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A COMPARATIVE STUDY ON THE EFFECT OF EGYPTIAN AND LIBYAN CRUDE OIL EXTRACTS ON THE SYNTHESIS OF SOME METABOLITES OF *DUNALIELLA BARDAWIL*

Allam, M.M, Khaleafa, A.F. and El-Salihin, H*.

Botany Department, Faculty of Science, Alexandria University, Egypt
** Biology Department, Faculty of Science, Omar El-Mokhtar University, Libya*

Abstract

The effect of different concentrations of water soluble fractions of crude Egyptian and Libyan oil was tested on the synthesis of some metabolites of *D. bardawil* – a marine green unicellular alga- that used mainly for fish feeding. The results obtained cleared that toxic stress of crude oil extracts depends mainly on: type and source of oil, concentration of the extract and duration of culture period. Lower concentrations of both oil extracts stimulated synthesis of most measured metabolites while higher concentrations hampered their synthesis or even killed the organism. Libyan crude oil extract is more toxic than Egyptian one. Glycerol was the only metabolite that increased by increasing the concentration of the extract from 25% up to 75% of both types of oils. Total proteins, total carbohydrates and total amino acids content decreased quantitatively by increasing the concentration of extract and period of culture. At higher extract concentrations for both types of crude oils the number of gel bands for protein profile differed also according to the type of oil and concentration of extract. The zymogram obtained for the four studied isozymes cleared that toxicity effect of Libyan oil was more prominent than Egyptian oil.

Key words: amino acids, carbohydrates, crude oil, *Dunaliella bardawil*, glycerol, isozymes, proteins, protein profile.

Introduction

Pollution in its broad sense, is the addition of either harmful or excess harmless substances to the environment which in turn may cause disturbance in the web balance (Sulbivan and Currin 2000; Pichler *et al*. 2003). Oil pollution is the main serious problem along the coast of seas and oceans. It become one of the main causes of aquatic pollution because of its versatile use, over exploitation and rapid development of industries in the world (Panigrahi and Konar 1992).

Marine microalgae are considered as one of the most important microscopic algae, since it considered being the principle organisms for productivity of any aquatic ecosystem. Owing to the increase of different crude oil fractions in Oceans and seas and the expected effect of these pollutants on growth and metabolism of microalgae, the scientists paid great attention to the expected effects of these pollutants. Tukaj (1987) suggested that oils may occur in different forms and fractions in water which could be measured as total petroleum

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hydrocarbons. Consequently, the water soluble fractions of oil will be incorporated in the food chain and will exert toxic effects on aquatic biota. So great attention was paid to the effect of crude oil and its derivatives on growth and metabolism of aquatic plants- mainly photosynthesis - (Singh and Gaur 1990; Chen *et al*. 1992).

Kauss and Hutchinson (1975) found that toxicity of oil resulting in a lag phase perior to the onset of growth. Hanan (2006) proved that duration of the lag phase depending on nature and concentration of the oil extract, type of the tested species as well as duration of culturing period. The mechanism of inhibition was not well understood but Morales-Loo and Goutex (1990) concluded that toxic effects of the water soluble fractions disrupts the biosynthesis mechanism required for a fractional photosynthetic apparatus in sensitive algae, a phenomenon coupled to sterol accumulation in these species. Petkov *et al*. (1992) suggested that sterol concentration in the lipid membrane could be very important for algal survival in petroleum treated cultures. Sun and Wang (1990); Awad and Bakhadlag (1999) studied the effects of oil hydrocarbons on cellular organic constituent and found that low concentrations enhanced the photosynthesis leading to increase in its primary production.

So the intent of this study was conducted to find out the effect of the water soluble component of Egyptian and Libyan crude oil on the synthesis of certain metabolites (glycerol, carbohydrates, protein content, amino acids, protein profile, malate, succinate and glutamate dehydrogenase isozymes) of *Dunaliella bardawil* one of the main planktonic alga used mainly for fish feeding.

Materials and methods

The biological material chosen for this work was the axenic unicellular green alga *Dunaliella bardawil* obtained from the algal collection of Phycological laboratory, Faculty of Science, Alexandria University. The basal medium used for culturing was the MH medium (Johnson *et al*. 1968) as modified by Loeblich (1982). Antibiotic mixtures (Stein 1973) were occasionally used to facilitate the recovery and maintenance of axenic cultures. *Dunaliella bardawil* were grown in Erlenmeyer Pyrex-glass flasks (capacity 250 ml each) containing 50 ml basal medium with different concentrations of water soluble fractions of crude oil. The cultures were grown under controlled laboratory conditions of temperature (25 °C ± 3 °C) and light at 80μ mol m^{-2} s^{-1}. The photoperiod regime was 16h light/ 8h dark. The culture period lasted 16 days and harvested every 4 days by centrifugation at 7000 rpm for 20 min. The supernatants were discarded and the remaining pellets were used for determination of the tested metabolites.

Preparation of extract

Extracts of both crude oils were obtained by the method described by Boylan and Tripp (1971) and modified by Kauss and Hutchinson (1975). One part

of oil was added to 20 parts of sterile culture. The aqueous extracts were sterilized by filteration (Millipore ® HA 0.45 μm membrane filter) to reduce any bacterial contaminants (Kauss and Hutchinson 1975). Thus aqueous phase was designated as 100% oil extract. The four concentrations that have been used were 25, 50, 75 and 100% of both types of oils were prepared by using the culture medium itself for dilution to maintain the aqueous crude oil extract (ACOE) culture at the same medium constituent.

Determination of carbohydrates

Carbohydrate contents were estimated according to the method described by Dubois *et al*. (1959).

Determination of glycerol

Glycerol was determined according to the method recommended by Chitlaru and Pick (1989).

Determination of proteins

Protein was determined by the method described by Hartree (1972) which is the modification of the original folin- phenol method of lowry *et al*. (1951).

Determination of individual free and total amino acids

The individual amino acids, except for tryptophane, were extracted by the method described by Speckman *et al*. (1958) using a Bekmen 100cl amino acid analyzer. The response of the amino acid analyzer was checked by analyzing a standard mixture of seventeen commonly occurring amino acids in protein hydrolyzate and the recoveries were used to calculate the amounts of the amino acids in the sample.

Protein profile analysis

The discontinuous slab gel electrophoresis technique was applied according to Scandalias (1969) for protein and isozyme separation. The gel containing soluble proteins was stained with 0.04 PAGE blue G. 90 dye in 3.0% perchloric acid.

Isozymes analysis

For isozyme analysis the recovered gels were transferred directly to the appropriate staining reaction mixture as follows: Malate Dehydrogenase MDH (Shaw and Koen 1964), α-Esterase, Glutamate Dehydrogenase GDH and Succinate Dehydrogenase SDH (Shaw and Prasad 1970).

Statistical analysis

Data of the present study were subjected to standard one way analysis of variance (ANOVA)

Results and Discussion

A glimpse at the results obtained concerning the effect of different concentrations of Egyptian and Libyan crude oil aqueous extracts (table 1); on synthesis of glycerol by *Dunaliella bardawil* it is clear that at both extracts glycerol highly significant increased by increasing the concentration of extracts as well as the days of culturing but still less than control. Higher concentrations (75and 100%) were more toxic than lower ones (25 and 50%) especially for Libyan oil. However, under all concentrations and duration of culturing time the toxic effects were highly significant. The stress conditions of the water soluble extract of both oils caused a shift metabolism towards glycerol synthesis. Kaplan *et al*. (1980) and Taha (2002) revealed that shift metabolism towards glycerol formation is largely from stored carbohydrates. Also Degani *et al*. (1985) reported that excess glycerol is converted to storage product starch. Glycerol has been known for many years to be an early product of photosynthesis in *Dunaliella* (Jones and Galloway 1979; Wegmann *et al*. 1980). Also, Many authors cleared that glycerol has a unique physiological adaptation to external adaptation specially salt stress (Chitlaru and Pick 1991 and Taha 1997 & 2002)

Table (1): Effect of different concentrations of the water soluble fractions of Egyptian and Libyan crude oil on glycerol content (mg ml^{-1}) of *Dunaliella bardawil* after 4, 8, 12 and 16 days of culturing.

Time (days)	Control	Treatments							
		% Egyptian crude oil				% Libyan crude oil			
		25	50	75	100	25	50	75	100
4 days	**3.15**	2.33	2.89	3.20	2.13	1.78	2.78	2.99	1.67
8 days	**8.29**	6.29	7.73	7.92	5.89	4.95	6.78	7.26	4.26
12 days	**15.38**	10.94	11.26	12.54	9.08	11.52	13.85	12.79	11.26
16 days	**14.8**	10.12	10.94	11.95	8.25	10.50	12.67	12.35	10.33

F-value	(Glycerol $_{25,\ 50,\ 75\ \&\ 100\%}$)			
Time (days)	**	**	**	**
Treatment	**	**	**	**
Time x treatment	**	**	**	**

** = highly significant

On the light of the experimental results obtained for the effect of different concentrations of the water soluble fractions of Egyptian and Libyan crude oil on carbohydrates content of *D. bardawil* after 4, 8, 12 and 16 days culturing, it is clear from table (2) that after 4 days culturing the total carbohydrates highly significant decreased but soluble carbohydrates at concentrations 50 and 75% significantly increased compared to control. This figure could be clearly observed after 8 days culturing at all concentrations when the soluble carbohydrates at all concentrations increased compared to control. On the contrary at the same period of culturing the content of insoluble carbohydrates decreased at concentrations 75 and 100%. These data may prove that soluble carbohydrates increased gradually with the increase of oil concentration on the expense of the insoluble ones which decreased. This fact could be clearly seen after 16 days culturing. Statistical treatment proved that the toxic effect of water soluble fractions of Egyptian and Libyan crude oil was highly significant under all the period of culture used and concentration of extract except at concentration 50 % the effect was non significant. The whole map obtained proved that the water soluble fractions of Libyan crude oil could be considered more toxic than Egyptian crude oil. These results go parallel with those obtained by Soto *et al*. (1979 a & b) where they pointed out that starch grains increased in number in *Chlamydomonas augilosa* under the effect of low concentrations of some hydrocarbons and crude oil. In this work lower concentrations of oil increased the synthesis of carbohydrates while the reverse could be observed at higher concentrations. These results go in harmony with those obtained by Karydis (1979) who reported that under lower concentrations of oil the biosynthesis of polysaccharides and protein increased. Also, El-Naggar *et al*. (1998) and Hanan (2006) reported that low concentrations of crude oil raised the carbohydrates content, while the higher concentrations generally reduced the metabolic activities of *Chlorella homosphera* and *C. vulgaris*.

The impression from the obtained overall results concerning effect of either Egyptian and Libyan crude oil on the synthesis of protein by *Dunaliella bardawil* after 4,8,12 and 16 days culturing (table 3), is that content of total protein decreases by increasing either the concentration of oil or period of culturing. The only increase in total protein or in its fractions could be observed at concentration 25% for both Egyptian and Libyan crude oil, irrespective to the period of culture. Rabea (2002) reported that all nitrogen fractions soluble, insoluble and total in *Chlorella vulgaris* and *Phormidium autumunal* were found to increase at low concentrations of petroleum. On the contrary, the minimum accumulation of all nitrogen fractions in both organisms was recorded at the highest concentrations. The same author reported also that accumulation of all nitrogen fractions under lower concentrations of crude oil was the highest in *Phormidium* as compared with that in *Chlorella*. The same results were also obtained by Karydis (1979) for *Cyclotella cryptica*, *Pavlova lutheri* and *Skletonema costatum*. It was reported that low concentrations of crude oil

stimulated protein content while high concentrations had an inhibitory effect for *Chlorella homosphera* and *C. vulgaris* (El-Sheekh *et al*. 1999 and Hanan 2006).

Table (2): Effect of different concentrations of the water soluble fractions of Egyptian and Libyan crude oil on protein content (μg ml^{-1}) of *Dunaliella bardawil* after 4, 8, 12 and 16 days of culturing.

Time (days)	Parameters	Control	Treatments							
			% Egyptian crude oil				% Libyan crude oil			
			25	50	75	100	25	50	75	100
4 days	Soluble	25	23	26	25	14	14	15	13	10
	Insoluble	45	49	45	35	20	30	25	25	19
	Total	70	72	71	60	34	44	40	38	29
8 days	Soluble	86	87	74	64	45	80	65	70	50
	Insoluble	105	174	109	99	70	165	107	100	70
	Total	191	261	183	163	115	245	172	170	120
12 days	Soluble	137	142	100	87	74	133	105	91	80
	Insoluble	184	274	179	154	126	260	170	163	139
	Total	341	416	279	241	200	293	275	254	219
16 days	Soluble	143	130	105	108	104	129	90	74	71
	Insoluble	176	261	130	129	116	149	140	139	127
	Total	319	391	235	237	220	278	230	213	198

F-value (Protein)	Soluble (25, 50, 75 & 100%)	Insoluble (25, 50, 75 & 100%)	Total (25, 50, 75 &100%)
Time (days)	** ** ** **	** ** ** **	** ** ** **
Treatment	** ** ** **	** ** ** **	** ** ** **
Time x treatment	** ** ** **	** ** ** **	** ** ** **

** = highly significant

Table (3): Effect of different concentrations of the water soluble fractions of Egyptian and Libyan crude oil on carbohydrates content (μg ml^{-1}) of *Dunaliella bardawil* after 4, 8, 12 and 16 days of culturing.

Time (days)	Parameters	Control	Treatments							
			% Egyptian crude oil				% Libyan crude oil			
			25	50	75	100	25	50	75	100
4 days	Soluble	5.1	4.9	5.8	5.2	3.2	4.0	5.7	5.6	36
	Insoluble	14.3	13.0	11.3	9.7	6.7	11.0	9.1	9.0	6.9
	Total	19.4	17.9	17.1	14.9	9.9	15.0	14.8	14.6	10.5
8 days	Soluble	12.7	15.5	17.7	15.5	13.6	14.1	15.5	9.5	8.5
	Insoluble	33.2	44.4	35.1	28.6	23.9	41.4	22.6	15.6	14.0
	Total	45.9	59.9	52.8	44.1	37.5	55.5	38.1	25.1	22.5
12 days	Soluble	24.6	26.5	27.1	26.6	20.8	25.5	22.5	17.5	17.1
	Insoluble	65.1	72.6	51.4	49.7	28.3	65.4	47.5	31.4	21.0
	Total	89.7	99.1	78.5	76.3	49.1	90.9	70.0	48.9	38.1
16 days	Soluble	22.0	25.7	26.6	24.3	20.4	24.4	21.0	16.0	15.8
	Insoluble	62.0	65.1	49.7	46.0	27.3	63.3	43.1	29.0	18.3
	Total	84.0	90.8	76.3	70.3	47.7	97.7	64.1	45.0	34.1

F-value (Carbohydrates)	Soluble (25, 50, 75 & 100%)	Insoluble (25, 50, 75 & 100%)	Total (25, 50, 75 &100%)
Time (days)	** ** ** **	** ** ** **	** ** ** **
Treatment	** ** ** **	** ** ** **	** n.s. ** **
Time x treatment	** ** ** **	** ** ** **	** n.s. ** **

** = highly significant n.s.= non significant

The free amino acids of *Dunaliella bardawil* cultured at concentrations 50% and 100% water soluble fractions of Egyptian and Libyan crude oil (table 4) differed quantitatively but not qualitatively. El-Essawy *et al.* (1985) and Hanan (2006) reported that amino acids produced in algal cells mainly differed quantitatively according to the type of algae and conditions of cultivation. It is clear from these results that the free amino acids of the krebs cycle family represents the highest values among the other groups of amino acids. The free amino acids of this family in *Dunaliella* represented nearly 38.3 and 46.2% of total amino acids at concentrations 50 and 100% Egyptian crude oil respectively. While in case of Libyan crude oil they represented 43.9 and 50%, respectively. Taking into consideration the values obtained for the free amino acids of the triose pyruvic acid family, it is clear that they occupied the second position for the Egyptian and Lybian crude oil. Jones &Stiwart (1969) stated that glycine, glutamic acid, aspartic acid, alanin and serin were the most predominant amino acids in different algae. At 100% Egyptian crude oil it increased by 4.2% while at Libyan crude oil it increased by 13.1%. Concerning the essential free amino acids it is clear that arginine from the glutamate family and isolucine from the aspartate family were the only essential amino acids that increased by treating the organism with concentrations 50 and 100% Egyptian crude oil compared to control. Arginin increased by 1.3 and 1.2 fold at concentrations 50 and 100% while isolucine increased by 1.4 and 1.6 fold, respectively.

Anent the observed increase in the content of proline in *D. bardawil* cultured under the toxic effects of the water extract of both Egyptian and Libyan crude oil, it may be correlated to the stress effects of these extracts (Agrwal and Gupta, 1995). However, Verburuggen *et al*. (1993) and Mattioni *et al*. (1997) explained that activity of the enzyme pyrroline-5-carboxylate reductase which catalysis the last step of proline synthesis in stress-regulated which depends on the degree of stress agent. Proline is considered to be involved in protection of enzymes (Soloman *et al*. 1994), cellular structures (Van Rensburg *et al*. 1993) and to act as free radicle scavenger (Alia *et al.* 1995).

Inspection of the data recorded in table (4) concerning the effect of 50 and 100% of water soluble fractions of Egyptian and Libyan crude oil on conjugated amino acid fractions, it is clear that the krebs cycle family and triose pyruvic acid family are the dominant groups within both the control and the treated organism, the least one is the shikimik acid family. The total conjugated amino acids at 50 and 100% Egyptian crude oil decreased by 6.1 and 31.9%, respectively, while for Libyan crude oil they decreased by 22.8 and 43.4%, respectively compared to control. Salama and Abdel Basset (1987) stated that amino acids content declined in organisms grown under stress. A glance at these results it is obvious that Libyan crude oil is more toxic than Egyptian one however, the glutamate family greatly decreased in Libyan oil than in case of Egyptian one where it slightly decreased compared to control. The map concerning the data obtained for the content of total amino acid fractions recorded

Table (4): Effect of 50% and 100% concentrations of the water soluble fractions of Egyptian and Libyan crude oil on content of free, conjugated and total amino acid fractions (mg/100g fresh wt.) of *Dunaliella bardawil* cells. (*=essential amino acids)

Amino acids		Krebs cycle family											Total
		Glutamate family				Total	Aspartate family					Total	
		Glu.	Arg.*	Pro.	His.*		Asp	Thr.*	Lys.*	Isoleu.*	Meth.*		
Free amino acids													
Control		86.9	17.8	16.7	3.6	125.0	15.9	20.2	33.5	7.9	6.5	84.0	209.0
Egy	50%	44.2	23.0	5.7	2.5	75.4	14.1	11.6	30.5	11.4	4.0	71.6	147.0
	100%	44.1	21.6	8.0	3.5	77.2	17.8	15.0	31.4	12.6	3.2	80.0	157.2
Liby	50%	40.5	8.9	3.2	3.3	55.9	9.5	13.0	20.5	8.4	5.9	57.3	113.2
	100%	48.4	10.5	5.8	5.2	69.9	14.7	14.4	2.5	8.0	4.7	44.3	114.2
Conjugated amino acids													
Control		1046.1	778.2	573.3	435.4	283.0	1049.1	648.8	709.5	860.1	232.5	3500.0	6333.0
Egypt.	50%	931.8	792.0	424.3	274.5	2422.6	859.9	623.4	887.5	989.6	201.0	3561.4	5984.0
	100%	654.9	99.4	1337.0	267.5	2358.8	536.2	366.0	753.6	686.4	5.8	2342.2	4701.0
Libyan.	50%	250.5	552.1	333.8	269.7	1406.1	733.5	553.0	682.5	895.6	217.1	3061.7	4467.8
	100%	597.6	408.5	169.2	215.8	1391.1	584.3	321.6	447.5	577.0	133.3	2063.7	3454.8
Total amino acids													
Control		1133	796	590	439	2958	1065	669	743	868	239	3584	6542
Egypt.	50%	976	815	430	277	2498	874	635	918	1001	205	3633	6131
	100%	699	121	1345	271	2436	554	381	785	699	9	2428	4864
Libyan.	50%	291	561	337	273	1462	743	566	703	904	223	3139	4601
	100%	646	419	175	221	1461	599	336	450	585	138	2108	3569

Cont. Table 4

Amino acids		Triose pyruvic acid family									Shikimic acid family			Total
		Triose family			Total	Pyruvate family			Total	Total				
		Gly.	Ser.	Cyst.		Ala.	Val.*	Leu.*			Ph.al a.*	Tyr.	Total	
Free amino acids														
Control		15.7	9.3	1.2	26.2	68.7	9.5	15.9	94.1	120.3	9.4	7.6	17.0	346.3
Eg	50%	8.2	6.2	0.3	14.7	51.9	6.0	4.9	62.8	77.5	5.6	1.8	7.4	231.9
	100%	8.4	10.6	0.5	19.5	45.1	9.6	6.7	61.4	80.9	0.6	3.3	3.9	242.0
Li	50%	4.8	7.5	1.2	13.5	28.1	7.1	4.3	39.5	53.0	3.3	2.6	5.9	172.1
	100%	4.3	6.4	1.0	11.7	35.8	6.9	6.6	49.3	61.0	3.2	4.0	7.2	182.4
Conjugated amino acids														
Control		607.3	544.7	8.8	1160.8	850.3	851.5	1156.1	2857.9	4018.7	635.6	294.4	930.0	11281.7
Egyp	50%	476.8	474.8	24.7	976.3	693.1	1025.0	1040.1	2758.2	3734.5	595.4	281.2	876.6	10595.5
	100%	380.6	300.4	8.5	689.5	523.9	391.4	813.3	1728.6	2418.1	392.4	163.7	556.1	7686.0
Liby	50%	492.2	449.5	37.8	979.5	630.9	753.9	1134.7	2519.5	3499.0	474.7	248.4	723.1	8709.9
	100%	458.7	278.6	82.0	819.3	605.2	403.1	622.4	1630.7	2450.0	289.8	195.0	484.8	9389.6
Total amino acids														
Control		623	554	10	1187	919	861	1172	2952	4139	645	302	947	11628
Egyp	50%	485	481	25	991	745	1031	1045	2821	3812	601	283	884	10827
	100%	389	311	9	709	569	401	820	1790	2499	393	167	560	7928
Liby	50%	497	457	39	993	659	761	1139	2559	3552	478	251	729	8882
	100%	463	285	83	831	641	410	629	1680	2511	293	199	492	6572

in table (4) followed nearly the same trend as for free and conjugated amino acids. It is clear also that the decrease in the content of total amino acids specially at high concentrations of both types of oil may be due to the drastic effect of these compounds on the biochemical activities as well as the contents of several amino acids generated from either krebs acid family or pyruvate family in addition to shikimic one (Mona 2000 and Hanan 2006).

Most of the obtained 26 different bands concerning total soluble protein profile in gel plate of control and treated organism at 50 and 100% of the water soluble fractions of Egyptian and Libyan crude oil (photo 1 and table 5) were distributed between 93.0 and 21.3 kda in all lanes. It is clear that some of the bands that appeared in the control disappeared in the treated organism and others

appeared new in lanes of the treated organisms. Most of the bands that disappeared at 100% of both types of oil were in the region of high molecular weight. The appearance of new bands may be due to the degradation of some high molecular weight protein to lower ones. Salah El-Din (1994) reported that most of the algal species have similar physiological functions which are related to biosynthesis or biodegradation of the same macromolecules. The number of bands that appeared new at concentration 50% were 7 and 9 bands while at 100% the number were 3 and 7 bands for Egyptian and Libyan crude oil, respectively. This increase in number of new bands in Libyan than in Egyptian one may ascertain the hypothesis that Libyan oil is more toxic than Egyptian one.

It is clear therefore that extracts of both crude oil induced four types of modifications transient and permanent intensification of protein and transient and permanent decreases. Sinha and Hader (1996) found that *Anabaena* species cultured at stress conditions did not show any changes in protein pattern. On the contrary Fulda *et al*. (1999) found that composition of periplasmic proteins obtained from cells of *Synechocystis* species grown under stress showed clear differences. Also Maha (2003) reported that *Nostoc linckia* cultured under stress conditions showed the appearance of new bands which were not detected in the control culture. Hoyos and Zhang (2000) are in agreement with those obtained results. Also Exss-Sonne *et al*. (2000) and Awatif (2002) reported that tolerance of an organism to stress conditions could be achieved through synthesis or accumulation of new proteins. This conclusion seems to explain the different changes in the number of total soluble protein bands for *Dunaliella bardawil* grow under stress of the water soluble fractions of crude oil.

The most commonly used marker in differentiation between the response of an organism and the external stresses are the cytological traits. In this field an increase in the efficiency and resolving power could be achieved by using isozymes electrophoresis (Pontikis *et a*l. 1980). These isozymes form a banding pattern (zymogram) that is used in marker assisted characterization within a single species. Under these conditions (stresses or any cause) many variants of an enzyme may often be recognized (Rothe 1994 and Taha 2002). The appearance of bands on the gel reflex the conditions of stresses to which the organism was subjected (Abd El-Salam *et al*. 1981).

The results proved that zymograms of α – esterase as well as malate dehydrogenase were affected by the stresses created by oil than the other tested two isozymes (succinate and glutamate dehydrogenase) (photos 2-5 and tables 6-9). However, glutamate dehydrogenase was the only isozyme that slightly affected by the oil stresses. It is clear also from the obtained zymograms that some bands were common within the treated and the untreated cultures, others disappeared and still others newly formed. From the overall zymograms obtained for the four studied isozymes, it could be concluded that stress effect of Libyan crude oil is higher than that of Egyptian crude oil and malate dehydrogenase was greatly affected by the two types of oil.

Photo (1):

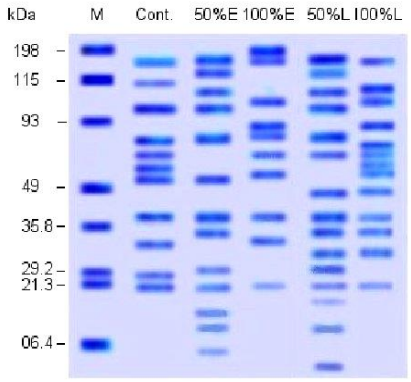

Table (5):

Band	Control	%Egyptian crude oil		%Libyan crude oil	
		50	100	50	100
1	-	-	-	+	
2	-	+	-	-	-
3	-	+	-	+	-
4	-	+	-		-
5	-	-	-	+	-
6	+	+	+	+	+
7	+	-	-	-	-
8	-	+	-	+	-
9	-	-	-	+	+
10	+	-	+	-	-
11	-	+	-	+	+
12	+	+	+	+	+
13	-	-	-	+	+
14	+	+	+	-	+
15	+	-	-	-	+
16	+	-	+	+	+
17	-	-	-	-	+
18	+	+	+	+	-
19	-	-	+	-	+
20	+	+	-	+	-
21	-	-	+	-	+
22	-	+	-	+	+
23	+	-	-	-	-
24	-	+	-	+	-
25	+	+	+	+	+
26	-	-	+	-	-

Effect of 50% and 100% concentrations of water soluble fractions of Egyptian (E) and Libyan (L) crude oil on soluble protein profile bands pattern of the studied *Dunaliella bardawil* .

Photo (2):

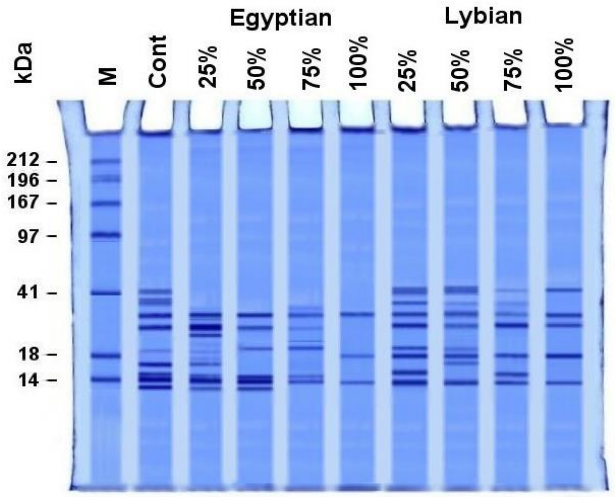

Table (6):

Bands	Control	% Egyptian crude oil				% Libyan crude oil			
		25	50	75	100	25	50	75	100
1	+	+	+						
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+					
4	+	+					+		
5					+	+	+	+	+
6	+	+	+	+		+	+		
7	+	+	+	+		+	+	+	+
8	+	+	+	+	+	+	+	+	+
9	+					+	+	+	
10	+					+	+	+	+

α- esterase bands pattern of *Dunaliella bardawil* under the effect of different concentrations of water soluble fractions of Egyptian and Libyan crude oil.

Photo (3):

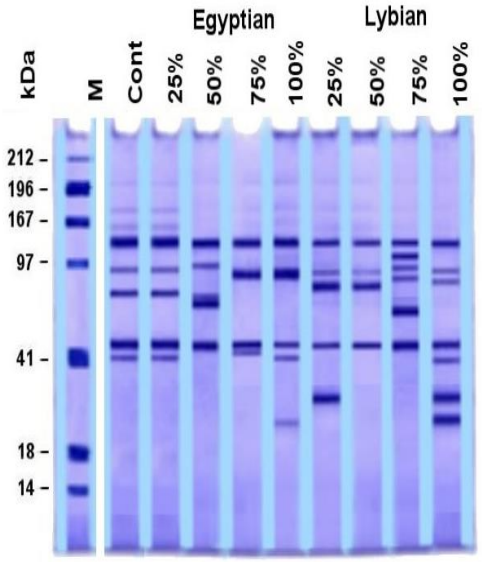

Table (7):

Bands	Control	% Egyptian crude oil				% Libyan crude oil			
		25	50	75	100	25	50	75	100
1					+				+
2						+			+
3	+	+			+				+
4				+					
5	+	+	+	+	+	+	+	+	+
6								+	
7			+						
8	+	+	+			+	+		
9								+	+
10						+	+		+
11	+	+		+	+			+	
12			+					+	
13	+	+	+	+	+	+	+	+	+
14	+	+							
15	+	+							

Malate dehydrogenase bands pattern of the studied *Dunaliella bardawil* under the effect of different concentrations of water soluble fractions of Egyptian and Libyan crude oil.

Photo (4):

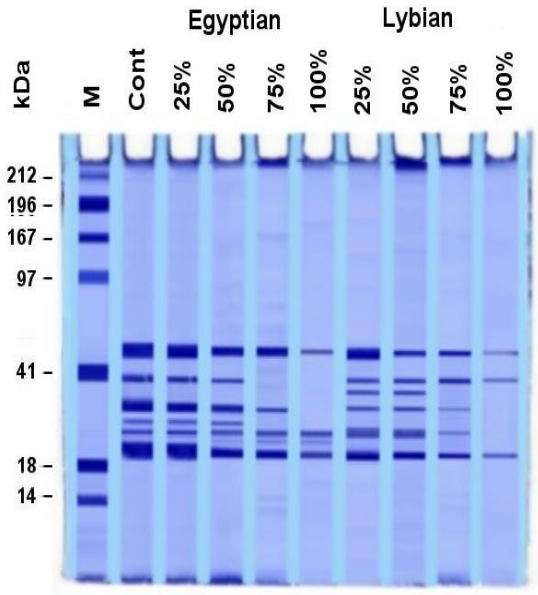

Table (8):

Bands	Control	% Egyptian crude oil				% Libyan crude oil			
		25	50	75	100	25	50	75	100
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	
3	+	+	+						
4	+	+	+	+		+	+	+	
5	+	+	+			+	+	+	+
6	+	+	+	+	+	+	+	+	+

Succinate dehydrogenase bands pattern of the studied *Dunaliella bardawil* under the effect of different concentrations of water soluble fractions of Egyptian and Libyan crude oil.

Photo (5):

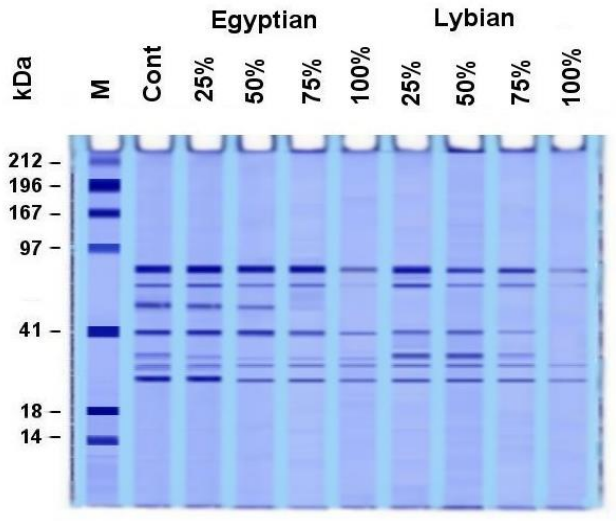

Table (9):

Bands	Control	% Egyptian crude oil				% Libyan crude oil			
		25	50	75	100	25	50	75	100
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	
4	+	+	+	+	+	+	+	+	
5	+	+	+	+					
6	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+

Glutamate dehydrogenase bands pattern of the studied *Dunaliella bardawil* under the effect of different concentrations of water soluble fractions of Egyptian and Libyan crude oil.

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دراسة مقارنة على تأثير المستخلص المائي لكل من البترول الخام المصري والليبي على بناء بعض المركبات الأيضية لطحلب دونياليلا بارداويل

مها ماجد علام – عبد الفتاح خليفة – حميدة الصالحين*

قسم النبات – كلية العلوم – جامعة الإسكندرية - مصر
*قسم الأحياء – كلية العلوم – جامعة عمر المختار- ليبيا

تم دراسة تأثير تركيزات مختلفة للمستخلص المائي لكل من البترول الخام المصري والليبي على بناء بعض المركبات الأيضية لطحلب دونياليلا بارداويل – طحلب وحيد الخلية يستخدم عادة في تغذية الأسماك. وقد أوضحت النتائج أن درجة الإجهاد السمي للمستخلص المائي للبترول الخام مرتبطة بنوع الزيت الخام ، تركيز المستخلص المائي و مدة الإستزراع. أثبتت النتائج أن التركيزات المنخفضة للمستخلص المائي لكلا النوعين من البترول حفز بناء معظم المركبات الأيضية التي تم قياسها وعلى العكس فإن التركيزات العالية للمستخلص أدت إلى انخفاض ملحوظ في بناء هذه المركبات الأيضية وأحيانا أدت إلى موت الكائن نفسه. ولقد لوحظ أن الجليسرول هو المركب الأيضي الوحيد الذي ازداد بناؤه تحت التركيزات المختلفة للمستخلص. أما البروتينات والكربوهيدرات والأحماض الأمينية الكلية انخفضت كميا مع زيادة تركيز المستخلص وفترة الإستزراع. كما أوضحت نتائج الفصل الكهربي للبروتين الذائب أن التركيزات العالية للمستخلص أدت إلى اختلاف كبير في عدد الحزم. وبخصوص الزيموجرامات التي تم الحصول عليها من الأربعة ايزوزيمات المستخدمة فقد لوحظ اختفاء بعض الحزم تحت تأثير التركيزات المختلفة وظهور حزم جديدة بالمقارنة بالتجربة الضابطة. وأن التأثير الأكثر سمية كان واضحا في حالة البترول الليبي بالمقارنة بالبترول المصري.