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Research Article
Biochemical and Yield of Flax in Responses to Some Natural Antioxidant Substances under Sandy Soil Conditions

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Abstract
Background and Objective: Abiotic stress (drought and the low nutrients of sandy soils) affecting on plant growth and yield. The foliar application of natural antioxidants compounds considered one of the methods to avoid the deleterious effects of such stress conditions. The present work implemented to find out the effect of spraying aspartic acid, coumarin and benzoic acid at different concentrations on growth, yield, some physiological criteria and quality traits of flax seed yield grown under sandy soil conditions. **Materials and Methods:** The experiment conducted in randomized complete block design with three replications. The concentrations for foliar applications with aspartic and benzoic acid (50, 100 and 200 mg L^{-1}) and coumarin (25, 50 and 100 mg L^{-1}) were distributed at random in the plots. **Results:** The studied growth criteria and photosynthetic pigments increased significantly with the application of aspartic acid, coumarin and benzoic acid. Aspartic acid at 100 mg L^{-1} was the most effective treatment followed by 50 mg L^{-1} benzoic acid in most cases of the tested parameters. The highest value was observed in response to benzoic acid in total soluble sugars, proline and free amino acid. Aspartic acid spraying induced the highest values of total phenols, IAA, DPPH% and total soluble protein. Aspartic acid was the superior in affecting yield, yield components and the nutritive value of flax followed by coumarin and benzoic acid. **Conclusion:** The obtained results suggested that the use of aspartic acid at 100 mg L^{-1} was promising in alleviating stress on flax and improved the yield and nutritive value of its seeds.

Key words: Aspartic acid, benzoic acid, coumarin, flax, pigments, enzymes, seed yield and quality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recently with excessive increases in Egyptian populations accompanied by great loss of agricultural soils, Egyptian scientists paid their attention towards compensating this decrease by reclaiming and cultivating new sandy soils. Thus, due to the poor characteristics of new sandy soils the efforts made in two directions. First goal of cultivating new areas was selecting suitable crops for soil and climate conditions of the selected areas and the second goal was selecting crops to meet human needs and food shortage problems such as; oil plants. So, it became important to increase the productive potent of seed oil plants.

Flax (*Linum usitatissimum* L.) as one of the seed oil source economically important fiber crop (linseed) plant grown all over the world. Its importance because of its high nutritional contents of protein, water soluble fibre fractions[1], lignin content[2], mucilage, linamarin (a cyanogenic glycoside) and enzymes[3]. Today flax mainly grown for its oil content[4]. A ratio of 1:3 for omega-3 to omega-6 fatty acids found to have beneficial effects, but it was not found in other oil-seed plants[5]. Flax seed oil characterized by its richness in linoleic (omega-6) and linolenic (omega-3) polyunsaturated fatty acids (PUFA), which are essential for humans, thus it is needed to be provided via food and has several human health benefits. Linseed oil has anticancer effects on breast, prostate and colon cancers[6]. Traditionally, flax and its oil are used for several purposes, e.g., manufacture of paints, varnishes and linoleum, nutraceutical, pharmaceutical, animal feed and as a human food or a food ingredient in processed foods[7].

The uses of amino acids and phenolic compounds are natural secondary plant metabolites that can promote and develop plant growth by improving plant tolerance against biotic and abiotic stresses (salinity, nutrient shortage, low availability of water and changes in temperature)[8]. Amino acids also contributed in regulating some plant processes that influences growth and development, ion uptake, ion transport and membrane permeability[9]. Amino acids considered as precursors and constituents of proteins[10] (stimulator for cell growth). They act as buffers due to its content of both acid and basic groups that help to preserve the suitable pH value inside the plant cell[11]. Also, amino acids known as bio-stimulants that influence the physiological activities in the plant that have positive effects on plant growth, yield and significantly mitigate the abiotic stresses injuries[12,13]. Taiz and Zeiger[14] stated that other amino acids can be formed from glutamate, glutamine and aspartate which are the main ones synthesized by plants. Moreover, Maeda and Dudareva[15] reported that amino acids act as stress-reducing agents, source of nitrogen and hormone precursors. Akladious and Abbas[16] found that aspartic acid positively enhanced growth, anthocyanin, α-tocopherol, ascorbic acid and antioxidant enzyme activities in plants as a defense system against the reactive oxygen species.

Phenolic compounds (Vanillic acid, gallic acid, salicylic acid, cinnamic acid, P-coumaric acid, coumarin and benzoic acid) act as defense molecules against biotic and abiotic stress[17,18]. Furthermore, these biomolecule substances may donate to soil and conservation of water, weed management and mineral element sustenance. Among plant phenolics, coumarin is naturally distributed in a wide range of plant communities and known to inhibit several physiological processes related to seed germination and growth of plants as well as development and photosynthetic pigments synthesis[19,20]. Recently, investigations reported the growth stimulating activity of coumarin[21,22]. In addition, Lupini *et al.*[21] suggested that a pathway of auxin-like behavior of coumarin interfered with auxin signaling. Accordingly, the influence of coumarin on plant growth seemed to be specified to some species, method of application and its concentration[23,24]. Benzoic acid is known to provide abiotic stress tolerance[25]. As reviewed and discussed by Abdallah *et al.*[26] using quinoa plants in sandy soils, benzoic acid as carboxylic acid naturally synthesized by plants. These compounds are exudates towards the rhizosphere to facilitate the assimilation of mineral nutrients thus increasing the rate of mineral uptake. Such action helped soybean plants to overcome the adverse effects of nutrients shortage in sandy soil and consequently improved its yield and quality[27].

Different environmental stress conditions e.g., nutrient shortage, water availability, heat and salt stresses are dominant in reclaimed sandy soils. Therefore, the current study aimed at increasing plant tolerance to such conditions via applying foliar spraying with aspartic acid, coumarin and benzoic acid at different concentrations during different growth stages for supporting plants in overcoming the adverse environmental conditions and ameliorating negative effects on the yield quantity and quality of flax.

MATERIALS AND METHODS

A field trial was carried out at the Experimental Station of National Research Centre, (latitude 30°30' 1.4' 'N, longitude 30°19' 10.9'' E and mean altitude 21 m above sea level) Al-Nubaria district, El-Beheira Governorate, Egypt, (North Africa represent arid or semi-arid region) during two successive summer of 2017/2018 and 2018/2019 seasons. To study the effect of aspartic acid, coumarin and benzoic acid on growth,

Table 1: Some physical and chemical characteristics of the experimental soil

	2017/2018		2018/2019	
Item season depth (cm)	00-30	30-60	00-30	30-60
Coarse sand (%)	40.7	38.2	38.7	36.5
Fine sand (%)	44.6	43.0	42.6	38.1
Silt (%)	10.7	13.8	13.7	17.8
Clay (%)	4.0	5.0	5.0	7.6
pH	7.84	7.89	7.95	7.85
Electrical conductivity (dS m^{-1})	11.76	5.79	1.59	1.81
Saturation (%)	32	27	23	25
$CaCO_3$ (%)	1.00	6.00	1.90	1.30
Organic matter (%)	0.40	0.07	0.38	0.32
Anions (meq. L^{-1})				
CO_3^-	-	-	-	-
HCO_3^-	0.50	0.60	0.32	0.45
Cl	8.40	8.00	12.70	15.40
SO_4^-	1.11	1.40	1.98	2.15
Cations (meq. L^{-1}):				
Ca^{++}	1.80	2.10	4.00	5.60
Mg^{++}	0.90	1.50	1.80	2.00
Na^+	7.10	6.20	9.00	10.20
K^+	0.20	0.20	0.20	0.20

yield and its components as well as seed quality of flax under sandy soil conditions. Soil of the experimental site was sandy soil. Mechanical, chemical and nutritional analysis of the experimental soil was presented in Table 1 according to Chapman and Pratt[28].

The experimental design in Randomized Complete Block Design (RCBD) with three replications. The concentrations for foliar applications with aspartic and benzoic acid (50, 100 and 200 mg L^{-1}) and coumarin (25, 50 and 100 mg L^{-1}) were distributed at random in the plots.

Flax (*Linum usitatissimum* L.) seeds (cultivar Sakha2) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt and were sown at rate of 2000 seeds/m^2 on mid of November in both seasons in rows 3.5 m long with distance between rows of 20 cm apart. Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). Pre-sowing, 360 kg ha^{-1} of calcium superphosphate (15.5% P_2O_5) was added. Nitrogen in the form of ammonium nitrate 33.5% N was applied after emergence at the rate of 180 kg ha^{-1} in five equal doses. Potassium sulfate (48% K_2O) was added at two equal doses of 120 kg ha^{-1}.

Irrigation was carried out using the new sprinkler irrigation system once every 5 days. Foliar application of different treatments of the tested materials were applied twice after 30 and 45 days from sowing. Plant samples were taken after 60 days from sowing for measurements of growth and some biochemical parameters (Morphological measurements and chemical analysis), while yield attributes were considered when full maturity signs detected on plants.

Growth measurements: The morphological measurements were shoot length (cm), root length (cm), fresh and dry weight of shoot (g/plant) and fresh and dry weight of root (g/plant).

Yield measurements: When signs of full maturity stage showed measurements for yield and its components (Plant height (cm), technical stem length (cm), fruiting zone length (cm), seed yield/plant, biological yield/plant, no. of fruiting branches/plant, no. of capsules/plant, no. of seeds/capsule, 1000-seed weight, straw, biological, seed and oil yields per hectare) were estimated.

Chemical analysis: Some biochemical aspects were determined including photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) in fresh leaves which were estimated using the method of Lichtenthaler and Buschmann[29]. Indole acetic acid content were extracted and analyzed by the method of Larsen *et al.*[30]. Phenolic content was measured as described by Danil and George[31]. Free Amino Acids (FAA) was extracted according to Vartainan *et al.*[32] and determined with the ninhydrin reagent method of Yemm *et al.*[33]. Proline content was extracted and calculated according to Bates *et al.*[34]. Total Soluble Sugars (TSS) were extracted according to Prud'homme *et al.*[35] and assayed according to Yemm and Willis[36]. The antioxidant activity (DPPH radical scavenging) was determined by using the method of Liyana-Pathiranan and Shahidi[37]. Determination of total carbohydrates was carried out according to Sadasivam and Manikam[38]. Total flavonoids were assayed using the method of Chang *et al.*[39]. Total protein as well as Total Soluble Protein (TSP) and oil concentrations were determined according to the methods of AOAC[40]. Seeds oil fatty acids profile were determined quantitatively by Gas Liquid Chromatography according to Fedak and De La Roche[41].

Statistical analysis: The combined analysis of two growing seasons were statistically analyzed according to Snedecor and Cochran[42]. Means were compared by using Least Significant Difference (LSD) at 0.05 level of probability.

RESULTS AND DISCUSSION

Growth criteria: The data presented in Table 2 showed the effect of aspartic acid, coumarin and benzoic acid on shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of flax plants. The highest significant ($p<0.05$) increase was obtained in shoot and root

Table 2: Effect of foliar spraying with different concentrations of aspartic acid, coumarin and benzoic acid on growth criteria of flax plants cultivated in sandy soil (combined analysis of two seasons)

Materials	Concentration ($mg\ L^{-1}$)	Shoot length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Root length (cm)	Fresh weight of root (g)	Dry weight of root (g)
Control	0	63.33±0.87	3.21±0.31	0.84±0.05	9.17±0.02	0.34±0.002	0.16±0.002
Aspartic acid	50	86.00±1.35	4.28±0.25	1.29±0.18	10.25±0.02	0.46±0.02	0.19±0.02
	100	93.75±1.11	7.51±0.65	2.21±0.36	11.75±0.07	0.82±0.04	0.30±0.01
	200	87.50±1.85	5.61±0.50	1.44±0.36	8.50±0.03	0.35±0.01	0.15±0.004
Coumarin	25	90.63±0.65	4.68±0.87	1.60±0.20	8.25±0.03	0.35±0.01	0.14±0.003
	50	91.00±0.41	3.49±0.63	1.24±0.22	10.63±0.09	0.33±0.02	0.22±0.03
	100	82.00±1.32	3.20±0.25	1.08±0.19	8.38±0.04	0.28±0.02	0.11±0.001
Benzoic acid	50	87.75±0.48	6.52±0.25	2.11±0.47	11.50±0.02	0.72±0.03	0.26±0.04
	100	85.75±0.95	4.54±0.80	1.32±0.10	9.63±0.09	0.40±0.01	0.18±0.003
	200	83.25±1.50	4.44±0.29	1.21±0.29	9.50±0.12	0.40±0.003	0.17±0.004
LSD (0.05)		2.70	0.71	0.15	1.32	0.06	0.05
Effect of tested materials							
Aspartic acid		82.65±1.27	5.15±0.43	1.45±0.11	9.92±0.45	0.49±0.06	0.20±0.021
Coumarin		81.74±1.32	3.65±0.23	1.19±0.07	9.11±0.45	0.33±0.01	0.16±0.016
Benzoic acid		80.02±0.74	4.68±0.33	1.37±0.14	9.95±0.41	0.47±0.05	0.19±0.016
LSD (0.05)		2.03	0.49	0.09	NS	0.03	0.01

NS: Not significant, Each value represents the Mean±standard error

fresh and dry weights of flax plants sprayed with aspartic acid as compared to plants sprayed with coumarin. On the other hand, spraying flax with benzoic acid produced the highest root length without significant differences among the three tested materials.

Data also showed that the effect of different concentration of aspartic acid (50, 100, 200 mg L^{-1}), coumarin (25, 50, 100 mg L^{-1}) and benzoic acid (50, 100, 200 mg L^{-1}) on growth parameters of flax grown on sandy soil. The results showed significant ($p<0.05$) increases in plant height, root length and fresh and dry weights of shoot and root as compared with the control plants. The most pronounced ($p<0.05$) increase in all morphological criteria was observed with foliar application of aspartic acid at 100 mg L^{-1}.

These results agreed with Akladious and Abbas[16] who indicated that treating tomato plant under both non-saline and saline conditions with aspartic acid increased its growth measurements. Also, Sadak *et al.*[43] found that foliar application of different concentrations of amino acid mixture significantly ameliorate the reduction effect of salinity stress in growth parameters and faba bean plant contents of photosynthetic pigments, total carbohydrates, polysaccharides, DNA and RNA. El-Awadi *et al.*[44] observed that, aspartic acid treatment led to the significant increases in growth parameters in wheat cultivars grown in sandy soil conditions.

Amino acids may play an important role in plant metabolism and protein assimilation necessary for cell formation and consequently increase fresh and dry matter[45,46] on potato and strawberry, respectively. Shehata *et al.*[47] indicated that spraying celeriac plants with amino acids significantly increased plant height, leaf number and fresh and dry weight of leaves.

Saleh *et al.*[48] found that application of coumarin increased significantly the growth parameters of *Vicia faba* plants. They explained that the increase in growth may be due to the increases in endogenous growth promoters (IAA and GA_3) and also the increases in osmoregulators (TSS, total soluble protein and total phenols) due to coumarin treatment. The increase in growth parameters of flax plant in response to coumarin is in consistency with results on various crops reported by Lupini *et al.*[21] and Al-Wakeel *et al.*[22]. Abdallah *et al.*[26] and Khairy and Roh[49] found that the growth parameters of quinoa and tobacco plant increased due to benzoic acid foliar spraying.

Photosynthetic pigments: Data in Table 3 revealed that the effect of aspartic acid, coumarin and benzoic acid on chlorophyll a, chlorophyll b, carotenoids and total pigment contents. In general, the highest ($p<0.05$) values were recorded when plants sprayed with aspartic acid over that recorded with either coumarin or benzoic acid.

All exogenous application of the previous material induced a significant ($p<0.05$) increase in all photosynthetic pigments. The maximum increases of the photosynthetic pigments were obtained by foliar application with aspartic acid at the rate of 100 mg L^{-1}. The enhancement of the effect of aspartic acid on photosynthetic pigments was on line with those reported by Haroun *et al.*[50] on *Phaseolus vulgaris* and El-Awadi *et al.*[44] on wheat.

Table 3: Effect of foliar spraying with different concentrations of aspartic acid, coumarin and benzoic acid on photosynthetic pigments (mg g^{-1} fresh weight) of flax plants cultivated in sandy soil (combined analysis of two seasons)

Materials	Concentration (mg L^{-1})	Chl. a	Chl. b	Carotenoids	Total chlorophyll
Control	0	12.88±0.04	2.18±0.02	3.86±0.05	18.75±0.19
Aspartic acid	50	17.53±0.08	3.67±0.01	7.00±0.09	28.10±0.23
	100	18.40±0.23	4.13±0.04	7.64±0.13	31.21±0.70
	200	17.90±0.53	3.69±0.07	7.70±0.19	29.52±0.24
Coumarin	25	16.26±0.10	3.44±0.03	5.92±0.02	25.60±0.30
	50	17.78±0.04	3.58±0.03	6.47±0.13	27.82±0.12
	100	17.27±0.11	3.83±0.01	6.50±0.05	27.59±0.10
Benzoic acid	50	17.62±0.18	3.80±0.04	6.93±0.19	27.04±0.51
	100	15.63±0.22	3.74±0.02	6.33±0.09	25.71±0.16
	200	15.55±0.14	3.53±0.10	5.59±0.05	24.46±0.24
LSD (0.05)		0.64	0.12	0.32	0.93
Effect of tested materials					
Aspartic acid		16.68±0.21	3.42±0.08	6.55±0.13	26.89±0.50
Coumarin		16.05±0.23	3.26±0.06	5.69±0.20	24.94±0.37
Benzoic acid		15.42±0.35	3.31±0.05	5.68±0.10	23.99±0.41
LSD (0.05)		0.20	0.06	0.13	0.72

Each value represents the mean±standard error, Chl. a: Chlorophyll a, chl. b: Chlorophyll b

Table 4: Effect of foliar spraying with different concentrations of aspartic acid, coumarin and benzoic acid on some compatible solutes, total phenols and indoles of flax plants cultivated in sandy soil (combined analysis of two seasons)

Materials	Concentration (mg L^{-1})	TSS	Proline	FAA	TSP	Total phenols	IAA	DPPH (%)
		(mg/100 g fresh wt.)					(μg g^{-1} fresh wt.)	
Control	0	181.00±3.40	118.20±3.85	454.19±2.15	239.53±3.52	218.74±1.07	20.49±0.18	27.44±0.39
Aspartic acid	50	213.73±4.13	257.87±4.02	532.64±2.48	339.54±1.36	261.51±2.11	30.31±0.09	36.37±0.96
	100	357.65±0.76	264.51±4.15	811.05±3.21	394.93±3.19	322.06±0.57	34.42±1.90	49.96±0.34
	200	294.66±1.94	133.14±4.01	579.27±2.60	308.29±4.01	318.55±1.05	20.52±0.10	46.72±0.72
Coumarin	25	214.27±3.03	332.38±3.09	697.39±3.13	262.21±3.80	233.52±1.69	28.25±1.00	33.92±0.44
	50	275.85±1.08	483.30±3.04	1035.36±3.18	277.18±4.14	271.51±3.29	19.32±0.39	49.23±1.58
	100	240.50±3.12	142.97±4.64	528.28±0.25	255.58±4.95	258.69±3.59	18.48±0.80	31.13±1.09
Benzoic acid	50	377.07±4.13	500.37±3.70	1071.26±3.40	265.32±3.95	237.85±3.72	29.98±1.32	36.55±0.24
	100	350.35±1.52	401.77±4.50	999.92±0.60	299.34±4.94	245.91±2.18	26.50±0.35	34.35±0.49
	200	174.41±3.98	329.68±0.86	970.73±0.24	337.864.26±	278.93±2.11	25.18±0.01	28.47±0.91
LSD (0.05)		19.61	30.43	18.43	49.39	7.40	2.44	2.02
Effect of tested materials								
Aspartic acid		261.76±20.87	193.43±22.75	594.29±43.12	320.57±18.84	280.22±9.84	26.44±2.13	40.13±2.00
Coumarin		227.91±9.46	269.21±49.44	678.805±90.45	258.63±6.90	245.62±5.77	21.64±1.61	35.43±2.95
Benzoic acid		270.71±32.13	337.51±24.46	874.03±15.04	285.51±11.05	245.36±6.43	25.54±1.11	31.70±1.24
LSD (0.05)		2.45	20.12	2.80	24.80	1.27	1.54	0.25

Each value represents the mean±standard error

Regarding the effect of coumarin on photosynthetic pigments, Saleh *et al.*[48] study on *Vicia faba* plants found that, application of coumarin increased significantly its photosynthetic pigments content.

The positive effect of benzoic acid on photosynthetic pigments are in harmony with Anjum *et al.*[51] on soybean. They attributed the improvement effect of benzoic acid to the increase in gas exchange, stomatal conductance, transpiration and photosynthetic rates.

Also, Khairy and Roh[49] reported the same trend in salinity-stressed tobacco plant (*Nicotiana tabacum*) when benzoic acid and p-coumaric acid resulted in remarkable increases in the contents of chlorophyll.

Changes in biochemical constituents: Table 4 showed the effect of aspartic acid, coumarin and benzoic acid on Total Soluble Sugar (TSS), proline, Free Amino Acid (FAA), Total Soluble Protein (TSP), total phenols, IAA and DPPH%. The highest value was observed in response to benzoic acid in TSS, proline and FAA. Meanwhile, aspartic acid spraying induced the highest ($p<0.05$) values of TSP, total phenols, IAA and DPPH%. Meanwhile, foliar application of aspartic acid (50, 100, 200 mg L^{-1}), coumarin (25, 50, 100 mg L^{-1}) and benzoic acid (50, 100, 200 mg L^{-1}) caused significant ($p<0.05$) increases in TSS, proline, FAA, TSP, total phenols, IAA contents and DPPH%. Moreover, the best concentration resulted in the highest values of most studied parameters were 100 and 50 mg L^{-1} for aspartic acid and both coumarin or benzoic, respectively.

Amino acids such as proline and asparagine are plant osmotic adjustment under saline conditions[52]. Tiwari *et al.*[53] stated that the increase in these compatible solutes in the treated plants can ameliorate the harmful effect of abiotic stress which make osmotic adjustment occurs in plants subjected to stress. They revealed also that such substances are vital in the adaptation of cells to various adverse conditions through raising cytoplasm osmotic pressure, stabilizing proteins and membranes and preserving the relatively high-water content. Also, at high drought stressed plants there is increase in amino acid protein decomposition.

The increase in IAA content in shoot tissues induce an improvement in growth rate (Table 2) indicated that this growth resulted from the role of endogenous hormones in stimulating cell division and/or enlargement[54]. In addition, amino acids play a key role in signaling stress response and secondary metabolism in plants[55]. Ashraf and Iram[56] attributed the accumulation of free amino acids in plants under water stress often to changes in biosynthesis and degradation procedures of amino acids and proteins. Amino acids foliar spraying increased TSS content on root of celeriac plants[47] and strawberry[46].

The asparagine and glutamine connect the two important metabolic cycles of the plant, the carbon and nitrogen cycles and they have an influence on both sugars and proteins. In plants, methionine, threonine and isoleucine are initiated from aspartate[57].

Saleh *et al.*[48] on *Vicia faba* plants found that application of coumarin increased significantly the total soluble and insoluble sugar, total soluble and insoluble protein and total phenols of the leaves. In this regard, the high content of soluble non-reducing sugars with coumarin treatments may be logical consequence of the GA_3 elevated level. The GA_3 known to promote the synthesis of sucrose in the leaves, since it stimulated fructose-1,6-bisphosphatase, sucrose synthase and sucrose phosphate synthase[58].

The improvement effect of benzoic acid on the growth of flax plants (Table 2) may be attributed to their positive effect on photosynthetic pigment biosynthesis (Table 3) and/or antioxidative activity (Table 4). These results came on line with the results observed by Tuna *et al.*[59] on maize and Anjum *et al.*[51] on soybean. Abdallah *et al.*[26] found that treatment quinoa plants with benzoic acid increased levels of total soluble sugar, proline, free amino acid, IAA and phenol contents. The increase in phenols content is in agreement with Mona and Sadak[60] who reported that the increase in total phenolic of canola was synchronized with the increase in its auxin content. They suggested that most of the phenolic compounds are diphenols and polyphenols, resulted from the increase in IAA. Moreover, these phenolic compounds may inhibit oxidase activity of IAA and lead to the accumulation of auxin which reflected in stimulating plant growth and yield.

Yield and yield components: The records presented in Table 5 revealed that aspartic acid produced the highest significant ($p<0.05$) values of most yield attributes (plant height, technical stem length, fruiting zone length, biological yield/plant, number of fruiting branch, number of capsule/plant, number of seeds/capsule, seed yield/plant, 1000-seed weight and seeds yield/ha) followed by coumarin treatment which was the highest ($p<0.05$) in number of seeds/capsule, seed yield/plant and seed yield/ha. These data indicated for the superiority of aspartic acid treatment followed by coumarin and benzoic acid in affecting yield parameters of flax grown in sandy soils.

Foliar application with different concentrations of aspartic acid, coumarin and benzoic acid exhibited the significant ($p<0.05$) effect in the studied yield traits (Plant height, technical stem length, fruiting zone length, biological yield/plant, No. of branches/plant, No. of capsules/plant, No. of seeds/capsule, 1000-seed weight and seed yield). All yield parameters showed a significant ($p<0.05$) increase in response to all treatments. The highest value was observed at 100 mg L^{-1} aspartic acid followed 50 mg L^{-1} coumarin.

El-Awadi *et al.*[44] demonstrated that aspartic acid treatment induced significant increase on yield and yield attributes in wheat grown in sandy soil condition. Moreover, Wang *et al.*[61] found that amino acid liquid fertilizer and liquid biological fertilizer increased cowpea yields compared to the control in presence of chemical fertilizer. Ramadan *et al.*[62] reported that foliar sprayed amino acid significantly improved head diameter, 100-seed weight, seed yield/plant of sunflower plants irrigated with either saline or non-saline water. The overall enhancement in the plant yield with amino acids treatment may be attributed to providing an easy source of growing substances. Also, the increases in energy conversion efficiency maximizes the plants growth ability and accordingly increases its productivity.

The present results with coumarin treatments supported with findings of Ramadan[63] who reported that exogenous application of coumarin to *Vicia faba* increased plant weight, number of pods/plant, number of seeds/plant and weight of 100-seeds.

The enhancement effect of benzoic acid on the yield may be attributed to its dynamic effect on improving the growth and yield of plants. These results are in agreement with those recorded by Abdel-Hakim *et al.*[64] on snap bean (*Phaseolus vulgaris* L.) and Anjum *et al.*[51] on soybean.

Table 5: Effect of foliar spraying with different concentrations of aspartic acid, coumarin and benzoic acid on yield and its components of flax plants cultivated in sandy soil (combined analysis of two seasons)

Materials	Concentration (mg L^{-1})	Plant height (cm)	Technical stem length (cm)	Fruiting zone length (cm)	Biol. yield/Plant (g)	No. of fruiting branches/plant	No. of capsules/plant	No. of seed/capsules	Seed yield/plant (g)	1000 seed wt. (g)	Seed yield (kg ha^{-1})	Straw yield (t ha^{-1})	Biol. yield (t ha^{-1})
Control	0	84.33±0.33	60.33±0.43	24.00±0.58	5.23±0.58	5.67±0.67	22.67±1.23	6.67±1.00	0.88±0.01	6.54±0.09	668±6.16	4.38±0.10	5.05±0.11
Aspartic acid	50	85.67±1.76	61.67±1.27	24.00±0.63	7.49±0.21	9.67±0.35	28.67±0.67	7.67±0.34	1.05±0.02	7.28±0.11	1038±18.53	7.64±0.10	8.68±0.09
	100	112.00±1.53	81.33±0.59	32.00±1.15	10.99±0.44	12.67±0.39	30.33±0.33	10.00±0.30	1.76±0.01	7.88±0.16	1581±6.72	9.61±0.04	11.19±0.04
	200	102.00±0.05	78.67±0.39	23.33±0.24	8.39±0.08	8.67±0.33	28.33±0.29	9.00±0.33	1.13±0.06	6.94±0.04	1099±47.06	8.33±0.06	9.43±0.11
Coumarin	25	91.67±0.33	68.00±0.61	23.67±0.39	7.59±0.26	8.33±0.31	25.67±0.57	9.33±0.67	1.12±0.04	7.18±0.06	1093±28.80	7.64±0.07	8.73±0.04
	50	107.00±0.58	79.33±0.41	27.67±0.75	10.03±0.16	10.67±0.43	33.67±0.81	8.67±0.88	1.43±0.06	7.40±0.27	1326±46.68	9.71±0.22	11.04±0.27
	100	96.67±1.20	71.33±0.90	25.33±0.34	6.39±0.08	7.67±0.45	29.67±0,31	10.00±0.30	1.49±0.04	7.24±0.09	1369±31.81	6.29±0.17	7.66±0.15
Benzoic acid	50	106.00±1.00	80.00±1.24	24.67±0.37	10.04±0.31	9.33±0.40	30.67±0.30	8.67±0.67	1.49±0.01	7.84±0.04	1369±1.78	9.42±0.18	10.79±0.18
	100	97.00±0.58	76.00±0.54	21.00±0.58	8.71±0.04	8.67±0.64	29.33±0.62	7.67±0.61	1.07±0.04	7.28±0.07	1056±13.47	8.73±0.07	9.78±0.09
	200	84.00±1.53	63.67±1.86	20.33±0.30	8.28±0.10	6.33±-0.33	26.67±0.30	7.33±0.33	0.94±0.02	7.12±0.06	962±1.10	8.69±0.16	9.65±0.16
LSD 0.05	3.20	2.67	1.95	0.99	1.55	2.10	1.27	0.09	NS	69.97	0.37	0.40	
Effect of tested materials													
Aspartic acid		96.00±3.90	70.50±2.98	25.83±1.44	8.03±0.54	9.17±0.62	27.50±0.31	8.34±0.58	1.21±0.11	7.16±0.13	1096.48±87.13	7.49±0.29	7.49±0.38
Coumarin		94.92±2.29	69.75±1.71	25.17±0.63	7.31±0.54	8.09±0.48	27.29±1.21	8.67±0.29	1.23±0.06	7.09±0.03	1114.13±46.62	7.00±0.50	7.00±0.51
Benzoic acid		92.83±3.24	70.00±2.70	22.50±0.71	8.07±0.51	7.50±0.51	27.34±0.65	7.59±0.26	1.10±0.08	7.20±0.12	1013.94±62.24	7.80±0.14	7.80±0.19
LSD (0.05)		1.16	1.41	0.33	0.35	0.61	1.06	0.60	0.08	0.03	55.51	0.18	0.20

NS: Not significant, Each value represents the mean±standard error

Table 6: Effect of foliar spraying with different concentrations of aspartic acid, coumarin and benzoic acid on yield quality of flax plants cultivated in sandy soil (combined analysis of two seasons)

Materials	Concentration (mg L^{-1})	Carbohydrate (%)	Flavonoids (%)	DPPH (%)	Oil (%)	Protein (%)	Oil yield (kg ha^{-1})	Protein yield (kg ha^{-1})
Control	0	24.98±0.40	23.31±0.15	34.85±0.11	31.88±0.51	17.15±0.10	214.80±1.87	115.560±0.98
Aspartic acid	50	28.48±0.34	24.86±0.14	67.13±0.68	34.42±0.28	18.74±0.20	357.43±1.96	196.152±1.23
	100	34.33±0.32	31.39±0.20	62.21±0.55	38.24±0.19	23.51±0.003	609.65±2.08	374.808±1.86
	200	33.14±0.39	28.60±0.57	51.67±0.52	37.00±0.41	21.88±0.21	409.99±2.65	242.448±1.08
Coumarin	25	29.87±0.46	26.30±0.03	52.09±0.90	34.42±0.48	22.82±0.35	379.37±2.04	251.520±1.54
	50	34.30±0.36	28.77±0.59	56.06±0.33	36.21±0.50	20.53±0.35	484.30±1.65	274.584±1.52
	100	27.81±0.23	25.35±0.51	45.90±0.52	38.05±0.22	19.42±0.63	525.26±1.74	268.080±1.06
Benzoic acid	50	33.00±0.20	24.13±0.12	51.04±0.37	38.93±0.22	25.27±0.55	537.62±2.87	348.980±1.36
	100	28.10±0.69	25.43±0.20	52.74±1.52	39.29±0.21	25.38±0.29	418.27±2.39	270.190±1.67
	200	26.30±0.45	25.01±0.70	66.70±0.78	34.38±0.53	21.22±0.69	333.65±1.88	205.940±0.87
LSD (5%)		1.22	1.02	1.12	1.07	12.08	5.928	3.80
Effect of tested materials								
Aspartic acid		30.23±0.97	27.04±0.54	53.97±1.51	35.31±0.55	20.32±0.51	397.97±9.88	232.250±26.9
Coumarin		29.24±0.91	25.93±0.57	47.23±2.30	35.14±0.64	19.98±0.74	400.95±16.95	227.450±4.14
Benzoic acid		28.10±1.03	24.47±0.29	51.33±2.53	36.12±0.81	22.26±0.74	376.08±14.06	235.150±20.68
LSD (5%)		0.82	0.72	1.32	0.80	0.73	4.02	2.536

Each value represents the mean±standard error

The stimulatory effect was correlated with the increase in endogenous promoters content (particularly IAA) and activity levels which are known to induce linear growth and development of plant organs[26]. They added that, this improvement might be due to a marked increase in the number of fruiting branches per plant which gave a chance to the plant to carry more flowers and hence more capsules. The increase in seeds weight may be due to the increase in photosynthetic pigments content which could led to increase in photosynthesis, resulting in greater transfer of photo-assimilates to the seeds. Moreover, the increase in yield and its attributes may be due to the effect of antioxidants on improving protein synthesis and delaying senescence.

Nutritive value of seeds: The data presented in Table 6 revealed that aspartic acid treatment was superior over both coumarin and benzoic acid effects which caused significant increasing ($p<0.05$) in flax seeds nutritive value (carbohydrate %) and antioxidant substances (flavonoids and DPPH percentages). On the same side, benzoic acid gave the highest significant ($p<0.05$) values of oil and protein percentage in the seeds of tested plants. It was also showed that 100 mg L^{-1} aspartic acid produced the highest ($p<0.05$) values of all the studied parameters, except for DPPH which came higher with 50 mg L^{-1} treatment. Coumarin treatment at the rate of 50 mg L^{-1} was effective in increasing significantly ($p<0.05$) carbohydrate, flavonoids and DPPH (%), while the best value of oil in the seeds resulted from plants treated with 100 mg L^{-1} of coumarin. The lowest concentration (25 mg L^{-1}) produced the highest ($p<0.05$) protein values in the tested seeds. Benzoic acid treatment at the rate of 100 mg L^{-1} gave the best values and significant ($p<0.05$) values in most cases. The increase in seed quality (carbohydrate, oil and protein %) due to aspartic acid supported with the results of other investigators; Sadak *et al.*[65] on sunflower when amino acids treatments were applied and increased total carbohydrates and protein%. Abd El-Monem[66] concluded a close relationship between the effect of amino acids and the stimulation of the photosynthetic output as well as the contents of soluble sugars, polysaccharides and total carbohydrates. The increase in total carbohydrates obtained herein is in accordance to those obtained by El-Awadi *et al.*[44] on wheat cultivars plants. The promotive effect of aspartic acids on protein formation may be due to their role of organic nitrogenous compound (amino acids) on the synthesis of proteins and enzymes[66].

Khodary[67] postulated that the increase in different constituents of carbohydrate accumulation may be referred back to the increase in photosynthetic pigments contents and stimulation of Rubisco activity. In previous work, Al-Wakeel *et al.*[22] observed that priming seeds with different concentrations of coumarin improved carbohydrates accumulation in sunflower leaves.

Regarding the effect of benzoic acid, it is known to provide abiotic stress tolerance[25]. Abdallah *et al.*[68] found that application of phenolic acids affected the nutritive value of wheat grains in terms of carbohydrate, protein and some macro-element contents. Dawood *et al.*[69] found that foliar spraying plants with benzoic acid increased oil% and carbohydrate% of the produced flax seeds. In addition, the increases in oil% could be attributed to the increase in the growth in vegetative parameters and nutrient uptake[70,51]. The induced improvement on yield quantity and quality might be

Table 7: Effect of foliar spraying with different concentrations of aspartic acid, coumarin and benzoic acid on fatty acids profile of flax oil (combined analysis of two seasons)

		Aspartic acid			Coumarin			Benzoic acid		
Fatty acid (%)	Control	50	100	200	25	50	100	50	100	200
Palmitic acid (C16:0))	12.16	8.75	8.70	9.01	8.76	8.67	8.73	11.25	9.29	8.28
Stearic acid (C18:0)	10.10	3.49	3.59	3.43	4.44	3.38	3.57	8.96	3.64	3.88
Oleic acid (C18:1) (MUFA)	11.49	12.58	11.18	11.59	11.42	11.01	11.63	15.09	11.34	11.98
*Linoleic acid (C18:2) (PUFA)	16.20	14.67	14.84	15.48	15.52	16.06	14.87	17.23	15.08	16.80
**Linolenic acid (C18:3n3) (PUFA)	49.90	60.40	61.69	60.49	59.86	60.89	61.08	47.47	60.51	59.06
Arachidic acid (C20:0)	0.15	0.11	0.00	0.00	0.00	0.00	0.11	0.00	0.14	0.00
Total unsaturated	77.59	87.65	87.72	87.56	86.80	87.96	87.58	79.79	86.93	87.84
Total saturated	22.41	12.35	12.29	12.44	13.22	12.05	12.41	20.21	13.07	12.16
TUS/ TS	3.462	7.1	7.14	7.04	6.57	7.30	7.06	3.95	6.65	7.22

*Omega 6, **Omega 3, PUFA: Polyunsaturated fatty acid, MUFA: Monounsaturated fatty acid

attributed to different treatment effects on activity of enzymes and translocation of the metabolites to the produced seeds.

Abdallah *et al.*[26] showed that foliar application of benzoic acid on quinoa increased significantly antioxidant compounds (flavonoid, phenolic and DPPH-radical scavenging capacity) compared to their controls. The increase in antioxidants in the present investigation may be due to the increase in the synthesis of carbohydrate (Table 6).

Changes in fatty acid composition of the seed yield: The results of gas liquid chromatographic analysis of fatty acids methyl esters in oil of the yielded flax seeds are presented in Table 7. Palmitic and stearic acids were the most predominant saturated fatty acid (12.16 and 10.10%) in control plants, while oleic, linoleic and linolenic acids were the main unsaturated fatty acids (11.49, 16.20 and 49.90%, respectively).

Treating flax plant with different concentrations of aspartic acid, coumarin and benzoic acid exhibited marked decreases in the levels of palmitic acid and total saturated fatty acids. Meanwhile, linolenic acid markedly increased and consequently total unsaturated fatty acids. It is worth to mention that the polyunsaturated fatty acids (Linolenic; omega-3) increased by different used treatments. The most effective treatment was aspartic acid despite the used concentrations, since it gave the highest increase in total unsaturated fatty acids (87.64% on average), while it markedly decreased total saturated fatty acids (12.36% on average) followed by coumarin and benzoic acid treatments, respectively.

The obtained results are quite similar to those obtained by Devi *et al.*[71], Bakry *et al.*[72] and Sadak*et al.*[73]. Abdel Rahim *et al.*[74] reported that the increase in percentage of unsaturated fatty acids accompanied by the decrease in saturated fatty acids proved the quality of oil. Polyunsaturated fatty acids are essential for human diets because of lowering the risk of heart diseases related to cholesterol oxidation. In addition, consumption of Oleic, Linoleic and Linolenic acids lowers the level of Low Density Lipoprotein (LDL) in human blood. These results are in accordance with those obtained by Ramadan *et al.*[62] who stated that, from consumer health point of view, the sunflower plants treated with amino acids increased its unsaturated fatty acids content. They added that this means an increase in omega-3 PUFA which would benefit human health due to its valuable influence on blood LDL level.

CONCLUSION

The obtained results suggested that the use of aspartic acid at the rate of 100 mg L^{-1} was promising in alleviating stress conditions on flax plants. It also improved the yield and its components as well as the nutritive value of seed yield under the current experimental site conditions. Moreover, the treatment with aspartic acid generally improved the polyunsaturated fatty acid:saturated fatty acid ratio followed by coumarin and benzoic acid treatments, respectively.

SIGNIFICANCE STATEMENT

With increasing global health interest, this work considers one of the efforts for the use of natural substances instead of chemicals in plant treatment to overcome adverse effects of environmental stress conditions. These attempts needed to be carried out by plant scientists for increasing plant productivity for increased human consumption demand without neglecting human health issues.

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