--
Epoxy	69	7,8-Epoxylanostan-11-ol, 3-acetoxy	0.71	--
Porphin derivative	70	3-[18-(3-Hydroxy-propyl)-3,3,7,12,17-pentamethyl-2,3,22,24-tetrahydro-porphin-2-yl]propan-1-ol	0.34	--
Flavonols	71	4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	0.54	0.68

**Arthrocnemum macrostachyum* from Burj Alarab, Egypt
#Arthrocnemum macrostachyum from Manzala, Egypt

greater phytotoxicity than the Manzala sample. This result suggested the role of high content of bicyclo[3.3.1]nonane-2,4-dione,3-(2,2-dimethyl propylidene) and 6,8-nonadien-2-one,6-methyl-5-(1-methy lethylidene) for the inhibitory effects of Burj Alarab extract on *E. sativa*. Wang *et al.* (72) identified the presence of bicyclic compounds in the extracts of *Ambrosia trifida* L., which inhibited the germination and growth of maize and wheat. However, the decrease in the phytotoxic effects of the Manzala extract may be related to the low content of both compounds and the presence of the antioxidant 3,4-dihydro-2h-1,5-(3"-t-butyl) benzodioxepine (9.86 %), which may play a vital role in mitigating the toxicity of other allelochemicals. Draganić and Lekić (17) reported that priming seeds with antioxidant compounds promoted the germination and growth in sunflowers under unfavourable

conditions. According to our results, similar phytotoxic effects were obtained with both *Halocnemum* specimens. Hexadecanoic acid and octadecanoic acid were the main components of the specimen from Burj Alarab, and the same compounds were identified as the major allelochemicals in the halophyte *Spartina alterniflora* (78). In contrast, the phytotoxic effects of the Manzala specimen may be due to thieno[3,4-c]pyridine, 1,3,4,7-tetraphenyl, benzothieno[2,3-c]quinolin-6(5h)-one, 2-methoxy and 15-methyltricyclo [6.5.2(13,14).0(7,15)] pentadeca-1,3,5,7,9,11,13-heptene. Furthermore, Elqahtani *et al.* (23) found benzothieno[2,3-c]quinolin as an allelochemical in the extract of *Heliotropium bacciferum* L. Additionally, 15-methyltricyclo [6.5.2(13,14).0(7,15)] pentadeca-1,3,5,7,9,11,13-heptene was detected as a component in the extract of *Nardostachys jatamansi* (70). These results suggested that habitat is an important controlling factor in the diversity and content of allelochemicals

Table 2. Chemical constituents of shoot extracts of *Halocnemum* from Manzala and Burj Alarab.

Compound type		Compound Name	H-M area %&	H-B area %$
Alicyclic	1	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-y	--	8.66
	2	Methyl 2-(3',3'-dimethyl-1'-butyn-1'-yl)-1-cyclohexene carboxylate	--	2.76
	3	13-Hexyloxacyclotridecan-2-one	--	1.82
	4	1-Chloromethyl-1-cyclopentyloxy-1-silacyclohexane	--	1.72
	5	Bicyclo[3.2.0]heptan-2-one,5-(ethoxycarbonylmethyl)-6-hydroxy-3,3-dimethyl-6-(oxacyclopropyl)	--	1.69
	6	7-epi-cis-sesquisabinene hydrate	--	0.95
	7	4-(2,4,4-Trimethyl-7-oxa-bicyclo[4.1.0]hept-2-en-3-yl)-pent-3-en-2-one	--	0.75
	8	Cyclononasiloxane, octadecamethyl	2.53	--
	9	5,5-Dimethyl-2-[2'-(trimethylsilyl)ethynyl]cyclohex-2-enone	1.67	--
	10	Olean-12-ene-3,15,16,21,22,28-hexol, (3á,15à,16à,21á,22à)	1.57	--
	11	2,5,5-Trimethyl-3,11- thiabicyclo[5.3.1(1,7)] deca-1,7-diene	1.05	--
	12	25-Norisopropyl-9,19-cyclolanostan-22-en-24-one,3-acetoxy-24-phenyl-4,4,14-trimethyl	0.87	--
Organic ester	13	Hexadecanoic acid, methyl ester	4.10	27.91
	14	17-Octadecenoic acid, methyl ester	7.18	24.62
	15	11-Eicosenoic acid, methyl ester	--	3.64
	16	Tetradecanoic acid, methyl ester	--	3.23
	17	Cyclopentanetridecanoic acid, methyl ester	--	1.32
	18	9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	--	1.23
	19	Methyl-9,9,10,10-d4-octadecanoate	--	0.95
	20	5,8,11,14-Eicosatetraenoic acid, methyl ester	1.74	0.64
	21	9,12,15-Octadecatrienoic acid, 2,3-is[(trimethylsilyl)oxy]propyl ester,(z,z,z)	3.55	--
	22	Docosanoic acid, 8,9,13-trihydroxy-,methyl ester	1.08	--
	23	palmitic acid, 2-(tetradecyloxy)ethylester	0.94	--
Organosilicon	24	Cycloheptasiloxane, tetradecamethyl	7.80	--
	25	Cyclooctasiloxane, hexadecamethyl	4.34	--
	26	Cyclohexasiloxane, dodecamethyl	3.62	--

	27	Cyclotrisiloxane, hexaphenyl	1.16	--
Organic acid	28	4-Oxo-4-(para-tolyl)-butyric acid	--	1.45
	29	5-Heptenoic acid, 6-methyl-4-[(4-methylphenyl) sulfonyl	1.37	--
	30	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	0.83	--
Condensed	31	Thieno[3,4-c]pyridine, 1,3,4,7-tetraphenyl	19.91	--
heterocyclic	32	Benzothieno[2,3-c]quinolin-6(5h)-one,2-methoxy	11.43	--
Alcohol	33	1-Eicosanol	2.03	--
	34	2-Hexadecanol	0.94	--
Phenolic	35	Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl	0.68	--
	36	2, 6-di-tert-butyl-4-methoxymethylphenol	0.45	--
Xanthin	37	Astaxanthin	1.84	--
derivative	38	Dihydroxanthin	0.74	--
Epoxy	39	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo [5.1.0.0(2,4)]octane	--	1.00
compound	40	2-Methyl-cis-7,8-epoxynonadecane	--	0.88
	41	15-Methyltricyclo [6.5.2 (13,14).0(7,15)] pentadeca-1,3,5,7,9,11,13-heptene	10.79	3.68
Benzopyran	49	Cromolyn	--	2.38
Aromatic	48	Benzene, 1,2,3,4-tetramethyl-4-(1-methylethenyl	--	2.26
Terpenoids	46	Limonen-6-ol, pivalate	--	2.21
Adamantane	47	Adamantane, 2-hydroperoxy-2-(2-oxiranyl)	--	2.16
Carbohydrate	50	d-xylitol, pentaacetate	1.76	--
Fatty acid	44	Oleic Acid	1.17	--
	42	3,4-secolanosta-4(28),8-diene-3-nitrile, 24-hydro	1.12	--
Spirocompound	45	Spirosolan-3-ol, 28-acetyl-, acetate (ester), (3á,5à,22á,25s	1	0.99
Organometallic	43	Hahnfett	0.76	--
Epoxide	51	Trans-Z-à-Bisabolene epoxide	--	1.19

[$] *Halocnemum strobilaceum* from Burj Alarab, Egypt.
[&] *Halocnemum strobilaceum* from Manzala, Egypt

Overall, our results demonstrated that the *Arthrocnemum* specimen from Burj Alarab was more inhibitory than other specimens. This may be attributed to the following; (i) Due to the sandy soil of Burj Alarab, the plant communities that inhabit this area, are exposed to greater drought stress than those in Manzala. This agrees with other researches suggesting that drought plays a vital role in the enhancement of the allelopathic potential of plants (25,48,68). (ii) Burj Alarab may be nutrients deficient than Manzala. Kong *et al.* (38) reported that the allelochemical levels of *Ageratum conyzoides* were enhanced under nutrients deficiency than nutrients-sufficient conditions. (iii) *Arthrocnemum* specimens occur in communities of low diversity in Burj Alarab, whereas they inhabit communities of high species diversity in Manzala. Many authors have reported that the inhibitory effects of allelopathic species increases and is dominant in low-diversity communities (40,73). Nonetheless, the reduction in allelochemical production under competition stress may be due to the lack of nutrients availability and that plants prefer to use these nutrients for growth enhancement to acquire light rather than for allelochemical production (12,52,56).

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