International Research Journal of Plant Science (ISSN: 2141-5447) Vol. 3(4) pp. 54-62, May 2012
Available online http://www.interesjournals.org/IRJPS

Full length Research Paper

The effect of sublethal concentrations of crude oil on the metabolism of Jojoba (Simmodsia chinensis) seedlings

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Accepted 24 April, 2012

The effect of crude oil contaminated soil at various sublethal concentrations (1%, 2%, and 3% v/w) on the metabolism of Jojoba (Simmodsia chinensis) seedlings was studied. The results showed that crude oil induced environmental stress in the seedlings. This is indicated by the increase in total carbohydrates, total proteins, amino acids and proline in shoot and a decrease in chlorophyll contents of the leaves of 85-days-old seedlings. Meanwhile, there were decreases in total carbohydrates in roots. Antioxidant enzyme activities were assessed in leaves. Increased the activities of peroxidase, ascorbate peroxidase and super oxide dismutase may contribute to limiting stress tolerance at the early stages of development in Jojoba leaves. Catalase activity was inhibited by the various concentrations except at 1%, which showed stimulation relative to the control treatment. In general, the application of inorganic fertilizers (NPK) had more or less significant changes in the metabolic activities of Jojoba at all concentrations (1-3%).

Keywords: Crude oil, inorganic fertilizers, Simmodsia chinensis.

INTRODUCTION

Petroleum oil pollution results from human activities such as drilling, manufacturing, storing, transporting, waste management of oil and vandalizing of oil pipe lines. The massive and extensive pollution of the environments by petroleum industries constitute socioeconomic and public health hazards. Petroleum oil pollution exerts adverse effects on plants indirectly by making toxic minerals in the soil more available to plants. Petroleum, generally referred to as “crude oil”, is a mixture of hydrocarbons, oils and chemicals obtained below the sub-surface of the earth. Crude oil contains a mixture of complex hydrocarbon molecules (Hunt, 1996), and also small quantities of sulphur (up to 10%), oxygen (up to 5%) and nitrogenous compounds (up to 1%) bound in complex organic molecules. Several metallic elements such as vanadium, nickel, iron, aluminum, sodium, potassium, copper and uranium are present in traces (Bremmer and Tabalabai, 1973). The hydrocarbons are classified into the following: Normal alkanes, branched alkanes, cycloalkanes and the aromatics (benzene, phenol, toluene, xylene and catechol (Njoku, 2004). Crude oil varies in appearance and composition from one oil kind to another (Craig, 2003). The varying compositions of one crude oil from the other have diverse effects on different organisms within the same environment (Overton et al., 1994). Saudi Arabia is one of the leading oil producing and exporting countries in the world. The crude oil contains a number of organic compounds which are removed as solid wastes from the oil refinery and defined as sludge. Generally, the crude oil sludge and other waste products are disposed as landfills which are likely to creates some health and environmental hazards (Al-Qahtani, 2011). Chemical changes in plants associated with contaminated environment have been associated with contaminated environment have been

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established by an array of scientific investigation. These include heavy metal accumulation in the lower plants (Parakinen et al., 1978; Ruhling et al., 1987) reduction in chlorophyll, protein and carotenoid (Agrawal, 1992) and increase in total amino acids (Rowland et al., 1988; Schmeink and Wild, 1990). Alteration in chemical composition is one of the methods of monitoring effect of hydrocarbon contaminated soil in plants (Achuba, 2006). The effect of hydrocarbon contaminated soil at sublethal concentration on the chemical composition of peanut (Arachis hypogea) seedlings revealed that levels of total soluble sugars, proteins and free amino acids were found to be significantly ($p \leq 0.05$) higher in plants grown in hydrocarbon polluted soil, than those grown in control soil. However, the chlorophyll content was significantly ($p \leq 0.05$) lower in plants grown in petroleum treated soil relative to control plants (Peretiemo-Clarke and Achuba, 2007). Hydrogen peroxide is produced as a byproduct of many normal cellular reactions. If the cells do not breakdown the hydrogen peroxide, they would be poisoned (Timbrell, 1991). Increased activity of antioxidant enzymes could contribute to better cell protection from chemical toxins (Sevenian and Hochstein, 1985; Saltman, 1989) thereby improving the ATP production during photosynthesis. Accumulation of metabolites that act as compatible solutes is one of the probable universal responses of plants to changes in the external osmotic potential. Metabolites with osmolyte function like sugar alcohols, complex sugars and charged metabolites are frequently observed in plants under unfavorable conditions (Hasegawa et al., 2000; Sotiropoulos, 2007). Proline and glycine betaine are known to serve as compatible osmolytes, protectants of macromolecules and also as scavengers of ROS under stressful conditions (Hellman et al., 2000; Ashraf and Foolad, 2007). An important consequence of stress in plants is the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O-2), hydrogen peroxide (H2O2) and the hydroxyl radicals (OH-) particularly in chloroplasts and mitochondria (Mittler, 2002). Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) for protection against the damaging effects of ROS (Asada, 1992; Prochazkova and Wilhelmova, 2007). Jojoba (Simmodsia chinensis (Link) Schneider) is a perennial woody shrub native to the semiarid regions of southern Arizona, southern California and northwestern Mexico. Jojoba (pronounced ho-HO-ba) is being cultivated to provide a renewable source of a unique high-quality oil. Much of the interest in jojoba worldwide is the result of the plant's ability to survive in a harsh desert environment. The utilization of marginal land that will not support more conventional agricultural crops could become a major asset to the global agricultural economy (Benzioni and Forti, 1989). Jojoba yields a crop of seeds that contain mean 40-50 % oil (Clarke and Yermanos, 1980). The main objectives of the present study were to evaluate the metabolic changes and antioxidative systems in Jojoba (Simmodsia chinensis) as influenced by crude oil pollution. Further, we attempted to understand if the inorganic fertilizers can minimize the environmental hazards of the cultivation in crude oil contaminated soil.

MATERIALS AND METHODS

Sources of Seeds, Soil and Crude Oil

Seeds of Jojoba (Simmondsia chinensis) will be obtained from, Department of Arid Land Agriculture, Faculty of Meteorology, King Abdul Aziz University, Saudi Arabia. Arabian heavy crude oil obtained from petroleum company Saudi Aramco (Saudi Arabia). The soil will be obtained from Dammam zone near the Petroleum company in the east of Saudi Arabia.

Pollution of Soil and Addition of Inorganic Fertilizers

The soil will be dried in room temperature and then sieved through 2 mm mesh. Pot experiments will be used to achieve this project. Two main groups from pots will be prepared. The dried soil will be supplemented with various concentrations of crude oil (0 (control), 1, 2, 3, and 4% (crude oil / soil w/w). The soil and oil will be well mixed to make homogenized contaminated soil in the 1st group. In the 2nd group, the inorganic fertilizers [75 mg nitrate (NH4 NO3) + 30 mg phosphate (KH2 PO4) / kg] will be added to the soil before seeding (Rosenberg and Ron, 1996) at all treatments. The Seeds are surface sterilized with 0.01M HgCl2 for 3 minutes, washed thoroughly several times with distilled water and then divided into five sets. Eight uniform of the sterilized Jojoba seeds will be planted in each pot. Three replicates for each treatment will be prepared. The control samples will be prepared. The pots are kept in the growth chamber and the plants are subjected to constant 25ºC, 60% relative humidity, 14/10 light/dark. The pots are irrigated with water at 2 days intervals.

Harvesting

At the end of experiment (85 days) the plants were harvested, and split up into root and shoot systems. Leaves in each group were collected for enzyme activities and chlorophyll determination. Root system and shoot system were dried in oven at 80ºC for 48 hours for determination of carbohydrates and nitrogen.

Determination of Photosynthetic Pigments

Leaves from each of the plants were cut, separately weighed and homogenized in 80% acetone. The extract was centrifuged at 3500 rpm at room temperature 25º C for 20 mins. The solutions were diluted to known volumes and the content of chlorophyll determined by the method of Lichtenthaler (1987).

Determination of Carbohydrate Content

The methods adopted for extraction of the different carbohydrate fractions tested were essentially those described by Yemm and Willis (1954). Dry tissue samples were submerged overnight in 10 ml 80% (v/v) ethanol at 25°C with periodic shaking. The ethanolic mixture was filtered and the ethanolic filtrate was made up to a certain volume and kept in the refrigerator for analysis of the different sugar fractions. Carbohydrate content was determined by antherone sulphuric acid according to Thayermanavan and Sadasivan (1984).

Determination of Protein content

Protein concentration was determined spectrophotometrically at 595 nm using the Bio-Rad Protein Assay Dye Reagent Concentrate (catalogue number 500-0006) in a method based on Bradford (1976). Bovine gamma-globulin (0.25-1.4 mg ml-1) was used as a standard reference.

Determination of Total Free Amino Acids Content

Total Free Amino acids content were extracted and estimated by following the method of Moor and Stein (1948).

Determination of Proline Content

Free proline accumulation was determined using the method of Bates et al. (1973). 0.04 gm dry weight of roots and shoots was homogenized with 3% sulfosalicylic acid and after 72h that proline was released; the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 hour and then absorbance at 520 nm was determined by UV-visible spectrophotometer (Biochrom, 2100).

Assays of some Antioxidant Enzyme Activities

Enzyme extraction: The samples were prepared as described by Mukherjee and Choudhuri (1983). A leaf sample (0.5 g) was frozen in liquid nitrogen and ground using a porcelain mortar and pestle. The frozen powder was added to 10 ml of 100 mM phosphate buffer (KH2PO4 / K2HPO4) pH 7.0, containing 0.1 mM Na2EDTA and 0.1 g of polyvinyl pyrrolidone. The homogenate was filtered through cheesecloth, and then centrifuged at 15,000 rpm for 10 min at 4oC. The supernatant was recentrifuged at 18,000 rpm for 10 min, and then the resulted supernatant was collected and stored at 4oC for catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase activity (SOD) assays.

Assay of Catalase Activity

Catalase (EC 1. 11. 1. 6) Activity was assayed according to Aebi (1984). The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of H2O2 consumption (Havir and McHale, 1987).

Assay of Peroxidase Activity

Peroxidase (EC 1. 11. 1. 7). Activity was determined according to Maehly and Chance (1954) by the oxidation of guaiacol in the presence of H2O2. The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm (Klapheck et al., 1990).

Assay of Ascorbate Peroxidase Activity

The activity of ascorbate peroxidase (EC 1. 11. 1. 11) was assayed using the method of Chen and Asada (1992), by measuring the decrease in absorbance at 290 nm for 1 min caused by ascorbic acid oxidation.

Assay of Superoxide Dismutase Activity

Superoxide dismutase (EC 1. 15. 1. 1) activity was measured as described by Dhindsa et al., (1981). Absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

Statistical Analysis

All data were analyzed statistically by one-way ANOVA. Values in the tables indicate the mean values ± SD based on independent three determinations (n =3). Least significant difference (L.S.D) test was used to assess the differences between treatments; $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Researchers like Anoliefo and Vwioko, 1995 recorded that oil contaminated (polluted) soil generally causes

Table (1): Effect of crude oil contaminated soil (COCS, V/W%) and inorganic fertilizers (IF) on chlorophyll contents (mg g^{-1} FW) in leaves of Jojoba (*Simmondsia chinensis*) plant.

Treatments (COCS%)	Pigments				
	Chl. a.	Chl. b.	Chl. a+b	carotenoids	Total Pigments
0% (Control)	69.667±3.215	45.442±2.162	115.109±5.377	17.127±1.203	132.236±6.580
1%	59.589±4.341**	37.880±1.832**	97.469±6.173**	13.494±1.186**	110.963±7.359**
2%	49.230±3.522**	26.621±3.021**	75.851±6.543**	10.835±0.871**	86.686±7.414**
3%	43.591±2.973**	24.508±2.562**	68.099±5.535**	9.460±1.534**	77.559±7.069**
Cont. +IF.	68.762±2.735	43.674±1.943	112.436±4.678	14.747±2.136	127.183±6.814
1% +IF.	58.511±4.167**	38.629±1.368**	97.1405.535**	13.401±1.478**	110.541±7.015**
2% +IF.	55.782±3.368**	36.632±3.436**	92.414±6.804**	12.291±1.263**	104.705±8.067**
3% +IF.	41.351±2.767**	26.803±2.582**	68.154±5.348**	11.868±0.971**	80.022±6.319**
L.S.D. at 5%	5.9418	4.2326	10.0163	2.3897	12.2860
L.S.D. at 1%	8.1867	5.8318	13.8008	3.2927	16.9280

Values are given as mean ± SD of three replicates in each group.
*Significant differences (*P*=0.05), and ** Highly Significant differences (*P*=0.01) as compared with control (0% COCS)

Table 2. Effect of crude oil contaminated soil (COCS, V/W%) and inorganic fertilizers (IF) on carbohydrate contents (mg g^{-1} DW) in root and shoot of Jojoba (*Simmondsia chinensis*) plant.

Treatments (COCS %)	Carbohydrate contents (mg g^{-1} DW)					
	Root			Shoot		
	soluble	Insoluble	Total	soluble	Insoluble	Total
0% (Control)	6.7±0.65	41.7±2.0	48.4±2.7	55.7±5.6	49.6±0.7	105.3±4.8
1%	7.1±0.84	26.3**±2.5	33.4**±5.0	61.5±5.8	54.6*±2.2	116.1*±9.0
2%	9.3**±1.1	23.2**±1.5	32.5**±3.8	66.7*±5.7	53.5*±2.8	120.2**±8.3
3%	14.2**±0.10	09.7**±0.4	23.9**±2.2	77.8**±8.1	45.3*±3.1	123.1**±4.7
Cont. +IF.	8.7** 0.35	52.0**±1.2	60.7**±2.1	61.8±5.5	50.4±1.6	112.2±5.2
1% +IF.	10.9** 0.95	32.0**±2.7	42.9±2.7	74.9**±2.3	43.0**±1.1	117.9*±6.6
2% +IF.	15.1** ± 0.20	17.6**±2.2	32.7**±2.1	92.4**±5.9	42.1**±2.4	134.5**±5.7
3% +IF.	19.5** 0.89	14.1**±1.7	33.6**±3.0	97.2**±5.6	40.5**±2.4	137.7**±3.8
L.S.D. at 5%	1.2	3.3	5.5	9.9	3.8	10.8
L.S.D. at 1%	1.7	4.6	7.4	13.7	5.3	14.9

Values are given as mean ± SD of three replicates in each group.
*Significant differences (*P*=0.05), and ** Highly Significant differences (*P*=0.01) as compared with control (0% COCS)

delayed seed emergence and that of spent lubricating oil contaminated soil is not different, this is due to poor wettability of the soil (Isirimah et al., 1989), which means that, the plants was exposed to drought stress (Al-Moaikal et al., 2012) The effect of petroleum – contaminated soil on various biochemical parameters of Jojoba is shown in Tables (1-4) and Figures (1-4). The Jojoba seedlings growing in hydrocarbon – contaminated soil are under environmental stress is indicated by significant changes in these biochemical parameters. The content of chlorophyll a, chlorophyll b, carotenoids and total pigments decreased significantly as the concentration of crude oil increased either alone or in combination with inorganic fertilizer (Table 1). Reduction in chlorophyll content has been an indication of environmental contamination (Agrawal, 1992) and (Sharif et al., 2012). This may explain the lower level of chlorophyll in plants exposed to crude oil contaminated soil. The total pigment content of Jojoba from crude oil contaminated soils mixed with inorganic fertilizer was generally higher than that of Jojoba from soils with crude oil alone at 2% and 3%. This suggest that the difference in the chlorophyll content of the plants from contaminated soils with fertilizer and those from soils without fertilizer may only be due to improved soil condition by inorganic fertilizer. However, the content of soluble, insoluble and

Table 3. Effect of crude oil contaminated soil (COCS, V/W%) and inorganic fertilizers (IF) on protein contents (mg g^{-1} DW) in root and shoot of Jojoba (*Simmondsia chinensis*) plant.

Treatments (COCS %)	Protein contents (mg g^{-1} DW)					
	Root			Shoot		
	soluble	Insoluble	Total	soluble	Insoluble	Total
0% (Control)	08.1±0.7	09.0±1.0	17.1±1.2	133.3±4.3	39.1±2.7	172.4±7.6
1%	11.7*±1.3	06.7±0.5	18.4±1.3	166.8**±4.1	36.1±1.3	202.9**±7.1
2%	23.7**±1.7	11.7*±1.3	35.4**±3.6	177.9**±3.9	31.2**±1.4	209.1**±3.5
3%	42.8**±3.6	16.7**±3.0	59.5**±2.9	189.1**±5.1	24.0**±0.9	213.1**±7.6
Cont. +IF.	16.4**±1.3	9.4±1.1	25.8**±1.6	147.6**±3.9	43.1±2.0	190.7**±5.0
1% +IF.	27.1**±1.9	9.7±1.0	36.8**±2.4	186.4**±4.9	55.2**±4.3	241.6**±5.8
2% +IF.	36.1**±1.7	9.4±0.6	45.5**±3.5	185.4**±5.3	60.5**±1.7	245.9**±9.2
3% +IF.	57.8**±2.1	24.4**±1.4	82.2**±2.3	184.5**±5.6	65.8**±4.0	250.3**±7.1
L.S.D. at 5%	3.4	2.5	4.4	08.1	4.5	11.8
L.S.D. at 1%	4.7	3.4	6.1	11.2	6.2	16.3

Values are given as mean ± SD of three replicates in each group.
*Significant differences (*P*=0.05), and ** Highly Significant differences (*P*=0.01) as compared with control (0% COCS)

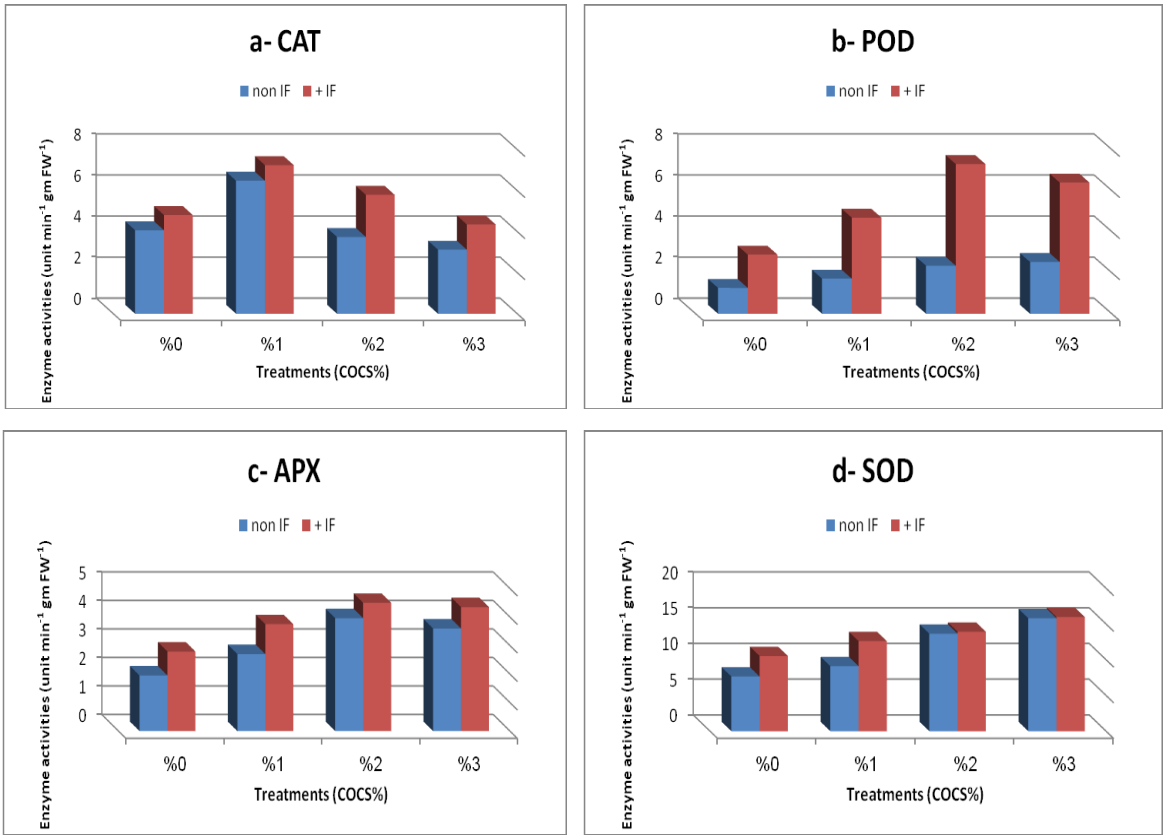
Table 4. Effect of crude oil contaminated soil (COCS, V/W%) and inorganic fertilizers (IF)) on total free amino acids and proline contents (mg g^{-1} DW) in root and shoot of Jojoba (*Simmondsia chinensis*) plant**.**

Treatments (COCS %)	Total free amino acids		Proline	
	Root	Shoot	Root	Shoot
0% (Control)	4.16±0.30	41.3±2.2	0.571±0.034	3.76±0.19
1%	3.05*±0.23	65.2**±2.6	0.666**±0.040	5.88**±0.61
2%	3.70*±0.28	88.1**±4.2	0.845**±0.040	6.61**±0.84
3%	6.60**±0.62	92.4**±3.0	0.940**±0.046	6.97**±027
Cont. +IF.	4.92±0.75	44.3±1.6	0.524±0.041	4.14±0.42
1% +IF.	6.48**±0.60	58.8**±1.9	0.547±0.040	8.15**±0.46
2% +IF.	6.94**±0.58	76.7**±4.8	0.405**±0.025	8.26**±0.16
3% +IF.	8.05**±0.77	85.6**±4.1	0.524±0.041	7.47**±0.34
L.S.D. at 5%	1.07	5.8	0.067	0.80
L.S.D. at 1%	1.47	8.0	0.093	1.10

Values are given as mean ± SD of three replicates in each group.
*Significant differences (*P*=0.05), and ** Highly Significant differences (*P*=0.01) as compared with control (0% COCS)

total sugar component increased in shoot with increasing crude oil concentration (Table 2) in crude contaminated soil alone and with inorganic fertilizer. The high significant increases were recorded at 2 and 3% crude oil. The increase in carbohydrate in Jojoba could be from amino acids derived from the breakdown of protein as shown in table 3. Stewart and Beevers (1967) demonstrated that increase in carbohydrate in germinating castor beans endosperm could be as result in gluconeogenesis from amino acids. Meanwhile, there was a significant decrease in total carbohydrate in root with increasing concentration of crude oil, owing to decreasing the insoluble sugars. The slow degradation of carbohydrate in roots indicated that the enzyme such as amylase whose activity is essential for providing energy for the growth might have been inhibited by crude oil. This suggests that the carbon skeletons for the growth through the respiratory breakdown of utilizable substrate are inhibited by crude oil. Several chemicals have been demonstrated to inhibit degradation of carbohydrate (Penner, 1966). Total carbohydrate recorded a high concentration in shoot and root in crude contaminated soil supplemented with inorganic fertilizer than those without fertilizers.

In table 3 there was a high significant increase in total and soluble protein in root of contaminated soil at 2% and 3% and in crude oil contaminated soil with inorganic fertilizer at all concentration, while insoluble protein shows a high significant increase only at concentration 3% in both treatments. Generally in shoot, there were a high significant increase in soluble, insoluble and total protein content which is proportional to the increase in percentage of crude oil contamination. This agreed with



Figure(1): Effect of crude oil contaminated soil (COCS, V/W%) and inorganic fertilizers (IF) on the activities (unit min^{-1} gm FW^{-1}) of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) in leaves of Jojoba (*Simmondsia chinensis*) plant.

Gbadebo and Adenuga (2012) who claimed that the percentage of protein content in cowpea plants were higher in oil contaminated soil than in uncontaminated soils. The changes in Total free amino acids and proline in both roots and shoots (Table 4) was more or less similar to that change in total protein. Among amino acids, the accumulation of proline is frequently reported in many plants or tissues in response to a variety of abiotic stresses (Hare and Cress, 1997). In maize primary root, for example, the proline level increases as much as a hundred fold under a low water potential (Voetberg and Sharp, 1991). However, the precise role of proline accumulation is still elusive. Whether it is to act as an osmo–regulator (Delauney and Verma, 1993) an osmo-protector (Csonka, 1989) or a regulator of the redox potential of cells (Bellinger and Larher, 1987) has not been decided. These results were in accordance with those obtained by Achuba (2006) on Cowpea (Vigna unguiculata) seedlings where, the results showed that the crude oil induced environmental stress in the seedlings. This is indicated by the increase in free sugar, total protein and amino acids and a decrease in chlorophyll contents of the leaves of 12-day-old seedlings .Crude oil induced changes in the activities of catalase (CAT), peroxidase (POX), ascorbic peroxidase (APX) and superoxide dismutase (SOD) in leaves of Jojoba as shown in table (5) and figure (1). Catalase activity (CAT) was measured in Jojoba leaves compared with the control; there was significantly higher CAT (Tab.5 & Fig.1a) activity upon exposure to 1% in oil contaminated soil and in 1% and 2% contaminated soil with fertilizers. No significant differences were observed at 2% and 3% in oil contaminated soil and at 3% in contaminated soil with fertilizers. The enhanced scavenging ability for H2O2 inhibited the accumulation of ROS and thus protected the plants from lipid peroxidation of membrane systems and oxidative damages under due to crude oil treatments (drought stress) .However increases in catalase activity in response to drought stress suggest a prominent role for this enzyme in the protection of leaf tissue against oxidative damage .There is a fragile balance between ROS production and scavenging that defines the normal steady state level of intracellular ROS. The avoidance of ROS production during drought stress is also an important strategy that enables plants to cope with water shortage without extensive damage (de Carvalho, 2008).

Table 5. Effect of crude oil contaminated soil (COCS, V/W%) and inorganic fertilizers (IF) on the activities (unit min^{-1} gm FW^{-1}) of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) in leaves of Jojoba (*Simmondsia chinensis*) plant.

Treatments (COCS %)	Enzyme activities (unit min^{-1} gm FW^{-1})			
	CAT	**POD**	**APX**	**SOD**
0% (Control)	**4.07±0.21**	**1.26±0.10**	**1.95±0.19**	**7.65±0.92**
1%	**6.48******±0.85**	**1.71±0.15**	**2.70******±0.21**	**9.10*****±0.67**
2%	**3.74±0.91**	**2.34******±0.23**	**3.96******±0.30**	**13.65******±0.69**
3%	**3.12±0.67**	**2.52******±0.18**	**3.60******±0.24**	**15.80******±0.79**
Cont. +IF.	**4.81±0.42**	**2.88******±0.63**	**2.79******±0.24**	**10.52******±0.56**
1% +IF.	**7.24******±0.31**	**4.68******±0.19**	**3.75******±0.17**	**12.60******±0.45**
2% +IF.	**5.79******±0.80**	**7.29******±0.27**	**4.50******±0.29**	**13.92******±0.42**
3% +IF.	**4.35±0.21**	**6.39******±0.23**	**4.35******±0.28**	**15.97******±0.74**
L.S.D. at 5%	**1.06**	**0.50**	**0.43**	**1.17**
L.S.D. at 1%	**1.46**	**0.69**	**0.59**	**1.61**

Values are given as mean ± SD of three replicates in each group.
*Significant differences (*P*=0.05), and ** Highly Significant differences (*P*=0.01) as compared with control (0% COCS)

In environmental stresses conditions such as drought, high activities of CAT enzymes are important for plants to tolerate stresses. Catalase is essential for the removal of H2O2 produced in the peroxisomes by photorespiration (Noctor et al., 2000). Catalase, which degrades H2O2 into water and oxygen, is one of the major antioxidant enzymes (Scandalios et al., 1997). In order to analyze the changes of POX enzymes in Jojoba leaves under crude oil contamination, POX activity was increased significantly for all crude oil concentration at two treatments (Table 5 and Figure 1b). Treatment with inorganic fertilizers had significantly higher POX activity than treatment with crude oil only, whereas restricted water supply had a mild effect on this activity. It is widely accepted that AOS are responsible for various stress-induced damages to macromolecules and ultimately to cellular structures. Consequently, the role of antioxidative enzymes, such as POX and CAT becomes very important. Mafakher et al., (2011) stated that, even under normal growth conditions, many metabolic processes produce ROS in plants, such as superoxide (O2-), hydrogen peroxide (H2O2), and the hydroxyl radical (-OH) (Sudhakar et al., 2001). Meanwhile, plants possess efficient antioxidant defense systems for scavenging ROS (Zhu et al., 2004). CAT and POX are the major antioxidant enzymes. In this study, the activities of antioxidant enzymes super oxide dismutase (SOD) (Table 5 and Figure 1c) and ascorbate peroxidase (APX) (Table 5 and Figure 1d) showed a significant increase in Jojoba leaves at all crude oil concentrations applied (table 4) However, the activities of these enzymes were markedly higher treatment with crude oil supplemented with inorganic fertilizers than without fertilizers. For example, at 1% crude oil with inorganic fertilizers showed a significantly increased activity of SOD(12.6 units-1 gmFW-1)/ APX (3.75 units-1 gmFW-1) when compared to crude oil only (9.1 units-1 gmFW-1)/ APX (2. units-1 gmFW-1) respectively. SOD is considered as a key enzyme in the antioxidant defense system as it regulates the concentration of O2- and H2O2. This enzyme is present in all aerobic organisms and in all sub-cellular compartments susceptible to oxidative stress (Bowler et al., 1992). In several cases transgenic plants overexpressing SOD showed increased tolerance to oxidative treatments and became more resistant to photo inhibition when exposed to different abiotic stresses (Smirnoff, 1993). Different levels of SOD activity might occur depending on stress intensity, species or genotype, growth conditions, stress period, plant age (Sgherri et al., 2000). Anyhow, a higher degree of protection against oxidative damage should require a fast removal of H2O2 by other scavenging systems, thus minimizing H2O2 toxicity and the formation of the highly toxic hydroxyl radicals (Perl et al., 1993). The intercellular level of H2O2 produced under stress conditions is regulated by catalases and peroxidases. Ascorbate peroxidases (APX) can scavenge H2O2 that is inaccessible for catalase because of their high affinity for H2O2 and their presence in different subcellular locations (Noctor et al., 2002 and Abbaspour, 2012).

This culminates in the observed gross effects of environmental stress imposed by crude oil on contaminated vegetation. The biochemical basis of crude oil toxicity at low concentration was clear and the role of inorganic fertilizers is not yet clear will be the subject of another investigation.

ACKNOWLEDGMENT

This work has been supported by Scientific Research at University of Dammam Under Reference No: 2011094.

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