

Physiological and Biochemical Studies on Flax Plant Grew in Calcareous Soil Amended with Water Hyacinth Dry Manure

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Abstract: The effect of calcareous soil (collected from Tushki) on some metabolic aspects and on the fiber yield of Flax (*Linum usitatissimum*) was studied under normal field conditions, either alone or in combination with water hyacinth dry manure at three concentration levels (0.25%, 0.5% and 1%) to improve the growth and the fiber yield of flax. The results obtained, showed that, the calcareous soil caused a decrease in photosynthetic pigments, endogenous phytohormones like IAA, GA₃ and total Zeatin. A reversible situation was found in ABA and IAA oxidase activity. More, there was an increase in phenol, lipid peroxidation and antioxidant enzymes (CAT, SOD, PPO and POX) activities. The rate of transport of elements (J_i) for K, showed a significant decrease. Na and Ca showed an increase compared with the corresponding control. However, Tushki soil caused a variable changes in fiber properties. The inhibitory effects of calcareous soil on the metabolic activities and fiber yield of flax plants can be improved by addition of 1% dry manure

Key words: Calcareous soil, manure, elements, hormones, phenol, oxidative enzymes

INTRODUCTION

The serious problems of arid and semi-arid areas around the world were, their sensitivity to wind erosion and a high rate of water percolation as well as the decrease in soil nutrients^[24].

Calcareous soils show high quantities of bicarbonate as well as calcium^[25]. The soil is considered calcareous from point of view, when it is in equilibrium with excess of carbonate at partial pressure of the atmospheric CO₂^[2].

The calcium carbonate rich soils are widely spread in Egypt^[2]. The Ca may therefore injure the plants primarily because of the accompanying HCO₃, even when large quantities of Ca are taken up by the plant, they can usually be precipitated in the cell sap or excluded^[23]. Calcium play an important role as a second messenger and is an important element in cell as well as in membrane structure and stability^[50]. Ca deficiency or excess may lead to serious cell damage. The calcareous soils may increase the unavailability of some macro and micronutrients to the crop which in turn retard growth and development of the plants^[4]. Moreover, most of the reclaimed areas have some stress problems i.e. drought, salinity and unbalanced nutrient elements. Therefore, attempts are made to increase crops productivity in new reclaimed soils by many ways such as applying organic

manures^[21,42]. Recently, the use of natural materials such as manure is used as a substitute of the chemical fertilizer. Remediation of the soil with organic manure improves their physical, chemical and biological properties of the status of essential nutrients and soil fertility^[22].

Using *Eichhornia crassipes* as soil additive before sowing could improve the nutrient uptake and nullify the stress effects and consequently improved the growth and yield of the plants^[27,38,13].

It was also deduced that the dry weight and yield of radish, okra, soybean and maize increased with increasing rates of water hyacinth^[51]. Julio *et al*^[20] found that Nutribora compost is prepared with *Eichhornia crassipes*, cow manure and moriche grove soil increases tomato production when used at an optimum dose of 80 t ha⁻¹. This compost has high concentration of K, Fe and Mn.

Intrinsic antioxidant defense mechanisms for coping with these reactive oxygen species include lipid soluble antioxidants (e.g. α-tocopherol and B-carotene), water soluble reductants (e.g. ascorbic acid and glutathione) and enzymatic antioxidants (e.g. superoxide dismutase, catalase and enzymes of the ascorbate / glutathione cycle)^[55]. It was also reported that that superoxide dismutase and other antioxidant enzyme activities were more affected in stressed plants.

Enzymatic protection is partly performed by SOD, catalase and peroxidase that eliminates O_2 and degrade H_2O_2 influencing the level of lipid peroxidation^[36].

The present work was conducted to investigate the role of water hyacinth dry manure on nullifying the effects of Ca CO_3 salt of Tushki soil which is considered as a new virgin soil as well as to estimate the proper concentration of dry manure which can also be used as a fertilizer used for soil amelioration as well as to study its effects on the fibre yield of flax.

MATERIALS AND METHODS

The experimental materials used in this investigation were the seeds of flax (*Linum usitatissimum* L.) provided from the Agricultural Research Centre, Cairo, Egypt.

Two pot experiments were conducted during two successive winter seasons, 2004/2005 and 2005/2006 in the screen of National Research Centre, Cairo, Egypt.

The soil was collected from Tushki i.e. 1150 km south^[12]. The water hyacinth (*Eichhornia crassipes*) was collected from the Nile river Domietta branch. Then the water hyacinth was air dried and ground. Each concentration was mixed thoroughly to the Tushki soil before sowing. Control Tushki soil received no water hyacinth and the fifth group considered as control set (garden soil, sand clay 2:1). Ten replicates for each concentration were carried out.

A similar lot of flax seeds were surface sterilized with 0.001M HgCl_2 solution for three minutes and then washed thoroughly with distilled water. The sterilized seeds were divided into five sets. Each set was sown in each group. Irrigation was carried out according to the usual practice. Plants were exposed to normal day length with natural temperature (about $23 \pm 2^\circ\text{C}$).

Throughout the growth of plants sampling was collected for mineral estimation at vegetative stage (30 and 37 days old). However the biochemical analysis was determined in 37 days old plants. Twenty plants were collected randomly from each group for measuring of growth parameters, endogenous hormones (Indole acetic acid IAA, gibberellins (GAs) abscisic acid (ABA) and cytokinins. Lipid peroxidation, oxidative enzymes activities (peroxidases (POX), catalase (CAT), polyphenol oxidase (PPO) and superoxide dismutase (SOD), IAA-oxidase as well as total phenol. Parameters of fibre yield were also measured.

Analytical methods: Total soluble sugars and total carbohydrates were determined using methods described by Yemem and Willis^[52] and Herbert *et al*^[15] respectively. Total nitrogen and total soluble nitrogen were estimated by the conventional micro-Kjeldahl method^[34].

Identification and determination of acidic hormones (IAA, GAs and ABA) were carried out by Gas Liquid Chromatography (GLC). A weighed samples was extracted according to the method adopted by Shindy and Smith^[40] and the methylation process was carried out according to Vogel^[49]. Cytokinins were extracted as mentioned for the above acidic hormones and were quantified by HPLC^[32].

The photosynthetic pigments were determined by spectrophotometer method recommended by Metzner *et al*^[29].

The extraction and estimation of total phenol was carried out according to Danial and George^[10] and recommended by A.O.A.C.^[1].

The level of lipid peroxidation was measured by determining the levels of malondialdehyde. Malondialdehyde is a product of lipid peroxidation and that assayed by thiobarbituric acid reactive substances (TBARS) contents^[45].

The extraction of catalase (Cat, EC 1.11.1.6), Peroxidase (POX, EC1.11.1.7), polyphenol oxidase (PPO, EC1.10.3.1) and superoxide dismutase (SOD, EC1.12.1.1) and their activities were made according to the methods described by Cao *et al*^[8]. The extraction and the assay of the activities of indole acetic acid (IAA) oxidase was carried out according to the methods described by Mukherjee and Choudhuri^[31].

Cations were determined according to the method of Chapman and Pratt^[9]. Flame Emission Spectrophotometry was used for determining potassium and sodium while calcium was measured by atomic absorption spectrophotometry.

The rate of transport of elements from root to shoot was estimated as:

$$J_j = (M_{s2} - M_{s1}) / (W_{r2} - W_{r1}) \times \text{RGR}$$

Where:

J_j is the transport of ion J from root to shoot, $(M_{s2} - M_{s1})$ is the change in ion content of the shoot from time 1 to time 2, $(W_{r2} - W_{r1})$ is the change in dry weight of the root from time 1 to time 2.

RGR is the relative growth rate of the root on dry weight basis over this period. These were calculated according to Termaat and Munns^[46].

Data were first subjected to analysis of variance (ANOVA) at ($P < 0.05$). The least significant difference (LSD) was used to compare treatments^[44].

Soil sample collected was air dried, thoroughly mixed and passed through 2mm sieve to remove debris. Physical and chemical analysis were carried out according to Jackson^[17], (Table 1).

Chemical analysis of water hyacinth was represented in (Table 2)

Table 1: Chemical and physical data for Garden and Tushki soil.

Data	Garden (control)	Tushki
Chemical analysis:		
Organic carbon%	0.80	0.72
CaCO ₃ %	3.40	8.9
HCO ₃ ⁻ %	0.06	0.33
Cl %	0.018	0.015
NO ₄ ⁻ (mg/100gD.wt.)	14.5	2.86
SO ₄ ⁻ (mg/100gD.wt.)	0.08	0.064
NH ₄ ⁺ (mg/100gD.wt.)	4.21	0.077
Px10 ⁻³ (mg/100gD.wt.)	0.55	0.6
Exchangeable cations:		
Kx10 ⁻⁴ (Mm /g D.wt.)	19.2	9.2
Nax10 ⁻⁴ (Mm /g D.wt.)	7.8	55.6
Cax10 ⁻⁴ (Mm /g D.wt.)	336.5	349.1
Salinity (ppm)	121.6	116.5
pH	7.4	7.7
Physical analysis:		
EC m mohs Cm ⁻¹	0.19	0.18
W.H.C. %	33.4	17
Mean moisture content	27.3	1.25

Table 2: Chemical analysis of *Eichhornia crassipes*.

Character	
Carbohydrate contents (mg glucose/g⁻¹D.wt)	
Total soluble sugar	9.9
Sucrose	3.1
Polysaccharides	19.7
Nitrogen contents (mg NH₄-N/g D.wt.)	
Total soluble nitrogen	9.5
Protein	24.1
Hormonal contents (µg/g D.wt.)	
IAA	750.6
Gas	397.4
ABA	398.6
Cytokinins	224.0
Ionic contents	
Potassium (mM/g D.wt.)	0.36
Sodium (mM/g D.wt.)	0.10
Calcium (mM/g D.wt.)	1.01
Zinc (µm/g D.wt.)	0.187
Lead (µm/g D.wt.)	0.06
Cadium (µm/g D.wt.)	0.005
Iron (µm/g D.wt.)	0.133
Copper (µm/g D.wt.)	0.028
Nikal (µm/g D.wt.)	0.019

RESULTS AND DISCUSSIONS

Endogenous Phytohormones: Growing Flax plants in Tushki soil induces a reduction in the growth promoters levels (IAA, GAs and total zeatin) compared to those of garden soil (Table 3). The level of growth promoters was about two times higher in garden soil grown plants compared with that of the Tushki grown ones.

The levels of auxin, gibberellins and cytokinins were markedly increased in flax shoots grown in Tushki soil treated with different concentrations of water hyacinth dry manure (0.25%, 0.5% and 1.0%) compared with those of Tushki grown shoots. The magnitude of such response was much more pronounced in flax plants grown in the soil treated with 1% dry manure (Table 3).

The increase in the levels of the endogenous growth promoters might be attributed to the increase in their biosynthesis and / or decrease in their degradation and conjugation. Moreover, the increase in the endogenous growth promoters levels of manure treated shoots may be attributed to the higher promoters content of exogenously applied water hyacinth manure. In this connection, Vankova *et al*^[48] and Blagaeva *et al*^[6] reported that the exogenous application of cytokinins stimulated zeatin riboside and dihydrozeatin-7-glucoside production in soybean plants, sugars beet cells and radish respectively.

On the other hand, a reverse effect was observed regarding ABA. A higher ABA level was found in Tushki grown flax shoot as compared with garden soil grown plants as well as addition of dry manure induce marked decline in ABA levels in flax shoots. The decrease in ABA content could be attributed to the shift of the common precursor isopentenyl pyrophosphate into the biosynthesis of cytokinins and / or gibberellins instead of ABA^[16].

Photosynthetic pigments: A significant reduction in chlorophyll a and b as well as carotenoids levels were observed in flax plants grown in Tushki soil as compared to those of the plants grown in garden clay soil, (Table 4). Addition of water hyacinth dry manure at concentrations 0.25%, 0.5% and 1% to Tushki soil induced a marked increase in chlorophyll a and b contents as well as total corotenoids compared to those of Tushki soil grown plants (Table 4). The reduction in pigments levels of tuski grow plants may be attributed to the increase of elements in leaves to the toxic levels or may be attributed to Mg deficiency or closure of stomata^[35] which lead to the inhibition in the rate of photosynthesis under stress conditions either by salinity or by drought. On the other hand, Bavaresco^[5] suggested that under calcareous soil conditions, certain nutrient elements are rendered less available to the growing plants probably due to their transformation to more complicated form.

The increase in the photosynthetic pigments of flax leaves resulted from addition of water hyacinth dry manure are closely related to the obvious increases in the contents of certain elements (eg. N at Fe, table 2) as well as the levels of plant hormones particularly growth promoters (table 3). Several authors are of the opinion that iron and cytokinins functions in the synthesis of chloroplastic protein and so it may play a vital role in chlorophyll synthesis^[56].

It was reported that kinetin and BA treatments increased the photosynthetic pigments and promoted chloroplast developmen^[13,16].

Table 3: Effect of Tushki soil amended with different concentrations of *Eichhornia crassipes* on endogenous phytohormone contents of *linum ustatissinum* ($\mu\text{g}/100\text{g F.wt}$). Each value is a mean of three different replicates.

Treatment	IAA	GAs	ABA	Zeatin riboside	Zeatin	Total zeatin
Garden(control)	46.92	21.12	7.1	10.51	5.22	15.73
Amended Tushki soil						
0	22.41	10.31	12.12	5.51	1.53	7.04
0.25 %	35.45	12.54	9.4	8.16	4.33	12.49
0.50 %	37.28	17.31	8.1	8.52	4.64	13.16
1.00 %	47.3	20.52	7.12	9.85	4.91	14.76
L. S. D at 5 %	2.58	1.16	0.96	1.79	0.19	1.14
L. S. D at 1 %	4.15	1.69	1.31	2.16	0.27	1.66

Table 4: Effect of Tushki soil amended with different concentrations of *Eichhornia crassipes* on photosynthetic pigment contents of *linum ustatissinum* ($\text{mg}/100\text{ g D.wt.}$).

Treatment	Chl(a)	Chl(b)	Carotenoids	Total pigments
Garden(control)	690	261	191	1142
Amended Tushki soil				
0.0	653	240	153	1046
0.25 %	693	270	206	1169
0.50 %	714	293	212	1221
1.00 %	718	282	231	1231
L S D at 5 %	30.8	29.6	10.4	34.9
LS D at 1 %	44.8	38.8	15.1	50.75

Table 5: Effect of Tushki soil amended with different concentrations of *Eichhornia crassipes* on phenol, lipid peroxidation and antioxidant enzyme activities of *linum ustatissinum*.

Treatment	Garden (control)	<i>Eichhornia crassipes</i>				LSD 5 %	LSD 1 %
		0.0	0.25 %	0.50 %	1.00 %		
Total phenol $\text{mg}/100\text{gD.wt.}$	137.3	249	396.8	421.1	474.9	8.58	12.48
Malondialdehyde $\mu\text{m}/\text{g F.wt.}$	0.35	0.41	0.36	0.35	0.3	0.06	0.09
Enzyme Activity ($\text{mg}/\text{g f wt}/\text{h}$)							
IAA oxidase	169	548	421	345	237	10.25	14.92
CAT	675	742	695	684	670	9.96	14.49
SOD	25.6	40.2	37.8	33.6	27.3	1.22	1.78
PPO	8.13	19.42	14.02	8.63	8.12	0.82	1.21
POX	7.2	11.31	11.05	9.2	8.32	0.66	0.97

Total phenols: Addition of dry manure significantly increased the concentration of total phenols in flax plants compared to Tushki grown plants (Table 5). Such increase in total phenols may be attributed to the increase in carbohydrate synthesis^[54] or the increase in endogenous level of cytokinin as confirmed by Hassanien *et al*^[14].

The increase in total phenol contents in response to soil amendment was concomitant with the increase in

IAA content (Table 3) of flax plants and low activity of IAA- oxidase (Table 5) lead to growth stimulation and yield of flax plants (Table 8).

Lipid peroxidation: Calcareous soils may injure, kill or inhibit the growth of some plants^[18]. Stresses induced membrane injury which may therefore due to changes in the membrane lipids and proteins or both. So, the recent results showed that malondialdehyde production

Table 6: Effect of Tushki soil amended with different concentration of *Eichhornia crassipes* on ionic contents of *linum ustatissinum* through time 1 (21 days) and time 2 (30 days) old as (mM/g D.wt.).

Treatment Time 1 (21 days)		K		Na		K/Na		Ca	
Root		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Garden (control)		0.135	0.152	0.476	0.181	0.283	0.316	0.023	0.025
Amended Tushki soil	0.0	0.040	0.115	0.520	0.390	0.008	0.294	0.059	0.033
	0.25 %	0.216	0.121	0.348	0.215	0.620	0.562	0.175	0.057
	0.50 %	0.276	0.198	0.297	0.240	0.929	0.825	0.182	0.062
	1.00 %	0.334	0.205	0.254	0.229	1.314	0.895	0.290	0.080
LSD at 5%		0.021	0.021	0.021	0.007	0.084	0.084	0.007	0.007
LSD as 1%		0.031	0.031	0.031	0.010	0.123	0.123	0.010	0.010
Time 2 (30 days)		K		Na		K/Na		Ca	
Garden (control)		0.421	0.233	0.976	0.285	0.431	0.329	0.045	0.035
Amended Tushki soil	0.0	0.070	0.165	0.910	0.850	0.076	0.194	0.060	0.061
	0.25 %	0.539	0.299	1.836	0.424	0.293	0.723	0.232	0.070
	0.50 %	0.660	0.381	1.502	0.391	0.439	0.974	0.256	0.095
	1.00 %	0.814	0.481	1.373	0.283	0.592	1.699	0.379	0.151
LSD at 5 %		0.021	0.021	0.021	0.007	0.021	0.060	0.021	0.007
LSD as 1 %		0.031	0.031	0.031	0.010	0.031	0.090	0.031	0.01

increased significantly in the flax plants grown in Tushki soils compared with those of the plants grown either in garden soil or in the Tushki soil treated with dry manure (Table 5). The addition of water – hyacinth dry manure to Tushki soil markedly reduced the concentration of malondialdehyde as compared with that of Tushki grown plants. The reduction in MDA production is positively related with the concentration of manure added. The lowest level of malondialdehyde was recorded in plants grown in Tushki soil treated with 1% dry manure. The decrease in such effect was attributed to the antioxidative properties of water hyacinth leaves which scavenge the reactive oxygen species results from stress^[7].

It was suggested that, as AOS induce peroxidation of membrane lipids, resistance to environmental stress may depend on the inhibition of AOS production or the enhancement of antioxidant levels^[39,36].

Antioxidant enzymes: Intrinsic antioxidant defense mechanisms for coping with reactive oxygen species results from stress include lipid soluble antioxidants, water soluble reductants (e.g. ascorbic acid and glutathione) and enzymatic antioxidants^[55].

Superoxide dismutase (SOD) is the most efficient scavenger of the superoxide anion and an essential component of the ascorbate glutathione cycle required for the detoxification of toxic ROS^[37]. The increased SOD activity, Catalase and peroxidase (Table 5) might reflect the increase production of \bar{O}_2 ^[47]. It was suggested that calcium loading in root cells induces dramatic increase in \bar{O}_2 release during wound stress^[30]. It was observed a large increase in SOD, phenol per oxidase and phenol oxidase in the flax shoots grown in Tushki soil compared to control and all the other treatments (Table 5).

The increase in the previous enzymes activities was negatively related to the concentration of the dry manure added to Tushki soil. The enhanced effect of Tushki calcareous soil on the previous enzymes activities may buffer the free radical mediated lipid peroxidation of the membrane (Table 5). Levitt^[25] reported a large increase in peroxidase activity in NaCl-treated guard plants. He attributed this to the peroxidase role in the oxidation of the accumulated substances leading to melanin formation from tyrosine in the necrotic areas and the increased catalase activity indicating a toxic accumulation of H_2O_2 . Lopez *et al*^[26] reported an increase of peroxidase activity in salt- stressed radish cells. Higher activities

Table 7: Effect of Tushki soil amended with different concentration with *Eichornia crassipes* on the rate of transport of K(J), Na (J) and Ca(J) of *linum ustatissinum*.

Treatment		Transport of KJ k	Transport of Na J Na	Transport of Ca J Ca
Garden(control)		5.508	0.707	0.07
Amended Tushki soil	0.0	0.16	1.472	0.080
	0.25 %	1.104	1.558	0.086
	0.50 %	1.656	1.637	0.299
	1.00 %	2.024	1.956	0.521
L. S. D at 5 %		0.021	0.021	0.021
L. S. D at 1 %		0.031	0.031	0.031

of SOD, peroxidase and catalase were recorded in cultivated NaCl stressed species of *Lycopersicon pennellii*^[39].

The effect of calcareous soil on SOD activity might be attributed to the Ca carbonate. The increased SOD activity might reflect the increase production of \bar{O}_2 ^[47]. It was suggested that calcium loading in root cells induces a dramatic increase in \bar{O}_2 release during wound stress^[30].

Indole acetic acid oxidase activity was significantly increased in the Tushki grown flax shoots compared with those of garden soil grown ones. The increase in IAA oxidase activity reaches about three times of that of control plants (Table 5). Addition of water hyacinth manure to Tushki soil markedly reduced the activity of IAA oxidase as compared with that of Tushki grown plants. The reduction in the activity was positively related to manure concentrations. IAA oxidase and peroxidase play an important role in the enzymatic destruction of IAA in the plants. This result was concomitant with the levels of IAA in flax shoots as shown in table (3).

Finally, the $CaCO_3$ tolerance seemed to be correlated with the stimulation of antioxidant enzymes and the enhanced ability to remove AOS. So we could say that flax might be considered as a calciophiles plants which can develop avoidance mechanisms. So the plants can take up Ca and precipitated it in the cell sap or excluded it^[25].

Changes in ionic contents: It has been known that maintenance of the normal differential permeability of the cell depends on a balance between monovalent (K^+ , Na^+) and divalent (mainly Ca^{2+}) cations^[25]. In the present work low levels of K were observed in either shoots or roots of Tushki grown flax plants throughout the two stages (Table 6) as compared with those of plants grown in either garden soil or manure treated soils. This might be attributed to the

inhibitory effect of calcareous soils on the uptake and translocation of K. The rate of K transport from roots to shoots were markedly increased in the plants grown in dry manure soil. A more pronounced high rate was observed in plants grown in soil amended with 1% dry manure (Table 7). Similar results have been reached by Jouany *et al*^[19] and Bavaresco^[5]. The growing of plants in a soil amended by water hyacinth manure increased the levels of K contents of shoots and roots compared to those of Tushki and garden grown plants. The increase in potassium content of manure treated plants is attributed to the high potassium level of the dry manure (Table 2), so potassium becomes more available for roots. This result is in accord with those obtained by Shukry, *et al*^[42]. Also, Shaaban and Mobarak^[38] and Hamada^[13] found that there is an increase in K concentration that was attributed to the enhancement effect of organic composts on cation exchange capacity of soil.

The amendment of Tushki soil with different amounts of dry manure increased the ability of flax plants to absorb sodium ions and decrease its transport to the shoots (Table 7). The sodium levels in Tushki amended soils were significantly low compared with that of Tushki grown shoots (Table 6). In this regard Younis *et al*^[53] recorded that the increase in $Ca(NO_3)_2$ concentration would inhibit Na accumulation and transport. It was also deduced that elevated Ca concentration in the nutrient solution inhibited Na uptake^[3].

The calcium accumulated in flax roots more than those of shoots. Tushki grown plants contain high levels of Ca compared with that of garden grown plants (Table 6). Similarly, the rate of Ca transport is higher in all Tushki grown plants (Table 7). Addition of dry manure increases the Ca^{2+} level in flax root and shoot. Such effect might be attributed to the increase in Ca^{2+} proportion in the soil and addition of manure may induce a better distribution

Table 8: Effect of Tushki soil amended with different concentrations of *Eichhornia crassipes* on fibre yield, quality and composition of *linum ustatissinum*.

Treatment		Fibre length (cm)	Fibre diameter (micron)	Lignin mg/g D. wt.	Cellulose mg/g D. wt.	Cellulose / lignin
Garden (control)		49.1	22.4	17.7	68.6	3.88
Amended Tushki soil	0.0	38.8	49.5	20.9	64.9	3.11
	0.25 %	40.1	43.5	19.1	70.9	3.71
	0.50 %	43	38.5	18.6	71.2	3.83
	1.00 %	47.4	34.4	18.3	76.4	4.17
L.S.D at 5 %		3.01	2.7	0.75	2.67	0.28
L.S.D at 1 %		4.38	3.92	1.1	3.88	0.41

of the CaCO₃ particles already exist in the soil and consequently increase the calcium ions (present in the manure) and may also due to the increase in organic matter content in soil which enhances the availability of most nutrients to plant roots^[28]. In this regard Olk and Cassman^[33] found that utilization of organic manure in newly reclaimed sandy soil resulted in significant increase in soil organic matter which in turn increases element availability. Similarly, addition of different concentrations of water hyacinth significantly increases the rate of Ca transport from roots to shoots. The increase in Ca- transport rate is concentration dependent i.e. a greater rate was observed in plants grown in Tushki soil amended with 1% manure. Singh and Singh^[43] reported that Ca uptake of *Eucalyptus* increased with CaCO₃ concentrations in the soil. Finally, we concluded that addition of dry manure creates a nutrient balance in the rhizosphere helping the absorption mechanism of root to freely select the required nutrients and amounts.

Changes in fibre yield, quality and fibre composition of flax stem: Flax plant considered as a source of fibres. The fibre contents and fibre properties in terms of fibre length, were markedly reduced in flax plants grown in Tushki soil compared with those of all the other treatments (Table 8). The above mentioned data was in agreement with those obtained by Shukry^[41] who suggested that the percentage of fiber was low in Tushki soil comparing with the other soils and control. Amendment of Tushki soil with various amounts of water hyacinth dry manure (0.25%, 0.5% and 1.0%) increase fibre length while induced a reduction in fibre diameter compared with those of Tushki grown plants which showed the greatest magnitude of fibre diameter. The fibre length was greater in flax stems grown in 1% dry manure added to Tushki soil (Table 8).

On the other hand, the fibre diameter of flax grown in untreated and treated Tushki soil was much

higher compared with that of garden soil grown plants. The increase in fibre yield and quality of Tushki soil amended with various concentration of water hyacinth dry manure may be attributed to the increase in nutrients uptake and assimilation and / or the high endogenous levels of promoters of the dry manure. It was postulated an increase in fibre yield of roselle plants treated with GA₃ and / or BA^[11].

Cellulose percentages were markedly increased in fibres of amended soil grown plants compared with those of control (garden) and stressed plants. While Tushki grown plants have more lignin percentage compared with the other treatments, the increase in cellulose percentage could be attributed to the synthesis of chlorophyll which involved in increasing metabolites and lead to the accumulation of different fractions of soluble sugars in plant tissues. So plants directed the excess amount of organic compounds towards the different components of the yielded fibre. Shukry^[41] reported low fibre percentage in Tushki grown plants compared to that of garden grown ones. Finally, it seemed that Flax plant can be cultivated at Tushki soil amended with water hyacinth at concentration 1% for a good fiber properties.

REFERENCES

1. A.O.A.C., 1975. Official Methods of Analysis of the Association of Official Agricultural Chemists. Pub. A.O.A.C. Washington D.C., U.S.A.
2. Balba, A.M., 1980. Calcareous soils properties and management. Alex. Sci. Exch., 1:1-162.
3. Banuls, J., F. legaz and E. Primo Millo, 1991. Salinity calcium interaction on growth and ionic concentration of citrus plants. Plant and soil, 133: 39-46.
4. Barsoum, M.S. and Z.M. Nassar, 1995. Response of fodder beet to foliar application of N, K and Zn under calcareous soil conditions. J. Agric. Sci. Mansoura Univ., 20: 2701-2712.

5. Bavaresco, L., 1997. Relationship between chlorosis occurrence and mineral composition and of grapevine leaves and berries. *Commun. Soil sci. Plant Anal.*, 28: 13-21.
6. Blagaeva, E., P. Dobrev, J. Malbeck, V. Motyka., A. Gaudinova and R. Vankova, 2004. Effect of exogenous cytokinins, auxins and adenine on cytokinin Nglucosylation and cytokinin oxidase / dehydrogenase activity in de-rooted radish seedlings, *J. Plant Growth Regul.*, 44: 15-23.
7. Bodo, R., A. Azzouz and R. Hausler, 2004. Antioxidative activity of water hyacinth components *Plant Sci. Elsevier science Ltd, Oxford, UK*, 166: 893-899.
8. Cao, X., L.Q. Ma and C. Tu, 2004. Antioxidative responses to arsenic in the arsenic hyperaccumulation Chinese brake fern (*Pteris Vittatta* L.). *Environ. Poll.*, 128: 317-325.
9. Chapman, H.D. and P.F. Pratt, 1978. Methods of analysis for soils, plant and water. California Univ. Division Agric. Sciences., 4034 pp.50 and 169.
10. Danial, A.D. and C.M. George, Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. *J. Amer. Soc. Hort. Sci.* 17: 621-624.
11. El-Bassiouny, H.M.S., H.K.I. Khattab and M. Sadak, 2005. Synergistic effect of GA3 and BA on growth, photosynthetic pigments, metabolites, phytohormones and fibre yield of roselle plants. *J. Biotechnol.*, 21: 13-31.
12. El-Sirafy, Z.M., Kh.H. El-Hamdi, A.A. Taha and H.M. Abdel-Naby, 1989. Paper production on sandy soils affected by compost addition And nitrogen fertilization. *J. Agric. Sci. Mansoura Univ.*, 14: 1793-1802
13. Hamada, E. El-Sayed, 2003. Studies on some oil plants growing in different soils. M.Sc thesis Mansoura Univ.
14. Hassanien, R.A., H.K.I. Khattab, H.M.S. El-Bassiouny and M.S. Sadak, 2005. Increasing the active constituents of sepals of roselle (*Hibiscus sabdariffa* L.) Plant by applying gibberellic and benzyladenine. *J. Applied Sci. Res.* 2 : 137 - 146.
15. Herbert, D., P. Phipps and O. Strange, 1971. Determination of total carbohydrates *Methods in Microbial.*, 58, 209.
16. Hopkins, W.G. and N.P.A. Hüner, 2004 *Introduction to Plant Physiology.* 3rd Edition. John Wiley and Sons, Inc. USA.
17. Jackson, M.L., 1965. Soil chemical analysis constable and Co. LTD. London.
18. Johnson, M.K., E.J. Johnson, R.D. Mac Elrory H.L. Speer and B.S. Bruff, 1968. Effects of salts on the halophilic alga *Dunaliella viridis*. *J. Bacteriol.*, 5: 1561-1468.
19. Jouany, C., B. Colomb and M. Bosc, 1996. Long- term effects of potassium fertilization on fields and fertility status of calcareous soils of south west France. *European J. of Agron.*, 5: 287-294.
20. Julio, C.R.R., E.M.C. Angel and N. Montano, 2004. Chemical characterization of Nutribora compost and its combined use with a commercial fertilizer for the cultivation of tomato . *Interciencia. Asociacion Interciencia, Caracas, Venezuela*, 29: 267-273.
21. Keshta, M.M., M.A. El-Hawary and M.A. Haekal, 1999. Effect of farmyard manure, Bio and mineral phosphorous fertilizer on rape seed under salt affected soil. *AL-Azhar. J. Agric. Res.*, 29: 15-24.
22. Khalil, M.E.A., N.M. Badram and A.A. El-Emam, 2000. Effect of different organic manures on growth and nutritional status of corn. *Egypt. J. Soil Sci.*, 40: 245-263.
23. Kinzel, H., 1963. Zellsaft Analysen Zum P fanlichen Calcium-und Sauerstoffwechsel-und zum Problem der Kalk-und Silikat-pflanzen. *Protoplasma*, 57: 522-555.
24. Kurban, H., H. Saneoka, R. Adilla, S.P. Gnanasiri and K. Fujita, 1999. Effect of salinity on growth, photosynthesis and mineral composition in leguminous plant *Alhagi pseudoalhagi* (Bieb) . *Soil Sic. Plant Nutr.*, 45: 851-862.
25. Levitt, J., 1980. Responses of Plants to Enviromental Stresses: Water; Radiation, salt and other stresses, vol. 2. Academic Press New York.
26. Lopez, F., G. Vansuyt, F. Casse-Delbart and P. Fourcroy, 1996. Ascorbate peroxidase activity not the m RNA level is enhanced in salt stressed *Raphanus sativus* plants. *Physiol. plant*, 97: 13-20.
27. Marschner, H., 1995. *Mineral Nutrition of Higher plants*, 2nd ed. London, Academic press.
28. Mengel, K. and E.A. Kirkby, 1982. Potassium in physiology, in *Principles of Plant Nutrition*.
29. Metzner, H., H. Raum and H. Senger, 1965. Untersuchungen Zur Sychon-nisier-Barkeiteinze-Iner Pigmenman-gel. *Mutantenvo. Chlorella. Planta*, 65-186.
30. Minibayeva, F.V., O.P. Kolesnikov, L.K. Gordon, H. Asard and P. Navas, 1998. Contribution of a plasma membrane redox system to superoxide production by wheat root cells. *Protoplasma.*, 205: 101-106.

31. Mukherjee, S.P. and M.A. Choudhuri, 1981. Effect of water stress on some oxidative enzymes and senescence in vigna seedlings . *Physiol. plant*, 52: 1-37.
32. Muller, P. and W. Hilgenberg , 1986. Isomers of zeatin and zeatin riboside in club root tissue: Evidence for trans-zeatin biosynthesis by *Plasmadiophora brassicae*. *Physiol. plant*, 66: 245- 250.
33. Olk, D.C. and K.G. Cassman, 1995. Reduction of potassium fixation by two humic acid fractions in vermiculitic soils. *Soil Sci. Soc. Am. J.*, 59: 250-263 .
34. Pirie, N.W., 1955. In *Modern Methods of Plant Analysis*. Interscience Publishers Inc., New York.
35. Sanchez Rodnguez, J., P. Perez and R. Martinez-Carrasco, 1999. Photosynthesis, carbohydrate levels and chlorophyll fluorescence estimated intercellular CO₂ in water stressed *Casuarina equisetifolia* forst and forst plant. *Cell and Env.*, 22: 867-873
36. Santos, C.L.V., A.H. Cam pos, Azevedo and G. caldeira, 2001. In situ and in vitro senescence induced by KCL stress: nutritional imbalance. Lipid peroxidation and antioxidant metabolism. *J. Exp. Bot.*, 52: 351-360.
37. Sen Gupta, A., P.R. Webb, A.S. Holaday and R.D. Allen, 1993. Over expression of superoxide dismutase protects plant from oxidative stress. *Plant Cell Physiol.*, 103: 1067-1073.
38. Shaaban, M.M. and Z.M. Mobarak, 2000. Effect of some green plant materials as soil additive on soil nutrient availability growth yield and yield components of faba bean plants. *J. Agric. Sci., Mansoura Univ.*, 25: 2005-2016.
39. Shalata, A. and A. Tal, 1980. The effects of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt tolerant relative *Lycopersicon pennellii*. *physiol. plant*, 104: 169-174 .
40. Shindy, W.W. and O. Smith, 1975. Identification of plant hormones from cotton ovules. *Plant physiol.*, 55: 550-554.
41. Shukry, W.M., 2001. Effect of soil type on growth vigour, water relations, mineral uptake and contents of fatty acids and protein of yielded seed of *Linum usitatissimum*. *Pakistan J. Biol. Sci.*, 4: 1470-1478.
42. Shukry, W.M., A.M. Gaber, M.A. Abbas and R.E. El-Samahy, 2001. Effect of cattle manure on growth and some physiological aspects and yield of soybean grown in calcareous soil. *Bull. Fac., Assiut Univ.*, 30: 201-215.
43. Singh, K. and K. Singh, 1993. Seedling growth and mineral composition of Eucalyptus hybrid under calcareous soil conditions. *J. of the Indian Society of Soil Sci.*, 4: 810-813.
44. Snedecor, C.W. and W.G. Corchran, 1980. *Statistical methods*. 7 "edition". Iowa State Univ. Press. Ame. A. Iowa, U.S.A.
45. Stewart, R.C. and J.D. Bewley, 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.*, 65:245-248.
46. Termaat, A. and R. Munns, 1986. Use of concentrated macronutrients solution to separate osmotic from NaCl specific effect on plant growth. *Aust. J. Plant Physiol.*, 13: 509-522.
47. Thompson , J.E., R.L. Legge and R.F. Barber, 1987. The role of free radicals in senescence and wounding. *New Phytol.*, 105: 314-317.
48. Vankova, R., K.C. Hsiao, C.H. Bornman and A. Gaudinova, 1991. Effect of synthetic cytokinins on level of endogenous cytokinins and respiration patterns of Beta vlegaeis cells in suspension. *J. Plant Growth Regul.*, 10:197-199.
49. Vogel, A.J., 1975. *A text Book of Practical Organic Chemistry*, 3rd ed., Book Society and Longmans Growth Ltd., London.
50. White, P.J., 1998. Calcium channels in the plasma membrane of root cells. *Ann. of Bot.*, 81: 373-183.
51. Widjanto, D.W., T. Honmura and N. Miyauchi, 2003. Possible utilization of water hyacinth (*Eichbornia crassipes* Mart Solms), an aquatic weed as green manure in vegetables cropping systems. *Jap. J. Trop. Agric.*, 47: 27-33.
52. Yemm, E.W. and A.J. Willis, 1954. The estimation of carbohydrates in plant extracts by anthrone *Biochem. J.*, 57, 508.
53. Youins, M.E., M.A. Abbas and W.M. Shukry, 1994. Salinity and hormone interactions in affecting growth, transpiration and ionic relation of *Phaseolus vulgaris*. *Biolog. plant*, 36: 83-89.
54. Youssef, E.M., 1993. Rejuvenation in *Acacia saligna*. *Labill Wend Bull. Fac. Agric. Univ. Cairo*, 44:105-130.
55. Zhang, X. and R.E. Schmidt, 1999. Antioxidant response to hormone containing product in Kentucky blue grass subjected to drought. *Crops Sci.*, 39: 545-551.
56. Zude, M., A. Alexander and P. Ludders, 1999. Influence of FEDDHA and sugar derivatives on curing iron chlorosis in citrus. *Gesunde pflanzen*, 51: 125-129.