EFFECT OF HUMIC ACID AND SULFUR ON GROWTH, SOME BIOCHEMICAL CONSTITUENTS, YIELD AND YIELD ATTRIBUTES OF FLAX GROWN UNDER NEWLY RECLAIMED SANDY SOILS

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ABSTRACT

A field experiment was carried out at the experimental Station of National Research Centre, Al Nubaria district, El-Behira Governorate-Egypt, in 2013/2014 and 2014/2015 winter seasons to investigate the influence of three sulfur fertilizer levels and/or foliar humic acid on some morphological and biochemical parameters as well as yield quantity and quality of flax plant grown under newly reclaimed sandy soil conditions. Results indicated that, humic acid foliar treatment with 20 (mg/l) in addition to sulfur fertilizer at rate of (250 and 500 kg/fed) gave significant increases in seed, straw and oil yield / fed. These increases due to the recorded increases in morphological criteria (plant height and root length, fresh and dry weight of shoot and root), photosynthetic pigments, (chlorophyll a, chlorophyll b and carotenoids), carbohydrate constituents (total carbohydrates, total soluble sugars and polysaccharides), IAA, phenol, free amino acids and proline contents. Meanwhile, lipid peroxidation decreased significantly in response to the above mentioned treatment as compared with control plants. Interaction between humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) was the most effective treatment as it gave the highest increases in all morphological criteria, biochemical parameters, yield and yield attributes compared with the other treatments. Humic acid and sulfur fertilizer at all levels caused marked decreases in total saturated fatty acids accompanied by marked increases in total unsaturated fatty acids. The essential fatty acids (Linoleic acid C 18:2 + Linolenic acid C18:3) were increased by all applied treatments. It is worthy to mention that, the enhancement effects of humic acid (20 mg/l) and sulfur fertilizer (500 kg/fed) were the most pronounced treatment on flax plant.

Keywords: fatty acid, flax, humic acid, lipid peroxidation, sulfur fertilizer, yields.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is a source of two products; flax seed for oil and fiber for linen products. In Egypt, it is an old economic crop grown as a dual purpose crop for seeds and fibers which is used for the manufacture of linen. Linseed contains 30-48 % of oil abundant in unsaturated fatty acids, especially α-linolenic acid (40-68 %), where technical purity can reach 95%. Linseed oil-chemical industry in drying oil (for the production of printing and other inks, varnishes, paints and linoleum).

Environmental pollution due to excessive application of chemical fertilizer such as nitrogen fertilizer to agricultural lands, surface waters and groundwater is one of the most important environmental and social concerns throughout the world especially in developing countries (Parr *et al*., 1992). Soil organic matter has beneficial effects on soil quality and positive effects on crop productivity and also has the potential to sequester carbon. In addition, organic matters could reduce the application of industrial fertilizers in long term (Rasmussen and Parton, 1994). Humic acid (HA) is one of the main organic substances, which is an important component of humic substances. Humic acid is complex substances derived from organic matter decomposition. Humic acid are the most significant constituents of organic matter in both soils and municipal waste compost, and have a relevant role in the cycling of many elements in the environment and in soil ecological functions (Senesi *et al*., 1996). According to previous investigations, humic seem to have a particular favorable effect on the nutrient supply. Foliar sprays of these substances also promote growth, and increase yield and quality in a number of plant species (Karakurt *et al*., 2009) at least partially through increasing nutrient uptake, serving as a source of mineral plant nutrients and regulator of their release (Atiyeh, *et al*., 2002). Moreover, humic acid influence respiration-process, the amount of sugars, amino acids and nitrate accumulated (Boehme *et al*., 2005).

Sulfur (S) is an essential macroelement for plant nutrition, but its concentration in plants is the lowest of all the macronutrients. Sulfur is increasingly being recognized as the fourth major essential nutrient element after nitrogen (N), phosphorus (P), and potassium (K). Plants are able to assimilate sulfate and reduce it to essential amino acids, where S is involved in a range of metabolic functions, including protein synthesis. Greater attention needs to be paid to the role of S in balanced crop nutrition.

Sulfur plays an important role not only in the growth and development of higher plants, but is also associated with increased stress tolerance in plants (Nazar, *et al*., 2011 and Osman and Rady, 2012). S deficiency negatively affects the chlorophyll and N contents of leaves and photosynthetic enzymes (Lunde, *et al*., 2008), and consequently the reduction in yields and quality parameters of crops (Hawkesford, 2000 and Osman and Rady, 2012). Adequate S nutrition improves photosynthesis and the growth of plants, and has regulatory interactions with N assimilation (Scherer, 2008). S is required for protein synthesis, N assimilation, and is a structural constituent of several co-enzymes and prosthetic groups (Marschner, 1995).

Also, several reports indicated that S was beneficial for newly-reclaimed soils to alleviate the adverse effects of environmental stress and to improve sustainable crop productivity (Osman, and Rady, 2014).

In Egyptian soils, which are characterized by a rise in pH, S reduced soil pH values by the oxidation of S to sulphate through various species of soil microorganisms (El-Eweddy, *et al*., 2005 and Osman and Rady, 2012) Decreasing soil pH improves the availability of microelements (e.g., Fe, Zn, Mn, and Cu) (Hetter, 1985) and improves the chemical properties of alkaline soils as well as increasing yields and related characteristics (Kineber, *et al*., 2004 and Osman and Rady, 2012).

The aim of this study was to investigate the influence of three sulphur fertilizer levels and/or foliar humic acid on some morphological, physiological and chemical parameters as well as yield quantity and quality of flax plant grown under newly reclaimed sandy soil conditions.

MATERIALS AND METHODS

Two field experiments were carried out at the experimental station of National Research Centre, Al Nubaria district El-Behira Governorate-Egypt, in 2013/2014 and 2014/2015 winter seasons. Soil of the experimental site was sandy soil. Mechanical, chemical and nutritional analysis of the experimental soil is reported in Table-1 according to Chapman and Pratt (1987).

Table-1. Mechanical, chemical and nutritional analysis of the experimental soil. mechanical analysis.

Sample time	Sand		Silt 20-0µ %	Clay < 2µ %	Soil texture
	Course 2000-200µ%	Fine 200-20µ %			
Before sowing	47.46	36.19	12.86	4.28	Sandy
During growth	41.51	50.00	5.50	3.00	Sandy
During harvest	49.51	38.09	10.48	1.92	Sandy

Chemical analysis.

Sample time	pH 1:2.5	EC dSm^{-1}	$CaCo_3$	OM%	Soluble cations meq/l				Soluble anions meq/l			
					Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
Before sowing	7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89
During growth	7.46	0.21	4.5	015	0.57	0.18	2.10	1.3	0.0	2.45	0.45	1.25
During harvest	7.35	0.15	3.9	0.13	0.52	0.13	0.18	0.9	0.0	1.12	0.45	0.79

Nutritional analysis.

Sample time	Available nutrients						
	Macro element ppm			Micro element ppm			
	N	P	K	Zn	Fe	Mn	Cu
Before sowing	52	12.0	75	0.14	1.4	0.3	0.00
During growth	87	12.0	105	0.35	0.8	0.3	0.45
During harvest	53	11.5	75	0.35	1.2	0.3	0.95

An experimental design was split plot design with three replication, where the two humic acid levels (0.0 and 20 mg/l) occupied the main plots and the three sulfur fertilizer levels (0.0, 250 and 500 kg/fed) were allocated at random in sub plots. Flax seed of Opal cultivar were sown on the 17th November in both season in rows 3.5 meters long, and the distance between rows was 20 cm apart, plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The seeding rate was 2000 seeds/m^2). Pre-sowing, 150 kg/fed of calcium super-phosphate (15.5% P_2O_5) were used. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 75 Kg/fed in five equal doses. Potassium sulfate (48 % K_2O) was added at two equal doses of 50 kg/fed. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Foliar application of humic acid was carried out twice; where plants were sprayed after 45 and 60 days from sowing. Plant samples were taken after 75 days from sowing for measurements of growth characters and some biochemical parameters. Growth parameters in terms of, plant height (cm), shoot fresh and dry weight (g), roots length (cm), root fresh and dry weight (g). Plant samples were dried in an electric oven with drift fan at 70°C for 48 hr. till constant dry weight. Chemical analysis measured were photosynthetic pigments, carbohydrate constituents, IAA, phenolic, free amino acids, proline contents and lipid peroxidation. Flax plants were pulled when signs of full maturity were appeared, then left on ground to suitable complete drying. Capsules were removed carefully. At harvest, plant height, technical stem length, fruiting zone length, number of fruiting branches/plant, number of capsules/plant, seed yield/plant, biological yield/plant were recorded on random samples of ten guarded plants in each plot. Also, seed yield/fed, straw yield/ /fed, seed oil % and oil yield /fed) and fatty acid profile were studied.

Chemical analysis: Photosynthetic pigments contents (chlorophyll a and b and carotenoids) in fresh leaves were estimated using the method of Lichtenthaler and Buschmann, (2001). Determination of total carbohydrates was carried out according to Herbert, *et al*., (1971). Total soluble sugars (TSS), were extracted by the method of Homme, *et al*., (1992) and measured by Yemm and Willis (1954). Indole acetic acid content were extracted and analysed by the method of Larsen, *et al*., (1962). Total phenol content was measured as described by Danil and George (1972) Free amino acid content was extracted according to the method described by Vartainan, *et al*., (1992) (and determined with the ninhydrin reagent method (Yemm, *et al*., 1955). Proline was assayed according to the method described by Bates *et al*., (1973). The level of lipid peroxidation was measured by determining the malonadialdehyde (MDA) contents. Malonadialdehyde is the product of Lipid peroxidation and that assayed by thiobarbaturic acid reactive substance (TBARS) contents (Stewart and Bewley, 1980). The oil of flax seeds were extracted according to Kates and Eberhardt (1957), Fatty acid determination was estimated by the method of (Harbone (1984)).

The data were statistically analyzed on complete randomized design system according to Snedecor and Cochran (1980). Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5%.

RESULTS AND DISCUSSIONS

Effect of Humic acid

Effect of Humic acid on growth parameters

Data in Table-2 show the effect of foliar treatment with humic acid on some growth parameters of flax plant. The results illustrated that flax plant of Opal cultivar treated with humic acid (20 mg/l) recorded significant increases of shoot and root length, fresh and dry weight of shoot and root compared with control plants. These obtained results are in agreement with those obtained by Peymaninia *et al*., (2012) on wheat, Bakry, *et al*., (2013) on flax and El-Bassiouny *et al*., (2014) on wheat plant. These obtained increases in response to humic acid due to that, humic acid is considered to increase the permeability of plant membranes and enhance the uptake of nutrients (Piccolo, *et al*., 1992). It could be concluded that this increases may be due to the role humic acid in increasing endogenous hormone as IAA (Table-4) and the role of these hormones in stimulating cell division and/or the cell enlargement and this in turn improve plant growth (Abdel Mawgaud *et al*., 2007). Furthermore, humic acid increases the porosity of soil and improve growth of root system which leads to increase the shoot system (Garcia, *et al*., 2008). Mataroiev (2002) describe the role of humats in improving soil physical and chemical characteristics by reaction with soil minerals then improving watery, aerial soil characteristics and nutrient mineral adsorption.

Table-2. Effect of humic acid on growth parameters of flax plant grown under newly reclaimed sandy soil. (Combined analysis of 2013/2014 and 2014/2015 seasons).

Humic acid (mg/l)	**Plant height** (cm)	**Fresh weight** (g)	**Dry weight** (g)	**Root length** (cm)	**Root fresh weight** (g)	**Root dry weight** (g)
0	40.22	2.38	0.63	11.00	0.38	0.21
20	42.33	2.91	0.82	13.00	0.53	0.25
LSD 0.05	1.20	0.43	0.15	0.39	0.12	0.02

Effect of Humic acid on biochemical characters

Photosynthetic pigments

Data in Table-3 indicate that humic acid with (20 mg/l) caused significant increases in chlorophyll a, chlorophyll, b, carotenoids and total pigments compared with control treatment. These values were obtained from foliar application of humic acid with (20 cm/l) on flax plants (1.56, 0.49, 0.54 and 2.59) for chlorophyll a, chlorophyll b, carotenoids and total pigments, respectively. The increases in photosynthetic pigments content due to humic acid application are in agreement with those obtained by (Ameri and Tehranifar, 2012; Bakry *et al*., 2013; El-Bassiouny *et al*., 2014) on different plants. This positive effect of humic acid on photosynthetic pigments could be attributed to an increased in CO_2 assimilation and photosynthetic rate which increased mineral uptake by the plant (Ameri & Tehranifar, 2012). These increases may be due to that, HA probably caused an increase in the synthesis of the chlorophyll and/or delayed chlorophyll degradation in the leaves of flax plant (Bakry *et al*., 2012a).

Table-3. Effect of humic acid on photosynthetic pigments (mg/g fresh wt) and total carbohydrates polysaccharides and total soluble sugar (mg/g fresh wt) as mg/g dry wt of flax plant.

Humic acid (mg/l)	**Chlorophyll a**	**Chlorophyll b**	**Carotenoids**	**Total pigments**	**Total carbohydrates**	**Polysaccharides**	**Total soluble sugar**
0	1.16	0.36	0.43	1.95	295.18	286.27	8.91
20	1.56	0.49	0.54	2.59	489.90	478.85	11.05
LSD 0.05	0.22	0.08	0.03	0.24	9.06	10.89	0.50

Carbohydrate constituents

Data in Table-3 indicate that humic acid treatment caused significant increases in total carbohydrate, polysaccharides and total soluble sugars contents of flax plant. The percent of increases reached 65.97, 67.27 and 23.99% for total carbohydrate, polysaccharides and total soluble sugar, respectively as compared to control plant. Similar results to our obtained results were obtained by Bakry *et al*., 2013 and El-Bassiouny *et al*., 2014) on flax plant. The significant increases in total carbohydrates, in shoots of flax plant concomitantly with the increased growth rate led to the conclusion that the photosynthetic efficiency was increased in response to HA treatments and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of flax plants. This positive effect of HA has also been observed on the main photosynthetic metabolism in maize leaves, where a decrease in starch content was accompanied by an increase of soluble sugars (Ferrara and Brunetti, 2008). This change appeared to be mediated by variations of the activity of the main enzymes involved in carbohydrate metabolism (Merlo, *et al*., 1991).

IAA and phenolic contents

Results in Table-4 indicate that humic acid foliar application caused significant increases in IAA and phenolic contents compared with control plants. These obtained results are in good agreement with those obtained by (Bakry *et al*., 2013 and El-Bassiouny *et al*., 2014) on flax and wheat plant respectively. With regard to the stimulatory effect of humic acid on IAA content, some researchers indicated that humic acid can be used as a growth regulator to regulate hormone level, (Serenella, *et al*., 2002). In addition application of HA inhibit indoleacetic acid (IAA) oxidase, thereby hindering destruction of this plant growth hormone (Mato *et al*., 1972). With respect to the increases in phenol contents these increase may be due to total phenols role that play a significant mechanism in regulation of plant metabolic processes and consequently overall plant growth (Lewis and Yamamoto, 1990).

Table-4. Effect of humic acid on IAA, phenolic, free amino acids, proline and lipid peroxidation of flax plant.

Humic acid (cm/l)	**IAA** (mg/100g fresh wt)	**Phenol** (mg/100g fresh wt)	**Free amino acids** (mg/100g dry wt)	**Proline** (µg/100g dry wt)	**Lipid peroxidation** (µg/100g dry wt)
0	196.94	23.12	82.18	17.08	14.62
20	284.61	33.77	141.67	23.73	10.09
LSD $_{0.05}$	43.27	4.05	27.57	2.69	1.23

Free amino acid, proline and lipid peroxidation

Data in Table-4 indicate that humic acid foliar application caused significant increases in free amino acids, proline, while caused significant decrease in lipid peroxidation compared with control. These results of free amino acids and proline content increments are in agreement with those obtained by Farahat, *et al*., (2012) and El-Bassiouny *et al*., (2014). Many functions have been postulated for proline accumulation in plant tissues, proline and free amino acids could be involved in the osmotic adjustment of plants (Delavari, *et al*., (2010)) and could also be a protective agent of enzymes and membranes (Gzik, 1996). When plant subjected to unfavorable conditions as the newly reclaimed sandy soil, plants maintain their water content by accumulation of compatible organic solutes act as osmoprotectants, as proline, in their cytoplasm (Bandurska, 1993). This means that the inhibitory effect of unfavorable conditions on flax plant was alleviated by humic acid treatments through increasing proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein. With regard to lipid peroxidation, as malondialdehyde (MDA) contents the obtained data are in harmony with those obtained by Bakry *et al*., (2013) on flax plants. Higher level of this substance is found in plants subjected to high levels of oxidative stress. So any stress is known to damage the cell, and alter most of its components and functions (Harinasut, *et al*., 2000; Zhang and Kirkham, 1996). The reduction in MDA contents caused by humic acid treatment may be as a result of their role in removing the stress of peroxidative, as they affecting the antioxidant enzymes and lipids peroxidation, would protect flax plant against stress.

Effect of Humic acid on yield components and oil yield

Data in Table-5 indicate that, humic acid treatment increased significantly all yield and their related traits except technical length. Humic acid with the rate of (20 cm/l) gave the highest values of seed, straw and oil yields/fed. This superiority may be due to the increases in plant height, fruiting zone length, the number of fruiting branches/plant, number of capsules/ plant and seed yield/ plant. Moreover, the superiority in oil yield/fed may be attributed to the increase in seed yield/fed and the increase in seed oil %. The trend of these results are supported by of (Bakry *et al*., 2012; Unlu, *et al*., 2011; Shehata *et al*., 2012; Shafeek, *et al*., 2013 and Abdel-Razzak and El-Sharkawy, 2013). The effect of humic acid on flax yield components could be attributed to presence of plant growth regulators, which are produced by increased activity of microbes such as fungi, bacteria, yeasts, actinomycetes and algae (Chris *et al*., 2005). It could be summarized that humic acid caused an increment in total seeds yield and caused an enhancement in some physical properties of pods.

Table-5. Effect of Humic acid on yield and yield components and oil yield of flax plant grown under newly reclaimed sandy soil: Combined analysis of 2012/2013 and 2013/2014 seasons).

Humic acid (mg/l)	**Plant height** (cm)	**Technical length** (cm)	**Fruiting zone length** (cm)	**Number of fruiting branches /plant**	**No. of capsules /plant**	**Bio. yield/ plant** (g)	**Seed yield/ plant** (g)	**Straw yield/** (ton/fed)	**Seed yield/** (kg/fed)	**Oil %**	**Oil yield** (kg/fed)
0	59.50	49.05	10.45	3.73	12.96	1.77	0.29	3.07	431.30	34.40	151.88
20	62.88	47.36	15.52	5.26	14.67	2.60	0.38	3.89	665.77	38.76	258.62
LSD $_{0.05}$	2.11	1.63	3.15	1.12	1.31	0.35	0.02	0.31	50.33	2.21	4.23

Effect of sulfur

Effect of Sulfur on morphological characters

Data presented in Table-6 revealed that high significant difference among all sulfur fertilizer levels on all morphological characters. Sulfur fertilizer at rate of (500 kg/fed) gave the highest values of shoot and root length, fresh weight of shoot and root and dry weight of shoot and root compared with control plant. These results are in agreement with those obtained by El-Sheikh *et al*., (2006) on grapes and Chandra and Pandey (2014) on wheat plant and Ibrahim and Naz (2014) on black gram plant. The simulative effect of sulfur addition to soil is due to that the elemental sulfur might be oxidized to sulphuric acid by sulfur-oxidizing bacterium (Thiobacillus sp.) resulting in lowering the pH value of the soil. This acidification of the soil may increase the solubility of phosphorus and micronutrients originally present in the soil and those applied as fertilizers (Ryan and Stroehlein, 1979 and Sadiq, 1985). Also, the enhancement effect of sulphur on growth characters was due to stimulatory effects of sulphur on cell division which helped in leaf expansion and increased light interception improved the vegetative growth (Anitha, *et al*., 2004).

Table-6. Effect of sulfur on morphological characters of flax plant under newly reclaimed sandy soil. Combined analysis of 2012/2013 and 2013/2014 seasons).

Sulfur (kg/fed)	**Plant height** (cm)	**Fresh weight** (g)	**Shoot dry weight** (g)	**Root length** (cm)	**Root fresh weight** (g)	**Root dry weight** (g)
control	39.00	2.04	0.49	10.67	0.34	0.17
250	41.50	2.89	0.75	12.33	0.44	0.24
500	43.33	3.00	0.94	13.00	0.58	0.28
LSD $_{0.05}$	0.51	0.07	0.18	0.47	0.03	0.02

Effect of sulfur on biochemical characters

Photosynthetic pigments

Data presented in Table-7 indicated that there is significant increase in photosynthetic pigments with increasing sulfur fertilizer level in flax leaves under newly reclaimed sandy soil. Sulfur with (500 kg/fed) gave the highest values of chlorophyll a, chlorophyll, b, and total pigments compared with the other treatments. While, Sulfur with (250 kg/fed) gave the highest value of carotenoids. These results are in agreement with this obtained by (Hawkesford, 2000; Lunde, 2008; Osman ans Rady, 2012 and Chandra and Pandey, N., 2014), they found that, sulfur deficiency negatively affects the chlorophyll and N contents of leaves and photosynthetic enzymes and consequently the reduction in yields and quality parameters of crops. Increased leaf chlorophyll contents of flax by sulphur application were due to its role in chlorophyll synthesis (Marschner, 1995 and Anitha, *et al*., 2004) which might delay the leaf senescence Marschner, 1995 and Iqbal, 1988).

Table-7. Effect of sulfur on photosynthetic pigments (mg/g fresh wt) and total carbohydrates polysaccharides and total soluble sugar (mg/g fresh wt) as mg/g dry wt of flax plant.

Sulfur (kg/fed)	**Chlorophyll a**	**Chlorophyll b**	**Carotenoids**	**Total pigments**	**Total carbohydrates**	**Polysaccharides**	**Total soluble sugar**
control	1.027	0.302	0.347	1.676	249.72	241.60	8.11
250	1.415	0.469	0.577	2.460	415.69	405.31	10.37
500	1.647	0.519	0.534	2.700	512.22	500.76	11.45
LSD $_{0.05}$	0.105	0.031	0.032	0.135	10.88	20.05	0.33

Total carbohydrate, polysaccharides and total soluble sugar

Results presented in Table-7 indicated that increasing sulfur fertilizer rates increased significantly the total carbohydrate, polysaccharides and total soluble sugar compared with the control plants. Sulfur rate of (500 kg/fed) gave the highest values of carbohydrate constituents. This is may be due to positive effect of sulfur fertilizer on photosynthesis which is associated with the increase in total pigments content (Table-7), while total soluble sugar showed the same trend, since it was increased significantly and gradually with increasing sulfur levels. These results are in harmony with those reported by (Hawkesford, 2000; Osman and Rady, 2012; Bakry, *et al*., 2012a).

IAA and phenolic

Data presented in Table-8 indicate that sulphur fertilization with different levels significant increased in IAA and phenolic contents of flax plant. Sulfur rate of (500 kg/fed) gave the highest values of the above mentioned parameters.

Free amino acids, proline and lipid peroxidation

Data presented in Table-8 indicate that different concentrations of sulphur caused significant increases in free amino acids, proline contents of flax plant. Meanwhile decreases in lipid peroxidation with increasing sulfur fertilizer rate of flax plants under this trail. Sulfur rate of (500 kg/fed) gave the highest values of the above mentioned parameters, while, the control gave the highest value of lipid peroxidation. These results are in agreement with those obtained by [45, 46 & 56] Many functions have been postulated for proline accumulation in plant tissues, proline and free amino acids could be involved in the osmotic adjustment of plants (Gzik, 1996).

Table-8. Effect of humic acid on IAA, phenolic, free amino acids, proline and lipid peroxidation of flax plant grown under newly reclaimed sandy soil.

Sulfur (kg/fed)	**IAA** (mg/100g fresh wt)	**Phenol** (mg/100g fresh wt)	**Free amino acids** (mg/100g dry wt)	**Proline** (µg/100g dry wt)	**Lipid peroxide** (µg/100g dry wt)
control	194.49	20.67	72.99	15.61	15.35
250	204.10	27.35	118.13	19.80	12.78
500	323.74	37.32	150.64	25.79	8.93
LSD 0.05	5.12	6.72	12.63	3.41	2.11

Effect of sulfur fertilizer on seed, oil and straw yields and their components

Data in Table-9 show that seed, straw and oil yields and their components were significantly and gradually increased with increasing sulfur fertilizer levels application (from 250 to 500 kg/fed) compared with control plants. These increases are due to the increase in plant height (cm), technical stem length (cm), fruiting zone length (cm), number of fruiting branches/plant, number of capsules/plant, biological yield/plant (g), seed yield /plant (g), and oil %. These results are confirmed by the results of Rehman Hafeez *et al*., (2013) they found that sulfur was beneficial for newly-reclaimed soils to alleviate the adverse effects of environmental stress and to improve sustainable crop productivity (Kineber, *et al*., 2004 and Osman and Rady, 2012). Sulfur improves the chemical properties of alkaline soils as well as increasing yields and related characteristics. The increase in yield attributes by S application can be explained by its role in ferridoxin, a Fe-S protein in chloroplast (Havlin *et al*., 2004) which enhanced synthesis and maintenance of chlorophyll contents improving photosynthetic rate and assimilating efficiency. Increase in number of fruiting branches per plant, 1000-seed weight and seed yield by sulphur nutrition are also supported by (Itanna, 2005 and Rehman Hafeez *et al*., 2013).

With regard to oil content, sulphur application also improved the seed oil contents in flax plant. The increased oil with S nutrition in flax plant had been reported in canola cultivars (Iqbal, 1988 and Haneklaus *et al*., 1999) they reported that S is involved in synthesis of fatty acids and through synthesis of sulphur containing amino acids such as cysteine, cystine and methionine.

Table-9. Effect of sulfur fertilizer on yield and yield parameters of flax plant under newly reclaimed sandy soil (Combined analysis of 2013/2014 and 2014/2015seasons).

Sulfur (kg/fed)	**plant height** (cm)	**Technical length** (cm)	**Fruiting zone length** (cm)	**Number of fruiting branches /plant**	**No. of capsules /plant**	**Bio. yield/ plant** (g)	**Seed yield/ plant** (g)	**Straw yield/** (ton/fed)	**Seed yield/** (kg/fed)	**Oil %**	**Oil yield** (kg/fed)
control	59.82	49.24	10.58	3.16	11.44	1.88	0.28	3.18	425.70	34.03	150.45
250	64.83	51.58	13.26	4.70	13.84	2.30	0.35	3.39	569.50	37.16	213.44
500	58.93	43.80	15.13	5.63	16.17	2.38	0.39	3.88	650.40	38.56	251.87
$LSD_{0.05}$	0.56	1.15	1.33	1.10	1.23	0.03	0.02	0.10	15.33	1.15	6.91

Effect of interaction between humic acid and sulfur on

Morphological characters

Data in Table-10 indicated that there were synergistic significant increases of all interactions between humic acid and sulfur fertilizer levels on growth characters except shoot dry weight and root fresh and dry weight. The interaction of humic acid (20 cm/L) with sulfur at rate of (250 kg/fed) gave the tallest.

Table-10. Effect of interaction between humic acid and sulfur on morphological criteria of flax plant under newly reclaimed sandy soil.

Treatment		**Shoot**			**Root**		
Humic acid (mg/l)	**Sulfur fertilizer** (kg/fed)	**Length** (cm)	**Fresh weight** (g)	**Dry weight** (g)	**Length** (cm)	**Fresh weight** (g)	**Dry weight** (g)
0	control	38.33	1.70	0.20	10.33	0.32	0.03
	250	42.33	2.33	0.26	12.01	0.35	0.04
	500	39.67	2.86	0.26	12.33	0.34	0.04
20	control	42.67	3.06	0.31	13.67	0.30	0.03
	250	44.67	2.34	0.29	11.00	0.36	0.04
	500	42.00	2.71	0.28	11.67	0.42	0.04
LSD 0.05		1.30	1.01	n. s	1.20	ns	ns

Photosynthetic pigments

Results in Table-11 indicated that there were significant effects of all interactions between humic acid and sulfur fertilizer levels on photosynthetic pigments. The interaction between humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) gave the highest values of chlorophyll a, chlorophyll b and total pigments. These increases in the photosynthetic pigments might be due the enhancement effects of humic acid and sulfur on chlorophyll biosynthesis.

Table-11. Effect of interaction between humic acid and sulfur on photosynthetic pigments (mg/g fresh wt) and carbohydrate constituents (mg/g dry wt) of flax plant under newly reclaimed sandy soil.

Humic acid (mg/l)	**Sulfur fertilization kg/fed**	**Chlorophyll a**	**Chlorophyll b**	**Carotenoids**	**Total pigments**	**Total carbohydrates** (Mg/100g dry wt.)	**Polysaccharides** (Mg/100g dry wt.)	**Total soluble sugar** (Mg/100g dry wt.)
0	control	0.947	0.291	0.314	1.551	185.00	177.97	7.02
	250	1.107	0.314	0.380	1.800	314.44	305.24	9.20
	500	1.440	0.481	0.602	2.523	386.11	375.59	10.51
20	control	1.390	0.456	0.552	2.398	445.27	435.03	10.24
	250	1.540	0.431	0.552	2.523	455.00	443.61	11.38
	500	1.755	0.606	0.516	2.877	569.44	557.90	11.53
LSD 0.05		0.131	0.021	0.040	0.156	10.96	11.05	0.13

Total carbohydrate, total soluble sugar and polysaccharides.

Results in Table-12 indicated that there were significant effects of all interactions between humic acid and sulfur fertilizer levels on total carbohydrate, total soluble sugar and polysaccharides. The interaction between humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) was the most effective treatment as it gave the highest values of total carbohydrate, polysaccharides and total soluble sugar. These significant increases in total carbohydrates, in shoots of flax plants concomitantly with the increased growth rate led to the conclusion that the photosynthetic efficiency was increased in response to Humic acid and sulfur treatments and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of flax plants. Similar results to our obtained results were obtained by Serenella et al., (2002). This positive effect of Humic acid has also been observed on the main photosynthetic metabolism in maize leaves, where a decrease in starch content was accompanied by an increase of soluble sugars (Merlo, *et al*., 1991). This change appeared to be mediated by variations of the activity of the main enzymes involved in carbohydrate metabolism (Serenella, et al., 2002).

Table -12. Effect of interaction between humic acid and sulfur on total carbohydrate, total soluble sugar, polysaccharides and free amino acids of flax plant under newly reclaimed sandy soil.

Humic acid (cm/l)	**Sulfur fertilization kg/fed**	**Total carbohydrates** (mg/100g dry wt.)	**Polysaccharides** (mg/100g dry wt.)	**Total soluble sugar** (mg/100g dry wt.)
0	control	18500	17797	702
	250	31444	30524	920
	500	38611	37559	1051
20	control	44527	43503	1024
	250	45500	44361	1138
	500	56944	55790	1153
LSD 0.05		1096	1105	13

IAA, phenolic, free amino acids, proline and lipid peroxidation

Data in Table-13 indicated that there were significant increases of all interactions between humic acid and sulfur fertilizer levels on IAA, phenolic, free amino acids, proline. The interaction between humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) resulted in the highest values of IAA, phenolic, free amino acids, proline while gave the lowest value of lipid peroxidation compared with the other interactions.

Table-13. Effect of interaction between humic acid and sulfur on IAA, total phenol, proline and lipid peroxide of flax plant under newly reclaimed sandy soil.

Humic acid (mg/l)	**Sulfur fertilization kg/fed**	**IAA (**Mg/100g fresh wt.)	**Phenol** (Mg/100g fresh wt.)	**Free amino acids** (Mg/100g dry wt.)	**Proline** (µg/100g dry wt.)	**Lipid peroxidation** (µg/100g dry wt.)
0	control	186.77	15.44	64.33	11.61	16.39
	250	202.21	25.90	81.64	19.61	14.32
	500	201.84	28.03	100.55	20.02	13.16
20	control	206.36	26.67	135.70	19.59	12.41
	250	314.02	34.70	118.13	22.80	8.64
	500	333.46	39.94	183.16	28.79	9.22
LSD 0.05		2.37	0.27	1.25	16.82	0.42

Seed, oil and straw yields and yield parameters

Data presented in Table-14 revealed that high significant difference among all interactions between humic acid and sulfur fertilizer levels in seed, straw and oil yields and their related characters. The interaction between humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) give the highest values of seed yield (735.4 kg/fed), straw yield 4.20 ton/fed) and oil yield (292.84 kg/fed) compare with the other interactions. This superiority in seed and straw yields /fed may be due to the increases in fruiting zone length, the number of fruiting branches/plant, number of capsules / plant and biological and seed yields / plant. Moreover, the superiority in oil yield/fed may be attributed to the increase in seed yield /fed and the increase in seed oil %.

The superiority of humic acid (20 cm/L) with sulfur at rate of (500 kg/fed) over the other interactions could be attributed to the stimulatory effects of humic acid and sulfur on increasing chlorophyll concentration in leaves might be attributed to the lowering of pH value as well as increasing the activity of soil organisms to liberate more nutrients from the unavailable reserves. Such results are in agreement with these obtained by many investigators. Haneklaus *et al*., 1999 and Ferrara and Brunetti (2010) stated that, the increase in berry size as a consequence of HA-S application at full bloom is probably ascribed to the uptake of mineral nutrients by the grapevines, but the possible hormone like activity of the HA-S (i.e., auxin-, gibberellin- and cytokinin-like activity) should also be taken into consideration. HA were found to promote soil water holding capacity and reduce watering requirements for plants (MacCarthy, 2001).

Table-14. Effect of interaction between humic acid and sulfur fertilizer on seed, oil and straw yields and yield parameters of flax plant under newly reclaimed sandy soil.

Characters	**Humic acid** (mg/l)						**LSD 0.05**
	0			**20**			
	Sulfur fertilizer (kg/fed)						
	Control	**250**	**500**	**Control**	**250**	**500**	
Plant height (cm)	53.00	65.00	60.50	66.63	64.66	57.35	2.05
Technical length (cm)	44.5	54.49	48.15	53.98	48.66	39.45	1.43
Fruiting zone length (cm)	8.5	10.51	12.35	12.65	16.00	17.90	1.32
Number fruiting branches/ plant	2.75	3.85	4.6	3.57	5.55	6.65	0.22
No. of capsules /plant	11.2	13.35	14.33	11.67	14.33	18.00	0.13
Bio. yield/ plant (g)	1.55	1.85	1.92	2.21	2.75	2.83	0.06
Seed yield/ plant (g)	0.22	0.32	0.34	0.34	0.37	0.43	0.01
Straw yield (ton/fed)	2.72	2.95	3.55	3.64	3.82	4.20	0.12
Seed yield (kg/fed)	275.3	453.2	565.4	576.1	685.8	735.4	10.01
Oil %	30.30	35.60	37.30	37.75	38.72	39.82	0.33
Oil yield (kg/fed)	83.42	161.34	210.89	217.48	265.54	292.84	5.21

Fatty acid composition

Fatty acids profile was estimated without replications, therefore the results could not be statistically analyzed. Table-15 shows that oils extracted from flax plant characterized by the presence of nine fatty acids, including six saturated fatty acids (lauric, myristic, palmitic, stearic, behenic and lignoceric) and three unsaturated fatty acids (oleic, linoleic and linolenic). The predominant fatty acids were linolenic followed by oleic and linoleic. Results indicated that palmitic acid was the dominant saturated fatty acid in the oil of flax. Results also indicated that the humic acid foliar treatment increased olic acid and linolenic acid contents with variable changes of bthe other fatty acids. Humic acid treatment caused marked increases in total unsaturated fatty acid, total saturated fatty acids and total unsat/total sat ratio. Results also indicated that the sulfur fertilizer increased the levels of oleic acid and linolenic acid with the concentrations added meanwhile low concentration increased linoleic acid but high concentration decreased it. Two levels of sulfur increased total unsaturated fatty acids, total saturated fatty acid and the ratio between them. The increased oil with S nutrition in flax plant had been reported in canola cultivars (Ferrara and Brunetti, 2010, Rehman Hafeez *et al*., 2013 and Haneklaus *et al*., 1999) reported that S is involved in synthesis of fatty acids and through synthesis of sulphur containing amino acids such as cysteine, cystine and methionine. Results also indicated that the interaction between humic acid and sulfur fertilizer decreased the level of saturated fatty acid. The least saturated fatty acids content was found in the oil of plants treated with humic acid in addition to 500kg/fed sulfur fertilizer this means that this interaction improve the quality of the obtained oil. Results in (Table-15) indicated that the range of total unsaturated fatty acids increased with humic acid treatment. It increased from 81.95% to 85.08 %. Sulfur fertilizer increased unsaturated fatty acid contents of oil of the yielded seeds. The most effective treatment was humic acid in addition with 500kg/fed sulfur fertilizer. Linolenic acid was the dominant unsaturated fatty acid in the oil of flax plant different treatment increased linolenic acid contents. Results in (Table-15) also indicated oleic acid was the another poly unsaturated fatty acid which increased by the different treatments of humic acid and sulfur fertilizer. These results are in general in accordance with Chris *et al*., (2005), Shehata and El-Khawas (2003). MacCarthy *et al*., (2001) and Bakry *et al*., (2013).

Table-15. Effect of interaction between humic acid and sulfur fertilizer on fatty acid composition of flax plant under newly reclaimed sandy soil.

Humic acid (mg/l)	**0**			**20**		
Sulfur (kg/fed)	**Control**	**250**	**500**	**0**	**250**	**500**
Lauric (C12:0)	0.69	0.27	0.27	1.16	1.51	1.12
Myristic (C14:0)	1.43	1.47	1.48	1.31	1.60	1.18
Palmitic (C16:0)	5.94	6.13	6.23	5.84	5.33	5.26
Stearic (C18:0)	3.95	3.69	4.06	3.27	2.26	2.12
Oleic (C18:1)	30.07	31.62	32.29	33.00	34.06	35.88
Linoleic (C18:2)	3.22	4.24	2.25	2.58	1.19	0.96
Linolenic (C18:3)	48.66	49.04	49.43	49.50	50.05	50.19
Beheric (C22:0)	0.49	1.02	0.05	1.19	0.69	0.65
Lignocenic (C24:0)	1.17	1.35	1.22	1.11	0.61	1.24
Total unsaturated	81.95	84.90	83.97	85.08	85.30	87.04
Total saturated	13.66	13.93	13.31	13.88	11.99	11.57
Total idetified	95.61	98.83	97.28	98.96	97.29	98.60
T Uns./TS	6.00	6.10	6.31	6.13	7.12	7.53

CONCLUSIONS

The obtained results revealed that foliar spraying flax plant with humic acid and/or sulfur fertilizer are very beneficial to crop growth and yield. It is worthy to mention that, flax plants grown under newly reclaimed sandy soil and similar growing conditions and sulfur fertilizer and/or foliar sprayed with humic acid at 20 mg/l to produce high quantity and good quality of flax seed yield and oil yield and fatty acid profile of the seed yield. Data presented that the increase of seed, oil and straw yields per feddan of flax plant under the conditions of this experiment by increasing sulfur fertilizer and humic acid levels caused gradual significant increases in the studied growth characters, increased photosynthetic pigment (chlorophyll a, chlorophyll b, carotenoids, consequently total pigments, total carbohydrates, polysaccharides, total soluble sugar, IAA, phenolic, free amino acids, proline and content of shoots, increases in values of total unsaturated fatty acids and yield components of the flax Opal cultivar compared with control. The data indicated that the best seed and straw yields were obtained by planting flax seeds and used (500kg/fed) from sulfur fertilizer with foliar sparing of (20 mg/L) from humic acid,

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