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Potential of Industrial Waste Water Use for Jatropha Cultivation in Arid Land

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ABSTRACT

A pot experiment was conducted in the greenhouse of the National Research Center, Dokki, Cairo, Egypt to investigate the effect of NKP foliar fertilizer and irrigation by Industrial Drainage Water (IDW) on Lipid Peroxidation (LP) and antioxidant enzyme activities [Catalase (CAT), Suproxide Desmatase (SOD) and Ascorbate Peroxidas (APX)] of jatropha plants. Plants fertilized with NPK fertilizers (N0g+P0g+K0g, N3g+P3g+K3g and N6g+P6g+k6g/pot) and irrigated by mixed varied levels of industrial drainage water (0, 25, 50 and 75% IDW). Data showed that concentration of LP increased as percentage of IDW increased up to 75% and tended to decrease with water contains zero IDW. A positive relationship was observed between the CAT, SOD and APX enzyme activities and the level of IDW in irrigation water. The maximum values of the three enzymes activities were obtained with application of NPK: 2:2:2 followed by application of NPK: 1:1:1 as compared with to the control. The highest lipid peroxidation were detected in leaves of non fertilized plants and irrigated by IDW, whereas the lowest values were detected in leaves of plants fertilized by NPK: 2:2:2 by fresh water.

Keywords: Jatropha (*Jatropha Curcus* L.), NPK, Combined Fertilizers with Industrial Drainage Water, Lipid Peroxidation, Antioxidant Enzymes Activities

1. INTRODUCTION

The genus Jatropha (Euphorbiaceae) comprises of about 170 species of woody trees, shrubs, sub shrubs or herbs in the seasonally dry tropics of the Old and the New World. They are used in medicinal folklore to cure various diseases of 80% of the human population in Africa, Asia and Latin America. Species from this genus have been popular to cure stomachache, inflammation, dysentery, vertigo, anemia, diabetis, as well as to treat HIV and tumor (Sabandar, 2013). They are also employed as ornamental plants and energy crops for production biodiesel (Sabandar *et al*., 2013).

Biodiesel is alternative fuel for diesel engines, are becoming increasingly important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fuelled engines (Raja *et al*., 2011). However, biodiesel is a locally-available source of energy that not only can provide energy to meet the increased energy demand derived from the economic development of developing countries. But, also contributes to climate change mitigation and rural development. Jatropha curcas (Fam. Euphorbiaceae) plant has been acknowledged as the preferred crop for the purpose which is drought resistant, perennial and fast growing on poor soil (Misra and Misra, 2010). Jatropha curcas L is a small tree with spreading branches and stubby twigs that grows to 20 feet high under favorable conditions. When propagated from seed five roots are formed-one tap root and 4 lateral roots. Plants propagated from cuttings normally develop only lateral roots with one perhaps developing into a psudo-tap root that may reach only 1/2 to 2/3 the length of a normal tap root. Jatropha has both male and female plants, which may produce different yields of nuts. Dormancy is induced by fluctuations in rainfall and temperature/light; nevertheless, not all trees respond simultaneously. In a hedge you may have branches without leaves beside ones full of green leaves. The life-span of Jatropha may

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be more than 50 years; however, termites are reported to attack older trees.

There is a growing interest in Jatropha curcas as a biodiesel "miracle tree" to help alleviate the energy crisis and generate income in rural areas of developing countries. Jatropha is becoming a poster child among some proponents of renewable energy and appropriate technology, especially as an oil-bearing, "drought resistant" tree for marginal lands for small farmers (Benge, 2006).

Several researches have been done for the possibility of use of poor quality water in trees production: (Pescod, 1992; Parsons *et al*., 2001; Achten, 2010). Under unflavored condition, reactive oxygen species such as superoxide radical, H_2O_2 and OH radicals have a role in lipid peroxidation that lead to membrane damage and degraded of bio molecules such as proteins, phospholipids and pigments. Antioxidant protection involves compounds such as carotenoids, ascorbic acid, a-tocopherol, phenolics and flavonoids (Abd El-Baky *et al*., 2010) and a number of enzymes including Superoxide Dismutase (SOD) and Ascorbate Peroxidase (APX), Catalase (CAT) and Glutathione Reductase (GR) are believed to play a crucial role in scavenge of different free radical (Abd El-Baky *et al*., 2008). Fertilization as a one from successful mean for depressing the damages caused from use of poor quality water was reported by many authors: (Hussein 2008; Segala *et al*., 2011; Mazhar *et al*., 2011). El-Kadi and Kamed (2004) stated that fertilizer led to effects in oxidative defense enzymes in fruit trees. Fertilizer reduction leads to production of oxygen radicals, which results to oxidative stress in the plant and the application of super absorbent polymer could conserve soil water and nutrients, making same available for plants to reduce oxidative stress and increase biomass accumulation, especially under reduced fertilization level (Zhang *et al*., 2011).

Therefore, this study aimed to study the effect of combined fertilizers and irrigation by mixed drainage water on enzymes of oxidative defense of jatropha plants.

2. MATERIALS AND METHODS

Pot experiments were carried out in a greenhouse during the 2011 summer season at the National Research Center (NRC), Dokki, Giza governorate, Egypt to evaluate the effect of NPK fertilizers and irrigation by different percentage of mixed industrial drainage water with fresh water on antioxidant enzyme activities in leaves of jatropha plants. The treatments were as follows:

- Jatropha plants irrigated with mixture drainage water: Fresh water, (25: 75, v:v), (50: 50, v:v) and (75: 25, v:v) and Jatropha plants irrigated with 100 % fresh water
- Application with fertilizer: The plants were fertilizer without mineral fertilizers, the plants were fertilizer with NPK at two different levels (1:1:1 and 2:2:2 w/w/w)

The experiment included 3 percentages of mixed drainage water in combination with three foliar fertilizer treatments i.e., 12 treatments in 6 replicates. A 36 pots of 35 cm in diameter and 50 cm deep were used, each pot were contained 30 kg of air dried clay loam soil (The physical (a) and chemical (b) properties is shown in **Table 1**). Jatropha seeds (*Jatropha curcus* L.) were sown at May, 1, 2011 in the summer season. Plants were thinned twice, the 1st days after sowing and the 2nd two weeks later to leave three plants/pot. Calcium super phosphate (15.5% P_2O_5) and potassium sulfate (48.5% KO_2) as treatments were added before sowing. Ammonium sulfate (20.5% N) as a treatment was added in two equal portions, the 1st after two weeks of transplanting and the 2nd two weeks later. Irrigation with mixed drainage water (the quality of IDW is shown in **Table 2**) at different concentrations was started 21days after sowing (one irrigation by mixed drainage water and the next irrigation by fresh water only alternatively.

2.1. Enzyme Activity Assay

2.1.1. Extraction of Cytosolic Fraction

A plant material (ca. 2 g) was excised and homogenized in 10 mL of ice-cold grinding buffer containing 0.4 M sucrose and 25 mM Tris (pH 7.2). The homogenate was passed through 4 layers of cheat cloth and centrifuged at 12,000×g for 15 min at 4°C. The resulting supernatant was used for determination of enzyme activities, lipid oxidation products and protein contents.

2.2. Enzyme Assays

The activities of Jatropha leaves cytosolic Superoxide Dismutase (SOD; EC, 1.15.1.1) was determined as described by Maehly and Chance (1954). The activity of Ascorbate Peroxidase (APX), (EC, 1.11.1.11) was assayed according to Nakano and Asada (1981). The activity of each enzyme was expressed on protein basis.

2.3. Determination of Lipid Peroxidation Products

The lipid peroxidation products in Jatropha leaves cytosolic fraction were estimated by the formation of Thiobarbaturic Acid Reactive Substances (TBARS) and quantified in term of Malonaldhyde (MDA) as described by Haraguchi *et al*. (1997). The lipid peroxidation was expressed as micromoles of MDA calculated using the extinction coefficient of 1.56×10^5 mM^{-1} cm^{-1}.

Table 1. Physical and chemical analysis of the soil used in pot experiment

Sand												
Course >200 µ	Fine 200-20µ		Silt 20-2 µ%		Clay < 2 µ%				Soil texture			
Soil physical analysis												
7.20	14.25		30.22		48.33				Clay			
					Soluble cations and anions meq/100 g soil							
pH	EC dSm -1	CaCO3%	CEC C mole g^{-1}	OM %	Na^{+}	K^{+}	Ca^{2+}	Mg2+	CO^{-3}	HCO^{-3}	Cl^{-1}	SO^{-2}-
Soil chemical analysis												
7.15	1.3	2.53	33.5	1.3	1.82	0.23	2.38	1.27	0.0	0.91	1.9	189
Available macro-nutrients %					Available micro-nutrients ppm							
N	P	K				Zn		Fe	Mn		Cu	
0.47	0.25	0.95				3.1		4.8	7.3		5.2	

Table 2. Industrial drainage water analysis

Parameters	Values
pH	7.19
EC (dS/m^{-1})	1.12
Soluble cations (meq/L)	
Ca^{++}	2.50
Mg^{++}	2.50
Na^{+}	6.50
K^{+}	2.00
Soluble anions (meq/L)	
Co^{3-}	--
HCO^{3-}	5.90
CL^{-}	5.00
SO^{4-}	0.80

2.4. Determination of Soluble Protein

The total protein content in wheat cytosolic fraction was determined at 595 nm, using Comassein blue G 250 as mentioned by Bradford (1979). Bovine Serum Albumin (BSA) was used as a protein standard.

2.5. Statistical Analysis

Results were statistical analyzed by as methods described by: Snedecor and Cochran (1989).

3. RESULTS

3.1. Affect of Nitrogen, Phosphorus and Potassium Ratio on Lipid Peroxidation and Oxidative Enzymes Activities in Leaves of Jatropha Plants

The activities of antioxidant enzymes Catalase (CAT), Ascorbate Peroxidase (APX) and Superoxide Dismutase (SOD) in leaves of Jatropha plant fertilizer with nitrogen, phosphorus and potassium by two ratios are shown in **Table 3**. The activities of the antioxidant enzymes CAT, SOD and APX presented differential response pattern to nitrogen, phosphorus and potassium by two ratios fertilization (NPK:1:1:1 and NPK2 :2:2). The highest enzymes activities of CAT, SOD and APX was occurred in leaves of jatropha plants fertilized by the highest ratio of NPK2: 2:2, with values 81.2, 45.5 and 39.98 µmoL g^{-1} FW/min, respectively. Both CAT and SOD activities in leaves plants increased as increasing the ratio of NPK fertilization.

Lipid Perioxidation (LP) gave its higher value (56.85 µmoL gfw^{-1}) when jatropha plant had no fertilizer NPK and tended to decreased leaves of jatropha plants fertilized by the highest ratio of NPK2:2:2 (42.54 µmoL gfw^{-1}) as shown in **Table 3**.

3.2. Effect of Irrigation by Industrial West Water on Lipid Peroxidation and Antioxidant Enzyme Activities

Lipid Perioxidation (LP) gave its higher value when Industrial West water (IDW) added in the rate of 75% and tended to decreased, but still more than the control. However the similar levels of LP were obtained in plants irrigated either 50% (64.03 µmoL gFW^{-1}) and 75% mixed water (75.0 µmoL gFW^{-1}) (**Table 4**).

The activities of antioxidant enzymes Catalase (CAT), Ascorbate Peroxidase (APX) and Superoxide Dismutase (SOD) in leaves of Jatropha plant irrigation by Industrial West water (IDW) are shown in **Table 4**. The activities of the antioxidant enzymes CAT, SOD and APX presented differential response pattern to irrigated of water conditions. The highest enzymes activities of CAT, SOD and APX was occurred in leaves of jatropha plants irrigated with the highest level of 75% IDW, with values 93.68, 57.14 and 39.90 µmol g^{-1} FW/min, respectively. Both CAT and SOD activities in leaves plants increased as increasing the levels of IDW in the water of irrigation up to the highest level used (75%).

Table 3. Affect of nitrogen, phosphorus and potassium ratio on lipid peroxidation and oxidative enzymes activities in leaves of jatropha plants

Fertilizer	LP TBARs µmol/gFW	CAT µmol/g FW/min	SOD µmol/g FW/min	APX µmol/g FW/min
Zero NPK	56.84	68.85	36.58	30.84
NPK 1:1:1	50.56	75.50	45.50	34.59
NPK2:2:2	42.54	81.20	45.50	39.98
LSD at 5%	N.S	N.S	4.83	N.S

Zero NPK = Without mineral fertilizer; NPK1:1:1 = 3g N+3g P+3g; K/pot NPK2:2:2 = 6N+6g P+6g K/pot

Table 4. Affect of Industrial drainage water on lipid peroxidation and oxidative enzymes activities in leaves of jatropha plants

Industrial drainage water (%)	LP TBARs µmol/gFW	CAT µmol/g FW/min	SOD µmol/g FW/min	APX µmol/g FW/min
Zero	56.84	44.62	27.83	24.66
25	57.79	75.39	38.53	30.49
50	64.03	87.04	51.79	35.81
75	75.00	93.68	57.14	39.90
LSD at 5%	N.S	N.S	4.830	N.S

Table 5. Effect of NPK fertilization and irrigation by mixed industrial drainage water on lipid peroxidation and oxidative enzymes activities in leaves of jatropha plants

Industrial drainage water (%)	Fertilizer NPK	LP TBARs µmol/gFW	CAT µmol/g FW/min	SOD µmol/g FW/min	APX µmol/g FW/min
Zero	Zero NPK	42.89	32.78	24.78	23.38
	NPK 1:1:1	44.67	43.62	32.17	26.08
	NPK2:2:2	41.27	57.47	26.55	24.52
25	Zero NPK	64.18	54.05	40.68	30.20
	NPK 1:1:1	58.77	65.78	59.23	49.91
	NPK2:2:2	50.41	76.34	65.68	54.94
50	Zero NPK	88.65	74.67	50.86	35.81
	NPK 1:1:1	76.31	89.59	55.86	54.94
	NPK2:2:2	64.87	96.85	78.66	77.64
75	Zero NPK	95.33	89.32	57.22	44.17
	NPK 1:1:1	87.98	92.11	73.28	64.74
	NPK2:2:2	76.54	109.43	81.93	88.87
LSD at 5%		2.370	N.S	4.83	N.S

Zero NPK = Without mineral fertilizer; NPK1:1:1 = 3g N+3g P+3g; K/pot NPK2:2:2 = 6N+6g P+6g K/pot

3.3. Influence of NPK Fertilizer Combined with Irrigation by Industrial Drainage Water on Lipid Peroxidation and Oxidative Enzymes Activities in Leaves of Jatropha Plants

The interactive effect between irrigation by mixed industrial drainage water and NPK fertilizer data showed in **Table 5.** Lipid Perioxidation (LP) gave its higher value when Industrial West Water (IDW) added in the rate of 75% (95.33 µ mo^{-1} gFW^{-1}) and tended to decreased, when irrigation with Industrial West water (IDW) combined with NPK fertilizer at level NPK2:2:2 (76.54 µmo^{-1} gFW^{-1}) but still more than the control (42.89µmoL gFW^{-1}). However the similar levels of LP were obtained in plants irrigated either 50% and fertilized with NPK1:1:1 (76.31 µmoL gFW^{-1}). Data in the same **Table 5** indicated that the positively affected of antioxidant enzymes (CAT, SOD and APX) activity under different irrigation levels of IDW treatment combined with NPK fertilizer at two levels. The highest antioxidant enzyme activities (CAT, SOD and APX) found when Jatropha plants irrigated with IDW at 75% and fertilized by NPK: 2:2:2 (109.43, 81.93 and 88.87 µmoL gFW^{-1}). Also, it was observed that the effect decreased parallel to the increase of IDW % in irrigation solution and the values of activity were higher with the addition of NPK fertilizer. Furthermore, CAT and APX clearly showed approximately similar

response of CAT enzyme activity except when plants irrigated with water contains 75% IDW which it was negatively responded.

4. DISCUSSION

Drainage water contains salts and heavy metals in varied degrees led to oxidative stress in plant irrigated with waters and cause several physiological changes due to chance in metabolic pathway (Lee *et al*., 2001; Panda and Upadhyay, 2003). Production of several Active Oxygen Species (AOS) increases in the presence of NaCl and has been stated to damage almost every macromolecule (Khan and Panda, 2002). However, in many plant cells exposed to oxidative stress the led to induction of both enzymatic (Superoxide Dismutase; (SOD), Catalase; (CAT), Ascorbate Peroxidase; (APO) and Glutathione Reductase; GR) and non-enzymatic (ascorbate, glutathione and alpha tocopherol) antioxidant defense systems, which help in detoxifying the AOS (Lee *et al*., 2001; Malencic *et al*., 2003; Akbari *et al*., 2011). Application of urea to NaCl-stressed lettuce plants can, at least, partially counteract the stress-induced damage, is also associate by detailed changes in wide array of metabolitic pathway including antioxidant enzymes (Abd El-Baky *et al*., 2010). Akbari *et al*. (2011) stated that NaCl stress increased the endogenous, nonenzymatic antioxidants and the activity of antioxidant enzymes, such as peroxidase, superoxide and catalase. Similarly, Golpayegani and Tilebeni (2011) and Abd El-Baky *et al*. (2008) could be induce in plants or microalgae in response to stressors such as salinity, to protect against oxidative stress, plant cells produce antioxidant enzymes such as SOD, POD, CAT and APX.

Ahn *et al*. (2005) suggested that antioxidant enzyme activities may be influenced by the availability of phosphorus, but are subject to considerable variation depending on the developmental stage and the season. Leja *et al*. (2007) found that activity against free radical was increased with nitrogen fertilizer from different sources (Ammonium nitrate, urea and ammonium sulfate) except calcium nitrate which led to decrease this activity. Nitrogen supply prevents oxidative stress in roots, but may improve root development and increase the uptake of Hg from the soil above safety consumption limits. This study highlights the importance of proper nitrogen fertilization towards future phyto-remediation applications with alfalfa plants (Gil *et al*., 2012). Cakmak and Horst (1991) indicate that increases in ROS production during both photosynthetic electron transport and NADPH-oxidizing enzyme reactions may be involved in membrane damage and chlorophyll degradation in K deficient plants. In good agreement with this suggestion, increases in severity of K deficiency were associated with enhanced activity of enzymes involved in detoxification of H_2O_2 (ascorbate peroxidase) and utilization of H_2O_2 in oxidative processes. Moreover, K deficient plants are highly light-sensitive and very rapidly become chlorotic and necrotic when exposed to high light intensity. In view of the fact that ROS production by photosynthetic electron transport and NADPH oxidases is especially high when plants are exposed environmental stress conditions, it seems reasonable to suggest that the improvement of K nutritional status of plants might be of great importance for the survival of crop plants under environmental stress conditions, such as drought, chilling and high light intensity. Several examples are presented here emphasizing the roles of K in alleviating adverse effects of different abiotic stress factors on crop production. Kant *et al*. (2007) mentioned that the NH_4^+/NO_3^- regime led to an increase in total N in control and saline treatments, but did not cause a large decrease in plant Na^+ content under salinity. Activities of GS (EC 6.3.1.2), GOGAT (EC 1.4.1.14), PEPC (EC 4.1.1.31) and AAT (EC 2.6.1.1) increased with salinity in roots, whereas there was decreased activity of the alternative ammonium assimilation enzyme GDH (EC 1.4.1.2). Several authors observed the interactive effect of fertilizer and moisture condition on metabolism of plants (Younis *et al*., 2008; Zhang *et al*., 2011; Mao *et al*., 2011).

Fertilizer reduction leads to production of oxygen radicals, which results to oxidative stress in the plant and the application of super absorben polymer could conserve soil water and nutrients, making same available for plants to reduce oxidative stress and increase biomass accumulation, especially under reduced fertilization level (Zhang *et al*., 2011).

5. CONCLUSION

Our results indicate that, even if oxidative stress is induced in jatropha plants irrigated with 25, 50 and 75% industrial west water, application of NPK could be provide protection against this oxidative stress by increase the antioxidant protective system, which involved as one of the factor responsible for salt tolerance of Jatropha plants. Therefore, the irrigation of Jatropha plants by mean of industrial west water at 75% (v/v) is possible when fertilized with NPK.

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