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Some Agro-Physiological Studies of Stigmasterol on Growth and Productivity of Some Flax Cultivars under Sandy Soil Conditions

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Abstract: Stigmasterol is the precursor of numerous secondary metabolites, including plant steroid hormones (Brassinosteroids) and plays an important role in plant growth and development. Therefore, two field experiments were carried out at the Experimental Station of National Research Centre, Al Nubaria district El-Behira Governorate-Egypt, in two successive winter seasons 2017/2018 and 2018/2019. The study aimed to evaluate the effect of foliar application of stigmasterol at rate of (0, 100, 200 & 300 mg/l) on growth, productivity and some biochemical aspects of seven cultivars of flax. Data show significant varietal differences among the tested cultivars (Sakha 1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Giza 11 and Amon) in growth, photosynthetic pigments, indole acetic acids (IAA), proline, free amino acids, yield and its components. Results clearly show the superiority of cultivar Sakha 6 in number of capsules/plant, seed yield/plant (g), seed yield (kg/fed) and oil yield (ton/fed), meanwhile Sakha 5 cultivar was superior in biological yield and straw yield/plant (g), biological yield and straw yield (ton/fed). The obtained data also show the enhancing role of stigmasterol treatment on growth and yield of the tested cultivars through improving different physiological aspects (photosynthetic pigments, IAA, proline, free amino acids) as well as, enhancing yield and its attributes. Flax oil%, carbohydrates%, flavonoids and phenolic contents as well as antioxidant activity expressed as (DPPH %) were promoted due to stigmasterol treatment. Stigmasterol at 200 mg/l mg/l recorded the highest values of plant height, fruiting zone length, technical stem length, number of fruiting branches, biological yield /plant (g), straw yield / plant (g) biological and straw yield (ton/fed). The chemical constituents carbohydrate%, oil%, flavonoids, phenolic contents and DPPH% in seeds were increased as a result of stigmasterol at 200 mg/l. Meanwhile, 300 mg/l stigmasterol treatment gave the highest number of capsules/plant, seed yield/plant, seed yield (kg/fed), biological yield (ton /fed) and oil yield (ton/fed). The interaction between cultivars and different concentrations of stigmasterol showed that 300 mg/l stigmasterol was the most effective treatment on Sakha 5 cultivar followed by Sakha 6 cultivar as it produced the highest increases in seed yield (610 kg/fed and 608.96 kg/fed respectively).

Key words: Flax · Stigmasterol · Osmoprotectants · Sandy soil · Seed yield · Oil

INTRODUCTION

One of dicotyledonous plant belongs to family Linaceae is linseed or flax (*Linum usitatissimum* L.) plant. Flax is grown in Egypt as a dual purpose plant (seeds and fibers). It is an important fibers and industrial oils source. Moreover, flax seeds has the potential of meeting edible oil and protein deficiencies [1]. Flax oil have a mixture of different fatty acids, it contain high amount of polyunsaturated fatty acids, especially omega 3 and omega 6 essential fatty acid (α- linolenic acid and linoleic acid). Omega 3 and omega 6 fatty acids play an important role in brain function and normal growth and development of humans [2, 3]. Flax plant is a winter plant

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so, its productivity is limited due to the competition with other economic winter plants resulting in a gap between production and consumption. So, it is important to increase flax production via using different strategies, such as expanding in flax cultivation in sandy soil and using the high yielding cultivars or using natural compounds as plant growth regulators, antioxidants, amino acids, vitamins ect to enhance its growth and yield under such soils

Brassinosteroids is an important hormone in plant cells as well as in animal cells. Brassinolide (BL) is a bioactive form of brassinosteroids (BRs) [4]. BRs are a class of polyhydroxylated steroidal phytohormone in plant and known as the sixth plant hormone after auxin, gibberellins, cytokinin, abscisic acid and ethylene [5]. Stigmasterol (StS) is a structural component of the lipid core of cell membranes and it is the precursor of numerous secondary metabolites, including plant steroid hormones, or as carriers in acyl, sugar and protein transport [6]. Moreover, stigmasterols have an important role in plant growth and development via regulating different physiological and biochemical processes as seed germination, plant growth, stomatal and vascular differentiation, cell division, cell elongation, root growth and photomorphogenetic process [3, 7] as well as, BRs play a crucial roles in free radical oxidation, biosynthesis of ethylene and root gravitropic response. Moreover, BRs mediate plant response to various stresses [8]. In this regard, Hashem *et al*. [9] found that soaking flax seeds in StS (200 ppm) treatment increased growth, endogenous hormones, yield and its components of flax plant. Khalil and Madany [10] stated that stigmasterols enhanced growth and some biochemical aspects of maize plant. In addition, El-Tantawy and Azoz [11] found that stigmasterol application improved growth and productivity of basil plant.

So, the objective of this investigation was to study the role of foliar treatment of stigmasterol on growth, some biochemical aspects and yield of some cultivars of flax plants grown under sandy soil conditions.

MATERIALS AND METHODS

A field experiments were carried out at Researches and Production Station of National Research Centre, Al Nubaria district El-Behira Governorate-Egypt, in 2017/2018 and 2018/2019 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 [12].

The experimental design was split plot design with three replicates, where the flax cultivars (Sakha 1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Giza 11 and Amon) occupied the main plots, while, concentrations of stigmasterols (0, 100, 200 and 300 mg/l) were allocated in the sub plots. Flax seed cultivars were sown on mid of November on both seasons 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 applied. 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 different levels of stigmasterol (0, 100, 200 and 300 mg/l) were carried out twice; where plants were treated after 30 and 45 days from sowing. Plant samples were taken after 60 days from

Table 1: Mechanical, chemical and nutritional analysis of the experimental soil

	Sand											
Mechanical analysis	Course 2000-200µ%			Fine 200-20µ %			Silt 20-0µ %			Clay < 2µ %		Soil texture
	47.46			36.19			12.86			4.28		Sandy
					Soluble cations (meq/l)				Soluble anions (meq/l)			
Chemical analysis	pH1:2.5	ECdSm^{-1}	$CaCO_3$%	OM%	Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
	8.25	0.11	0.9	0.9	0.7	0.02	0.1	0.3	0.0	0.2	0.8	0.12
	Available nutrients											
	Macro element (ppm)				Micro element (ppm)							
Nutritional analysis	N	P	K		Zn		Fe		Mn		Cu	
	12.9	3.6	52.9		0.12		1.98		0.46		0.06	

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. 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) as well as carbohydrates%, flavonoids, phenolics contents and DPPH activities of the yielded seeds.

Chemical Analysis: Photosynthetic pigments: Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*) and carotenoids were determined using spectrophotometric method described by [13]. Total phenol content was measured as described by [14]. Indole acetic acid content were extracted and analysed by the method of [15]. Proline and free amino acids were extracted as describes by [16] and assayed according to [17]. Free amino acids were estimated according to [18]. Determination of total carbohydrates of seeds was carried out according to [19]. Oil of linseed seeds were extracted according to [20]. Total flavonoids were determined using the method reported by [21]. The antioxidant activity (DPPH radical scavenging) was determined using the method of [22].

Statistical Analysis: The data were subjected to statistical analysis of variance of split plot design according to [23] Since the trend was similar in both seasons the homogeneity test Bartlet's equation was applied and the combined analysis of the two seasons was done according to the method. Means were compared by using least significant difference (LSD) at 1 and 5%.

RESULTS

Effect of Varietal Differences:

Changes in Growth Parameters: Shoot & root length (cm), fresh & dry weight of shoot and root (g), of seven flax cultivars are presented in Table 2. Data of growth parameters clearly show marked and significant differences among the seven flax cultivars tested, Sakha 1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Giza 11 and Amon in most growth criteria. Sakha 6 and Sakha 5 flax cultivars surpassed the other cultivars in shoot length, shoot fresh and dry weight and root length. Meanwhile Sakha 2 was the least growth parameters of all the tested flax cultivars.

Changes in Photosynthetic Pigments: Data presented in Table 2 demonstrated the effect of flax cultivars on photosynthetic pigments contents (*Chl* a, *Chl* b, carotenoids and total pigments). Data clearly show that there were significant differences ($P \geq 0.05 \geq$ and 0.01) among flax cultivars (Sakha 1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Giza 11 and Amon) on photosynthetic pigments contents. Sakha 6 surpassed the other cultivars in photosynthetic pigments constituents where Sakha 6 cultivar gave the highest values of pigmentation (*Chl a,* b and total pigments followed by Sakha 5 while the lowest total photosynthetic pigments was recorded by Giza 11 and Sakha 3 cultivars.

Changes in Phenols, IAA, Proline and Free Amino Acids: From the same Table the data showed that, there were significant and/or highly differences among flax tested seven cultivars in phenolic, IAA, proline and free amino acids contents. With the exception for the difference between the cultivars Giza 11 and Amon in phenolic content, Sakha 1 and 2 in proline contents and Sakha 2 & 5 in free amino acids contents the differences were non-significant. Sakha 6 surpassed the other flaxseed cultivars in all above mention studied characters.

Changes in Yield and its Components: Regarding to yield and its components (Table 2) the results show the effect varietal differences in plant height, fruiting zone and technical stem length, number of fruiting branches and capsules/plant, seed, biological and straw yield /plant, straw and biological yield (ton/fed), seed yield (kg/fed) and oil yield (ton/fed) of the seven tested flax cultivars. Data clearly show the presence of marked differences among various cultivars in yield and yield parameters. Sakha 6 cultivar surpassed the other tested cultivars in seed yield / plant (0.59 g) Seed yield (444.38 kg/fed) and oil yield (146.02 kg/fed), meanwhile Sakha 5 was superior in biological, straw yield/plant (1.73 g and 1.15 g) and straw and biological yield (3.39 & 3.82 ton/fed).

Table 2: Effect of varietal differences on growth parameters, biochemical constituents, yield, its components and nutritional value, antioxidant compounds and antioxidant activity in seeds (Data are means of two seasons)

Characters		Sakha-1	Sakha-2	Sakha-3	Sakha-5	Sakha-6	Giza-11	Amon	LSD 0.05	LSD 0.01
Growth parameters:										
Shoot length (cm)		55.00	50.33	60.92	61.17	61.17	60.33	51.75	1.40	1.96
Shoot fresh wt. (g)		3.60	2.92	4.02	5.57	5.53	4.24	5.12	0.18	0.25
Shoot dry wt. (g)		0.78	0.64	0.91	1.01	1.07	0.88	0.84	0.05	0.07
Root length (cm)		12.75	10.83	12.50	12.08	12.83	11.17	10.58	0.89	1.25
Root Fresh wt. (g)		0.43	0.32	0.54	0.76	0.47	0.54	0.51	0.03	0.05
Root dry wt. (g)		0.14	0.10	0.16	0.21	0.17	0.15	0.12	0.04	0.06
Chemical constituents:										
Chlorophyll a	mg/g fresh wt	1.10	1.56	1.06	1.67	1.99	0.97	1.52	0.01	0.02
Chlorophyll b		0.72	0.72	0.60	0.88	0.94	0.66	0.93	0.02	0.03
Carotenoids		0.31	0.36	0.38	0.61	0.62	0.37	0.53	0.01	0.02
Total pigments		2.13	2.65	2.03	3.16	3.55	2.00	2.98	0.02	0.03
Phenol	mg/100 g fresh wt	44.81	52.11	48.94	60.65	70.62	41.17	41.06	0.50	0.70
IAA		45.02	51.58	46.29	53.78	60.63	42.84	44.98	0.45	0.63
Proline	mg/100 g dry wt	38.65	38.91	39.30	40.58	57.66	52.93	55.57	0.40	0.56
Free amino acids		344.12	458.17	396.87	447.07	519.48	413.81	434.45	14.95	20.96
Yield and yield components:										
Plant height (cm)		73.42	59.00	76.75	82.67	79.25	75.25	74.33	3.82	5.36
fruiting zone length (cm)		24.92	18.58	21.17	26.08	27.17	26.92	24.00	1.67	2.35
Technical stem length (cm)		48.50	40.42	55.58	56.58	52.08	48.33	50.33	3.43	4.81
No of fruiting branches/ plant		4.50	4.50	3.83	4.42	5.42	4.75	5.00	0.35	0.49
No. of capsules/ plant		15.42	13.50	14.83	19.17	23.08	13.67	22.83	3.19	4.47
Seed yield/ plant (g)		0.57	0.54	0.55	0.58	0.59	0.47	0.55	0.03	0.05
Biological yield /plant (g)		1.61	1.16	1.24	1.73	1.53	1.37	1.59	0.13	0.19
Straw yield/ plant (g)		1.04	0.61	0.69	1.15	0.93	0.90	1.04	0.14	0.20
Straw yield (ton/fed)		2.90	2.68	3.04	3.39	3.17	2.87	2.60	0.03	0.04
Seed yield (kg/fed)		428.12	402.75	412.92	436.67	444.38	353.96	413.49	24.65	34.56
Biological yield (ton/fed)		3.33	3.09	3.45	3.82	3.62	3.22	3.01	0.04	0.05
Oil yield (ton/fed)		133.66	142.52	137.49	139.75	146.02	126.88	127.61	8.63	12.10
Chemical constituents:										
Oil %		30.96	35.24	33.09	31.79	32.75	35.40	30.45	0.25	0.35
Total carbohydrate %		32.48	32.28	32.97	32.41	32.96	31.92	30.48	0.29	0.41
Flavonoids (mg/100 g dry wt)		43.93	45.77	32.60	38.30	47.15	12.27	46.81	2.94	4.12
Phenolics (mg/100 g dry wt)		175.67	191.92	191.96	198.24	198.45	62.53	179.41	14.40	20.19
DPPH (%)		59.31	57.57	41.00	48.18	55.26	46.91	58.88	0.51	0.72

Changes in Nutritional Value, Antioxidant Compounds and Antioxidant Activity in Seeds: The changes in some nutritional value (carbohydrate% and oil %), antioxidant components (flavonoids and phenolic contents) and antioxidant activity (DPPH%) in seeds are presented in Table (2). Different flax cultivars show markedly the varietal differences effect of oil%, carbohydrate%, flavonoids and phenolics contents as well as antioxidant activities (DPPH %) of the seven tested flax cultivars. Data clearly show the superiority of Giza 11 cultivar in oil%, meanwhile Sakha 6 was superior over the above tested cultivars in total carbohydrate%, flavonoids and phenolics contents, Sakha 1 gave the highest DPPH activity.

Effect of Stigmasterol Concentrations

Changes in Growth Parameters: Table (3) show the effect of stigmasterol concentrations on growth criteria of flax plant. Data clearly show that different concentrations (100, 200 and 300 mg/l) of stigmasterol significantly and gradually increased different growth criteria (shoot length, shoot fresh and dry weight, as well as root length, fresh and dry weight) of l flax plant compared with untreated control plant. The most effective concentration was 300 mg/l on all studied growth criteria as compared with the other used concentrations. It increased shoot and root length, fresh weight and dry weight by 18.55%, 115.33%, 111.76%, 20.23%, 231.81% and 111.11% respectively as compared with control plants.

Table 3: Effect of stigmasterol concentrations (0, 100, 200 & 300 mg/l) on growth parameters, biochemical constituents, yield, its components and nutritional value, antioxidant compounds and antioxidant activity in seeds (Data are means of two seasons)

Characters		Concentrations (mg/l) 0	100	200	300	LSD 0.05	LSD 0.01
Growth parameters:							
Shoot length (cm)		50.57	58.90	59.52	59.95	0.85	1.13
Shoot fresh wt. (g)		2.61	4.72	4.78	5.62	0.19	0.25
Shoot dry wt. (g)		0.51	0.92	0.99	1.08	0.04	0.06
Root length (cm)		10.38	12.05	12.38	12.48	0.70	0.93
Root Fresh wt. (g)		0.22	0.54	0.56	0.73	0.03	0.04
Root dry wt. (g)		0.09	0.14	0.17	0.19	0.03	0.04
Chemical constituent:							
Chlorophyll a	mg/g fresh wt	1.18	1.37	1.50	1.59	0.02	0.02
Chlorophyll b		0.70	0.78	0.79	0.86	0.02	0.03
Carotenoids		0.40	0.45	0.47	0.49	0.00	0.00
Total pigments		2.28	2.59	2.76	2.94	0.03	0.04
Phenol	mg/100 fresh wt	37.52	48.03	56.94	62.85	0.29	0.39
IAA		34.72	45.11	55.28	62.11	0.33	0.44
Proline	mg/100 g dry wt	32.37	42.47	52.20	57.87	0.30	0.40
Free amino acids		319.91	406.38	476.80	519.19	8.47	11.32
Yield and yield components:							
Plant height (cm)		69.52	74.67	78.38	78.38	2.69	3.60
Fruiting zone length (cm)		20.19	24.19	27.38	24.71	1.23	1.64
Technical stem length (cm)		49.33	50.46	51.00	53.67	3.23	4.32
No. of fruiting branches/ plant		4.10	5.00	5.05	5.38	0.29	0.39
No. of capsules/ plant		11.43	18.38	18.86	23.33	1.30	1.74
Seed yield/ plant (g)		0.32	0.52	0.63	0.73	0.02	0.03
Biological yield /plant (g)		1.13	1.49	1.56	1.66	0.12	0.16
Straw yield/ plant (g)		0.80	0.97	1.03	1.13	0.12	0.16
Straw yield (ton/fed)		2.20	2.80	3.39	3.41	0.02	0.02
Seed yield (kg/fed)		255.32	374.73	468.16	554.52	16.50	22.05
Biological yield (ton/fed)		2.45	3.18	3.88	3.94	0.03	0.03
Oil yield (ton/fed)		79.10	122.89	159.25	183.87	5.51	7.37
Chemical constituents:							
Oil %		31.04	32.81	33.16	34.24	0.19	0.25
TCA		29.82	31.80	33.31	33.93	0.34	0.46
Flavonoids (mg/100 g dry wt.)		36.84	38.61	39.28	37.74	2.01	2.68
Phenolics (mg/100 g dry wt.)		180.29	173.53	167.14	161.43	10.02	13.40
DPPH %		46.34	51.59	56.38	55.47	0.24	0.32

Changes in Photosynthetic Pigments Contents: The effect of different stigmasterol used concentration (0, 100, 200 and 300 mg/l) on photosynthetic pigments (*Chl* a, *Chl* b, carotenoids and total pigments) are presented in Table (3). Different concentrations increased gradually and significantly photosynthetic pigments constituents as compared with untreated control plants. Foliar spray with 300 mg/l was the most effective concentration over the other used concentrations (100 and 200 mg/l).

Changes in Phenols, IAA, Proline and Free Amino Acids: Table (3) show the effect of different concentrations of stigmasterol on phenols, IAA, proline and free amino acids of flax plant. Treatment of flax plant with different concentrations (100, 200 & 300 mg/l) increased significantly the above mentioned studied parameters as compared with untreated control plants. Data also show that, increasing concentrations caused gradual increases in IAA, proline and free amino acids with increasing foliar applied stigmasterol concentrations. Moreover, the concentration of 300 mg/l gave the highest increases in different studied biochemical contents.

Changes in Yield and its Components: Yield and its components of flax plant treated with different concentrations of stigmasterol are presented in Table (3). Data show that foliar treatment of flax plant with different concentrations increased significantly different yield and its components (plant height, fruiting zone and technical

stem length, number of fruiting branches and capsules/plant, seed, biological and straw yields /plant, straw and biological yields (ton/fed), seed yield (kg/fed) and oil yield (ton/fed) as compared with untreated controls. 300 mg/l was the most effective concentration on all the studied parameters. It increased straw yield (ton/fed), seed yield (kg/fed), biological yield (ton/fed) and oil yield (ton/fed) by 55%, 117%, 60% and 10% respectively as compared with those control plants.

Changes in Nutritional Value, Antioxidant Compounds and Antioxidant Activity: The changes in some nutritional value (carbohydrate% and oil %), antioxidant components (flavonoids and phenolic contents) and antioxidant activity (DPPH%) as affected by different concentrations of stigmasterol are presented in Table (3). Different stigmasterol concentrations (100, 200 & 300 mg/l) significantly and gradually increased oil%, carbohydrate%, flavonoids and phenolics contents as well as antioxidant activities (DPPH %) as compared with untreated control. Data clearly show the superiority of 300 mg/l application of stigmasterol as compared with other treatments.

Interaction Effects

Changes in Growth Parameters: The effect of foliar treatment of stigmasterols with different concentrations (0, 100, 200 & 300 mg/l) on growth parameters of seven flax cultivars are presented in Table (4). Data clearly show that, foliar spraying of different concentrations of stigmasterols increased significantly most studied growth parameters of the seven tested cultivars as compared with untreated controls. Foliar spraying of flax with 300 mg/l was the most effective concentrations which increased different studied parameters. With the exception of, shoot length in Sakha 2 & Sakha 3, shoot fresh and dry weight of Giza 11, root length of Sakha 3, Sakha 6 and Giza 11 and root dry weight of Sakha 2 the most effective concentration was 200 mg/l.

Changes in Photosynthetic Pigments: The changes in pigmentation of different cultivars of flax plants in response to different stigmasterol concentrations are presented in Table (5). Data clearly show that, foliar treatment of stigmasterol with different concentrations (100, 200 & 300 mg/l) caused significant increases in photosynthetic pigment constituents (*Chl* a, *Chl* b, caroteinoids and total pigments) as compared with untreated controls. Increasing stigmasterols concentrations increased gradually different constituents of photosynthetic pigments of the seven studied cultivars (Table 5). The most effective treatment was 300 mg/l as it caused the greatest contents of different photosynthetic pigments (*Chl a, Chl b,* carotenoids nd total pigments).

Changes in Phenols, IAA, Proline and Free Amino Acids: Data in Table (6) show the interaction effect of different concentrations of stigmasterols and flax cultivars on phenols, IAA, proline and free amino acids. Foliar treatment with different concentrations of stigmasterols increased significantly and gradually the above mentioned parameters as compared with their corresponding untreated control plants. Data also show that, increasing concentrations caused increases in phenols, IAA, total soluble protein (TSP), proline and free amino acids. Data show that, 300 mg/l was the most effective concentration on increasing phenol, IAA, proline and free amino acids contents of the seven tested cultivars as compared with other concentrations. Except Sakha 1 cultivar application of 200 mg/l was the most effective treatment on free amino acids content (Table 6).

Changes in Yield and Yield Components: Foliar treatment of stigmasterol with different concentrations (100, 200 and 300 mg/l) caused significant and gradual increases in yield and its components (plant height, fruiting zone length, technical stem length, number of fruiting branches and capsules/plant and seed, biological and straw yield g /plant, straw, seed, biological and oil yield /fed) of different cultivars of flax plant grown under sandy soil conditions (Table 7 & 8). Data clearly show the superiority of cultivar Sakha 5 followed by Sakha 6 over the rest of cultivars with 300 mg/l foliar treatment stigmasterol both cultivars gave 0.81 seed yield (g/plant), meanwhile, seed yield 610.00 and 608.96 kg/fed and oil yield kg/fed were 198.27 & 200.47, of Sakha 5 and Sakha 6 respectively.

Changes in Oil%, Total Carbohydrate%, Flavonoids, Phenolic Contents and DPPH Activity (%): The changes of nutritional contents (oil% and carbohydrate%), antioxidant compounds (flavonoids and phenolic contents) as well as antioxidant activities as (DPPH%) in seeds of the seven tested flax cultivar in response to foliar treatment of stigmasterol with different concentrations (100, 200 & 300 mg/l) are presented in Table (9). Data clearly show the promotive effect of different stigmasterol concentrations in increasing significantly the above mentioned parameters as compared with their corresponding untreated controls.

Table 4: Effect of interaction between cultivars and different concentrations (0, 100, 200 & 300 mg/l) of stigmasterols on growth parameters of flax plants grown under sandy soil:

Cultivars	Conc.	Shoot length (cm)	Shoot fresh wt. (g)	Shoot dry wt. (g)	Root length (cm)	Root Fresh wt. (g)	Root dry wt. (g)
Sakha-1	0	50.67	1.90	0.41	11.33	0.17	0.05
	100	52.67	2.82	0.76	13.00	0.34	0.12
	200	58.00	4.29	0.96	13.33	0.45	0.13
	300	58.67	5.39	0.98	13.33	0.75	0.25
Sakha-2	0	47.00	1.50	0.28	7.67	0.11	0.04
	100	49.00	3.13	0.74	10.67	0.27	0.08
	200	56.00	3.39	0.77	12.33	0.44	0.14
	300	49.33	3.67	0.79	12.67	0.47	0.13
Sakha-3	0	56.00	2.06	0.64	11.33	0.23	0.12
	100	59.67	3.30	0.69	12.67	0.46	0.13
	200	64.00	3.95	0.85	13.00	0.51	0.15
	300	64.00	6.78	1.47	13.00	0.97	0.25
Sakha-5	0	49.33	1.94	0.35	8.33	0.11	0.03
	100	64.00	6.06	0.92	11.00	0.86	0.21
	200	64.33	6.06	1.19	12.67	0.88	0.26
	300	67.00	7.50	1.57	16.00	1.19	0.32
Sakha-6	0	55.67	4.45	0.80	11.67	0.15	0.10
	100	60.00	5.67	1.10	12.00	0.43	0.11
	200	64.00	5.78	1.19	14.33	0.43	0.20
	300	64.33	6.20	1.20	13.33	0.83	0.26
Giza-11	0	53.67	2.21	0.49	9.00	0.33	0.10
	100	61.67	4.48	0.82	9.67	0.45	0.12
	200	62.67	6.40	1.50	13.33	0.53	0.14
	300	63.33	3.98	0.72	12.67	0.86	0.22
Amon	0	41.67	4.20	0.63	10.00	0.36	0.08
	100	52.33	4.65	0.89	11.00	0.46	0.10
	200	53.00	5.37	0.89	11.00	0.48	0.12
	300	60.00	6.27	1.23	11.33	0.75	0.17
LSD 0.05		2.24	0.49	0.11	1.84	0.07	0.09
LSD 0.01		2.99	0.66	0.15	2.46	0.10	0.12

Table 5: Effect of interaction between cultivars and different concentrations (0, 100, 200 & 300 mg/l) of stigmasterols on photosynthetic pigments (mg/g fresh wt) of flax plants grown under sandy soil

Cultivars	Conc.	*Chl* a	*Chl* b	Carotenoids	Total pigments
Sakha-1	0	0.93	0.61	0.29	1.83
	100	1.02	0.72	0.30	2.04
	200	1.19	0.74	0.32	2.25
	300	1.26	0.81	0.34	2.41
Sakha-2	0	1.22	0.65	0.32	2.19
	100	1.55	0.70	0.35	2.61
	200	1.67	0.77	0.38	2.82
	300	1.79	0.78	0.39	2.96
Sakha-3	0	0.88	0.55	0.33	1.76
	100	1.06	0.59	0.37	2.02
	200	1.12	0.62	0.40	2.14
	300	1.18	0.64	0.41	2.22
Sakha-5	0	1.55	0.86	0.57	2.98
	100	1.68	0.92	0.63	3.23
	200	1.68	0.97	0.63	3.29
	300	1.77	0.97	0.64	3.38
Sakha-6	0	1.65	0.86	0.53	3.04
	100	1.98	0.91	0.61	3.50
	200	2.10	0.96	0.65	3.71
	300	2.22	0.99	0.66	3.87
Giza-11	0	0.80	0.55	0.31	1.67
	100	0.89	0.66	0.37	1.92
	200	1.07	0.68	0.38	2.12
	300	1.13	0.75	0.42	2.30
Amon	0	1.26	0.79	0.44	2.49
	100	1.40	0.94	0.52	2.86
	200	1.66	0.97	0.54	3.17
	300	1.77	1.06	0.59	3.43
LSD 0.05		0.04	0.05	0.01	0.07
LSD 0.01		0.06	0.07	0.01	0.10

Table 6: Effect of interaction between cultivars and different concentrations (0, 100, 200 & 300 mg/l) of stigmasterols on phenol, IAA (mg/100 g fresh wt), proline and free amino acids (mg/100 g dry wt) of flax plant grown under sandy soil

Cultivars	Conc.	Phenol	IAA	Proline	Free amino acids
Sakha-1	0	35.14	29.77	25.55	279.54
	100	43.97	39.90	34.25	325.38
	200	47.12	49.92	42.85	387.11
	300	53.00	60.51	51.94	384.45
Sakha-2	0	41.35	34.74	26.21	335.49
	100	47.27	43.92	33.13	424.16
	200	58.16	60.76	45.84	486.86
	300	61.66	66.90	50.48	586.17
Sakha-3	0	32.72	29.34	28.78	328.89
	100	43.25	43.50	35.61	385.65
	200	52.90	54.57	41.87	418.86
	300	66.90	57.74	50.94	454.08
Sakha-5	0	43.70	41.48	31.29	283.39
	100	57.86	51.62	38.95	420.11
	200	68.93	56.62	42.72	527.08
	300	72.10	65.42	49.36	557.69
Sakha-6	0	53.36	44.41	36.55	400.59
	100	68.25	54.94	54.19	498.58
	200	79.17	64.59	67.98	546.88
	300	81.70	78.59	71.93	631.87
Giza-11	0	27.65	29.32	36.22	283.19
	100	36.73	38.40	47.44	370.89
	200	48.42	50.09	61.88	483.76
	300	51.90	53.57	66.18	517.42
Amon	0	28.76	33.99	41.99	328.27
	100	38.90	43.47	53.70	419.88
	200	43.90	50.42	62.29	487.03
	300	52.70	52.04	64.29	502.62
LSD 0.05		0.78	0.86	0.79	22.40
LSD 0.01		1.04	1.15	1.05	29.95

Table 7: Effect of interaction between cultivars and different concentrations (0.0, 100, 200 & 300 mg/l) of stigmasterols on yield and yield components of flax plant grown under sandy soil

Cultivars	Conc.	Plant height (cm)	Fruiting zone length (cm)	Technical stem length (cm)	No. of fruiting branches/ plant	No. of capsules/ plant
Sakha-1	0	67.67	21.67	46.00	4.33	12.67
	100	73.00	24.33	48.67	4.33	12.67
	200	75.67	26.00	49.67	4.67	20.33
	300	77.33	27.67	49.67	4.67	16.00
Sakha-2	0	57.00	15.67	41.33	3.33	10.67
	100	61.33	20.00	41.33	5.33	13.00
	200	63.33	20.67	42.66	5.33	16.33
	300	59.33	18.00	41.33	4.33	14.00
Sakha-3	0	67.67	20.67	47.00	3.67	11.67
	100	6.33	33.33	49.00	3.67	15.00
	200	78.00	25.67	52.33	4.00	16.33
	300	75.00	23.00	52.00	4.00	16.33
Sakha-5	0	75.33	15.67	59.67	4.00	15.00
	100	82.33	28.00	54.33	4.33	20.33
	200	85.00	25.00	60.00	4.67	21.67
	300	88.00	25.67	62.33	4.67	19.67
Sakha-6	0	67.33	18.00	49.33	4.00	14.67
	100	81.00	28.67	52.33	6.33	25.67
	200	86.00	29.33	56.67	6.33	27.33
	300	82.67	32.67	50.00	5.00	24.67
Giza-11	0	66.00	19.33	46.67	4.67	6.33
	100	78.67	24.00	54.67	5.00	14.67
	200	82.67	28.67	54.00	5.33	18.67
	300	73.67	25.67	48.00	5.00	15.00
Amon	0	65.67	20.33	45.00	4.00	9.00
	100	77.00	25.33	51.67	6.33	26.33
	200	78.00	27.00	51.00		
	300	76.67	23.33	53.33	5.00	22.67
LSD 0.05		7.12	3.25	8.56	0.76	3.45
LSD 0.01		9.52	4.34	11.44	1.02	4.61

Table 8: Effect of interaction between cultivars and different concentrations (0, 100, 200 & 300 mg/l) of stigmasterols on yield and yield components of linseed plant grown under sandy soil

Cultivars	Conc.	Seed yield/ plant (g)	Biological yield /plant (g)	Straw yield/ plant (g)	Straw yield (ton/fed)	Seed yield (kg/fed)	Biological yield (ton/fed)	Oil yield (ton/fed)
Sakha-1	0	0.29	1.28	0.98	2.23	305.39	2.54	86.09
	100	0.62	1.44	0.83	2.62	345.00	2.97	108.97
	200	0.65	2.00	1.36	3.37	470.63	3.84	152.98
	300	0.73	1.70	0.98	3.37	591.46	3.96	186.58
Sakha-2	0	0.38	0.78	0.40	1.95	283.75	2.23	95.11
	100	0.54	1.07	0.53	2.62	405.21	3.02	142.20
	200	0.59	1.38	0.78	3.09	423.33	3.50	156.97
	300	0.66	1.41	0.75	3.08	498.71	3.59	175.81
Sakha-3	0	0.35	0.71	0.36	2.09	263.96	2.35	83.60
	100	0.52	1.33	0.81	2.99	389.58	3.38	127.68
	200	0.63	1.55	0.93	3.63	471.46	4.11	162.84
	300	0.70	1.38	0.67	3.43	526.67	3.95	175.82
Sakha-5	0	0.35	1.75	0.56	2.49	265.00	2.76	80.61
	100	0.50	2.01	1.14	3.45	376.88	3.83	120.17
	200	0.66	1.80	1.50	3.82	494.79	4.28	159.96
	300	0.81	1.38	1.40	3.79	610.00	4.43	198.27
Sakha-6	0	0.38	1.34	0.75	2.42	288.13	2.71	91.86
	100	0.52	1.46	0.80	3.01	388.33	3.40	127.81
	200	0.66	1.75	1.23	3.68	492.08	4.18	163.96
	300	0.81	1.56	0.95	3.58	608.96	4.19	200.47
Giza-11	0	0.23	1.02	0.79	2.13	172.29	2.31	57.37
	100	0.43	1.42	0.90	2.63	322.71	2.95	112.70
	200	0.55	1.45	0.92	3.33	413.54	3.75	154.14
	300	0.68	1.59	0.99	3.38	507.29	3.89	183.32
Amon	0	0.28	1.04	0.76	2.07	208.75	2.28	59.03
	100	0.53	1.42	0.90	2.28	395.42	2.68	120.69
	200	0.68	2.01	1.32	2.98	511.27	3.49	163.91
	300	0.72	1.89	1.17	3.07	538.54	3.60	166.81
LSD 0.05		0.06	0.31	0.31	0.05	43.65	0.07	14.58
LSD 0.01		0.08	0.42	0.41	0.06	58.35	0.09	19.50

Table 9: Effect of interaction between cultivars and different concentrations (0.0, 100, 200 & 300 mg/l) of stigmasterols on chemical constituents and antioxidant activity (DPPH %) in seeds of flax plants grown under sandy soil

				Flavonoids	Phenolics	
Cultivars	Conc.	Oil %	Total CHO%	---------- mg/100 g dry wt ----------		DPPH (%)
Sakha-1	0	28.19	30.03	41.38	171.87	56.40
	100	31.59	32.71	42.58	170.87	59.75
	200	32.51	34.96	44.69	177.51	62.40
	300	31.54	34.15	47.08	182.42	58.70
Sakha-2	0	33.52	30.60	42.73	184.94	53.75
	100	35.09	31.91	44.71	194.15	56.25
	200	37.08	33.44	48.73	201.26	61.30
	300	35.25	33.19	46.90	187.33	59.00
Sakha-3	0	31.68	30.73	26.03	184.99	32.75
	100	32.76	32.92	32.95	189.06	41.45
	200	34.54	34.54	37.35	200.71	46.99
	300	33.37	33.68	34.05	193.07	42.83
Sakha-5	0	30.46	29.36	33.30	191.29	41.89
	100	31.87	31.39	37.44	193.39	47.10
	200	32.33	33.31	41.88	198.90	52.68
	300	32.51	35.56	40.58	193.39	51.04
Sakha-6	0	31.86	30.85	44.83	195.46	52.05
	100	32.91	32.28	47.50	201.00	53.57
	200	33.32	34.97	49.61	203.96	56.22
	300	32.92	31.83	46.66	193.36	59.23
Giza-11	0	33.29	29.41	30.89	159.85	38.85
	100	34.93	31.37	37.36	186.54	42.97
	200	37.27	33.64	40.39	195.85	49.26
	300	36.13	33.25	40.45	198.87	56.56
Amon	0	28.25	27.77	38.73	173.60	48.72
	100	30.51	30.01	47.75	179.72	60.06
	200	32.06	32.49	52.31	185.75	65.80
	300	30.97	31.64	48.45	178.56	60.95
LSD 0.05		0.49	0.90	5.31	5.52	0.63
LSD 0.01		0.66	1.20	7.10	6.45	0.84

The most effective concentrations ranged between 200 mg/l in some cultivars and 300 mg /l in the tested other cultivars.

DISCUSSION

The collected data demonstrated the marked effect of cultivars namely Sakha1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Giza 11 and Amon on different growth parameters (shoot and root length (cm), fresh and dry weight of shoot and root (g)), photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments), phenolic, IAA, proline and free amino acids, as well as yield, its components, nutritional contents (oil% and carbohydrate %), antioxidant compounds (flavonoids and phenolics) as well as, antioxidant activity (DPPH%) of the seven tested cultivars of flax (Table 2). These differences might be expected due to the differences of these cultivars in origin and growth habit, where, these flax cultivars are grown for double purpose crop (oil and fibers) under the conditions of this trails. Moreover, these obtained results were in accordance with those obtained by, Kineber *et al*. [24], Khalifa *et al.* [25], Afifi *et al*. [26], Bakhoum *et al.* [27] and Dawood *et al* [28] on different plant species in many regions of the world. Moreover, the superiority of Sakha 6 and Sakha 5 might be due to the increased rate of quenching of chlorophyll fluorescence, which markedly increased plant biomass and this steady state was greater than the other cultivars. The superiority of these cultivars in seed yield and its components might be due to the superiority in plant height, fruiting zone length, branches and capsules number/plant, seed yield/plant.

The superiority of Sakha 6 cultivar over the other cultivars in oil yield/fed., may be due to its great number of capsules, fruiting branches, seed yield /plant, oil % and oil yield kg/ fed (Table 2). Dawood *et al*. [28] and Bakry, *et al.* [29] reported that, Sakha 5 surpassed significantly the other cultivars in the biological and straw yield (ton/fed) and these resulted from the superiority of Sakha 5 cultivar in plant height, technical stem length, biological yield/ plant and straw yield/plant.

Regarding to the promotive effect of stigmasterol on flax cultivars and the interaction effect of stigmasterol concentrations and flax cultivars on growth parameters. Data in the present work showed that different concentrations of stigmasterol improved growth of the tested seven cultivars of *Linum usitatissimum* plants (Table 3 & 4). This enhancement effect might be due to the role of stigmasterols on increasing the efficiency of water uptake and utilization, enhancing cell division and/or cell enlargement, resulting in longer shoots and roots and increasing leaf area which, consequently, increased the fresh and dry matter of root and shoots, presumably, as a result of larger surface area available for anabolic activities [30]. Similar results were obtained by Abd El-Wahed *et al*. [31], working on wheat, they stated that stigmasterol improved vegetative growth characteristics of wheat plant. Yu *et al.* [32] and Zhang *et al.* [33] observed that BRs application improved assimilation of carbon and nitrogen by the stabilization of membrane structures and also improved general growth and Moreover, Mahesh, *et al*. [34] stated that priming radish seeds in 24-epibrassinolide increased growth parameters of radish plant. Moreover, this effect might be attributed to the promotive role of stigmasterol treatment on soluble sugar levels which is necessary for the turgor and increasing the efficiency of water uptake and protecting the photosynthetic pigments. It could be concluded from these results that stigmasterol act as growth stimulants which may play a role in enhancing flax plant growth and development via improving certain metabolic activities [31].

Exogenous treatment of stigmasterol improved photosynthetic pigment contents of different tested cultivars of flax plant compared with those of the reference controls (Table 3 & 5). These results in leaves of treated flax plants were in good agreement with the increase in growth rate as well as to the increase in carbohydrate contents. In this respect, Abd El-Wahed *et al.* [31] found that, the contents of photosynthetic pigments (*Chl* a, *Chl* b and carotenoids) were increased in maize as sitosterol concentration increased. In addition, Khalil and Madany [10] Hassanein *et al*., [35] and Bassuany *et al*., [36] confirmed these promotive role of stigmasterol pigments of faba, flax and maize plants. The promotive role of stigmasterol on photosynthetic pigments contents of different flax cultivars are explained by Yuan *et al.* [37] they stated that exogenous application of BRs is increasing the capacity of carbon oxide assimilation in Calvin cycle using increased initial activity of RuBisCo.. In addition to, increased efficiency of photosystem II and activity of enzymes, such as RuBisCo, nitroreductase and glutamine synthesis [38, 39].

Exogenous treatment of stigmasterol increased phenolic contents of the tested flax cultivars (Table 3& 5). Phenolic contents are important protective components of plant cells. The potential of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet O_2 [40]. With respect to endogenous auxins as indole acetic acid, different concentrations of stigmaterols

foliar treatment increased significantly IAA contents of flax cultivars (Table 3 & 5), these increases in IAA may lead to enhancement of enzyme activity and, in turn, to an increase of the metabolic compounds which can also explain the increased growth parameters in stigmasterol-treated plants, as compared with untreated ones. In this respect, Shunquan *et al*. [41] showed that stigmasterol enhanced phytohormones (as IAA), which can play an important role as signals and regulators of growth and development of plants. In addition, Hashem *et al.* [9] and El-Greedly and Mekki [42] confirmed the promotive role of stigmasterol in maize and flax plants. Regarding to proline and free amino acids, proline can accumulate to high concentration without damaging cellular macromolecules. Therefore, it acts as a compatible osmolyte. Importantly, proline provides protection against membrane damage and protein denaturation during unfavorable environmental conditions. In our study, proline was accumulated in linseed cultivars treated with different stigmasterol treatment. There is a report in agreement with our findings revealing an increase in the proline content following application of BRs in sorghum and flax plants [43, 44].

Yield is a result of the integration of metabolic reactions in plants; consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield. Thus, in the present investigation all the used stigmasterol concentrations caused significant increases in different yield and its components of the tested seven flax cultivars (Tables 3, 7 & 8). In this connection, Hashem *et al*., [9] found that, flax yield and its components were significantly increased for plants which were twice sprayed by the stigmasterol and attributed these increasing to the increment of growth regulators (as IAA) which improved photosynthetic activities, consequently, this beneficially affects the number and weight of capsules and seed yield. In addition, El-Tantawy and Azoz [11] confirmed this promotive effect of stigmasterol on basil plant.

The obtained data of the effect of foliar application of stigmasterol on different cultivars of flax plant growing under sandy soil conditions show that different concentrations increased significantly oil, carbohydrates, flavonoids and phenolic contents as well as antioxidant activities of seed yield (Table 3 & 9). With respect to oil contents, Hashem *et al*. [9] and Bekheta *et al*. [45] concluded that application of stigmasterol, might increase the accumulation of oil of thyme and flax plants. Regarding to carbohydrates contents of seed yield, Hassanein *et al*., [35] confirmed our data on carbohydrate contents of faba plant.

Data presented in Table 3 & 9 indicated that stigmasterol treatments at (100, 200 and 300 mg/l) caused significant and pronounced increase in total flavonoids content. The importance of the flavonoids was known to possess significant antimicrobial activities and was utilized as natural plant protectants [46]. It could be suggested that flavonoids content may be an alternative to conventional fungicides in the control of storage seeds against some fungi.

The reactivity of flax seed yield was analyzed by DPPH, a stable free radical, under different treatments. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stoichiometrically correlated to the number of electrons gained [47]. The results of the DPPH assay revealed that DPPH radical scavenging activity of samples increased significantly with foliar treatment of different concentrations of stigmasterols of the tested flax cultivars. Yu *et al.* [48] concluded that significant levels of antioxidant activities and phenolic components have been detected in different plants and food products and suggesting that may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion. The increase in the scavenging activity can be considered an advantage of treatment used. This could be attributed to the increases in total phenols and total flavonoids [49].

CONCLUSION

Therefore, we would venture to recommend the use foliar spraying of different varieties of flax plants with stigmasterols at different concentrations as a natural and low-cost plant growth regulator for stimulating their growth with no discernible adverse effects and increase production of flax productivity under reclaimed sandy soil.

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