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Influence of biofertilizers on growth and some biochemical aspects of flax cultivars grown under sandy soil conditions

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Abstract

Background and objective: Flax (*Linum usitatissimum* L.) is an economic crop which has a dual purpose, for seeds and fibers. Biofertilizers have a significant effect on several metabolic processes; enhance plant growth and development via increasing of photosynthesis, endogenous hormones, ion uptake, nucleic acid, and protein synthesis. Thus this study deals with investigating the enhancing role of biofertilizers on the quality and quantity of three flax cultivars.

Materials and methods: A field experiment were carried out at the experimental Station of National Research Centre, Nubaria district, El-Behera Governorate, Egypt, during two successive winter seasons of 2015/2016 and 2016/2017 on flax cultivars (Line-3, Linola, and Sakha-1).

Results: Application of biofertilization treatments (Mycorrhiza, milk whey, yeast, and yeast extract as soil drench or foliar application, respectively) affects significantly most of the studied characters. Data show significant variations in vegetative growth parameters, photosynthetic pigments, carbohydrate, phenolic content, as well as seed yield, yield components, and nutritive value (oil, protein, flavonoid, and phenolic content) of the yielded seeds between three flax cultivars. Sakha-1 cultivar showed more adaptation to the conditions of sandy soil than the linola and Line-3 cultivars and reflected on the highest significant value of seed yield. All applied treatments caused significant increases in seed yield and its components of three flax cultivars under investigation. Sakha-1 cultivar showed the highest significant increase in seed yield/fed due to yeast extract treatment followed by milk whey treatment. Further, mycorrhiza treatment significantly increased seed yield of linola cultivar. Regarding Line-3, the highest significant increase in seed yield/fed was obtained by milk whey and yeast extract treatments respectively.

Conclusion: Yeast extract treatment is the most promising treatment that showed the highest significant increase in nutritive value of seed yield for the three flax cultivars under investigation. Yeast treatment caused the highest increase in total unsaturated fatty acid accompanied by the lowest decrease in total saturated fatty acid of three flax cultivars.

Keywords: Biofertilizers, Chemical composition, Fatty acids, Flax, Oil, Yield

Introduction

In Egypt, flax (*Linum usitatissimum* L.) is grown as a dual purpose crop (seed for oil and stem for fiber). Flaxseed oil is edible and has a very healthy fatty-acid profile, with low levels (approximately 9%) of saturated fat, moderate levels (18%) of monounsaturated fat, and high concentrations (73%) of polyunsaturated fatty acids (PUFAs). The by-product remaining after oil extraction—flaxseed meal—is a source of protein used in livestock feeds (Newkirk 2015). It is necessary to increase flax productivity per unit area which could be achieved by selecting high yielding cultivars and improving the agricultural treatments as well as treated plant with biofertilizers.

Recently, a great attention has been paid on the possibility of using natural safety substances to improve plant productivity and quality. Bio-stimulants have significant effect on several metabolic processes; enhance plant

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growth and development via increasing of photosynthesis, endogenous hormones, ion uptake, nucleic acid, and protein synthesis (Abbas 2013). In addition, biofertilization is very safe for human, animal, and environment to get lower pollution via decrease mineral usage fertilization as well as saving fertilization cost.

Active dry bread yeast is a natural safety biofertilizer which is usually added to soil or used as a foliar application on different crops because of their bioactivity and safety for human and the environment. It has been reported to be a rich source of phytohormones (especially cytokinins), carbohydrates, protein, vitamins, enzymes, amino acids, and minerals. It stimulates nucleic acid synthesis, chlorophyll formation, cell division, and enlargement and have protective role against different stresses (Shehata et al. 2012). Moreover, yeast also facilitates the growth of plants by inducing nutrient minerals absorption through improvement of soil pH to acidity (Pawte et al. 1985) and converting insoluble form of phosphorous into soluble on, thus enhancing phosphorous availability to plants (Abbas 2013). Moreover, Yeo et al. (2000) found that yeast extracts contain trehalose-6-phosphate synthase which is a key enzyme for trehalose biosynthesis. They suggested that the production of trehalose not only affects plant development but also improves stress tolerance. A diverse range of yeasts exhibit plant growth promoting characteristics, including pathogen inhibition (El-Tarabily and Sivasithamparam 2006), phytohormone production (Nassar et al. 2005), N and S oxidation (Falih and Wainwright 1995), phosphate solubilization, and stimulation of mycorrhizal-root colonization (Mirabal-Alonso et al. 2008).

Arbuscular mycorrhizal fungi (AMF) form symbiotic association with most plant species. These fungi enhance uptake of relatively immobile nutrients particularly phosphorus and other micronutrients and improve soil structure and quality (Soliman et al. 2012). Mycorrhizal fungi interact with a wide range of other soil organisms in the root, rhizosphere, and in the bulk soil. These interactions may be inhibitory or stimulatory; some are clearly competitive and others may be mutuality. These bio-elicitors increase producing primary productions and providing more resources for production of secondary metabolites through increase of available nutrient uptake (Garcia-Garrido and Ocampo 2002). AM fungi play an important role in regulation of water uptake (Marulanda et al. 2003), increasing antioxidant activity (Marulanda et al. 2007), osmotic adjustment (Wu et al. 2006), hormone relations (Estrada-Luna and Davies 2003), soil fertility, plant nutrition through enhancing the uptake, and translocation of mineral nutrients from soil to host plants (Ceccarelli et al. 2010), and plant protection against biotic and abiotic stresses (Smith and Read 2008).

Regarding whey milk, Hilshey (2014) mentioned that raw cow milk has been suggested as an effective bio-stimulant. It contains proteins and other compounds which have been observed to suppress plant disease and enhance plant tolerance to stress and enhanced nutrient uptake. Milk whey may be defined broadly as the watery part of milk remaining after the coagulation of milk and separation of the curd (Zadow 1994). Milk whey has low economic value and is composed of 93% water and 7% solids; it is rich in minerals, thus the use of milk whey is being important to enhance nutrient outflow to plants. Milk whey may be used as a biofertilizer to improve crop development and plant nutritional status (Demir and Ozrenk 2009; Erman et al. 2011; Ocak and Demir 2012).

This work aimed to investigate the physiological effect of biofertilizer (mycorrhiza; milk whey; yeast extract) on three flax cultivars grown under sandy soil conditions.

Materials and methods

Two field experiments were carried out at the experimental Station of National Research Centre, Nubaria district El-Behrea Governorate, Egypt, during two successive winter seasons of 2015/2016 and 2016/2017. Soil of the experimental site was sandy soil where mechanical and chemical analysis is reported in Table 1 according to Chapman and Pratt (1978).

The experimental design was split plot design with three replications, where flax seed cultivars (Line-3; Linola; Sakha-1) occupied the main plots, while the biofertilizers treatments were allocated at random in sub plots as follow:

1- Control
2- Mycorrhizal fungi (1 kg/fed) was added to the soil during seed sowing.
3- Milk whey (50 L/fed) was sprayed on plant after 45 days from sowing.
4- Yeast extract (1) (75 L/fed) was added to the soil after 45 days from sowing.
5- Yeast extract (2) (75 L/fed) was sprayed on plant after 45 days from sowing.

Chemical content of milk whey was determined according to Abdel-Rahman and Abo-Hamed (1992) and shown in Table 2.

Flax seeds were sown on 25th November in both seasons in rows 3.5 m 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 recommended agricultural practices for growing flax seed were applied and the seeding rate was 2000 seeds/m^2. Then, 150 kg/fed calcium super-phosphate (15.5% P_2O_5) was applied before sowing. Whereas, nitrogen was applied at five equal doses after emergence in the form of ammonium nitrate 33.5% at rate of 75 kg/fed. Potassium sulfate (48.52% K_2O) was added at two equal doses of 50 kg/fed.

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

A. Mechanical analysis											
Sand		Silt 20-0 μ%	Clay < 2 μ%	Soil texture							
Course 2000-200 μ%	Fine 200-20 μ %										
47.46	36.19	12.86	4.28	Sandy							
B. Chemical analysis											
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^{--}
7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89
C. Nutritional analysis											
Available nutrients											
Macro element ppm		Micro element ppm									
N	P	K	Zn	Fe	Mn	Cu					
52	12.0	75	0.14	1.4	0.3	0.00					

Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Plant samples were taken after 60 days from sowing for measurement growth characters (shoot height; root length; number of basal branch/plant; fresh and dry weight of shoot and root/plant). Photosynthetic pigments, total indole acetic acid (IAA), and phenolic content were determined in fresh leaf. Total carbohydrates, total soluble carbohydrates, and polysaccharides were determined in dry leaf.

At harvest, random samples of ten guarded plants/plot were collected to estimate the following characters: plant height, technical stem length, fruiting zone length, number of basic branch/plant, number of fruiting branches/plant, number of capsules/plant, seed yield/plant, weight of 1000 seeds, biological yield/plant (g), seed yield (kg/fed), and straw yield/ (ton/fed). Oil, protein, flavonoids, and phenolic contents were determined in the yielded seeds. The fatty acid profile was determined and identified in the yielded oil.

Chemical analysis

Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) of fresh leaves was determined according to Moran (1982). Total carbohydrates were determined calorimetrically according to the method of Dubois et al. (1956). Soluble carbohydrate was determined according to Smith et al. (1956). Polysaccharide was calculated by the difference between total carbohydrate and soluble carbohydrate. Indole acetic acid content were extracted and analyzed by the method of Larsen et al. (1962). Phenolic compounds were determined by using Folin Ciocalteu reagent as described by Makkar et al. (1997). Seed oil content was determined using Soxhlet apparatus and petroleum ether (40–60 °C) according to A.O.A.C (1990). The resultant defatted meal is used for determination of protein, phenolic compound, and flavonoids. The protein contents were determined by micro-kjeldahl method according to Miller and Houghton (1945). Total flavonoid contents were measured by the aluminum chloride colorimetric assay as described by Ordoñez et al. (2006). Methyl esters of fatty acids were prepared from an aliquot of total lipid according to Harborne (1984). Identification and quantitative determination of fatty acid were performed using gas liquid chromatography.

Statistical analysis

The obtained results were subjected to statistical analysis of variance according to method described by Snedecor and Cochran (1980), 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 calculated according to the method of Gomez and Gomez (1984). Means were compared by using least significant difference (L.S.D.) at 5% levels of probability.

Results

Variations in vegetative growth, biochemical constituents, and yield between three flax cultivars

Significant variations in most of vegetative growth parameters, photosynthetic pigments, carbohydrate, phenolic

Table 2 Chemical content of milk whey

Contents	Water	Lactose	Nitrogenous matter	Fat	Lactic acid	Ash	N	P	K
%	93.1	4.8	0.9	0.3	0.2	0.6	0.3	0.075	0.35

content, as well as seed yield, yield components, and nutritive value (oil, protein, flavonoid, and phenolic content) of the yielded seeds between three flax cultivars are shown in Tables 3 and 4.

Sakha-1 cultivar was characterized by highest shoot height, root length, chlorophyll b and polysaccharides, as well as seed and straw yield/fed, protein percentage. The highest significant values of carotenoid, total and soluble carbohydrate, IAA, and phenolic content appeared in linola cultivar. Whereas, the highest fresh and dry weight of shoot and root as well as total photosynthetic pigments, oil percentage, and flavonoid content appeared in Line-3 cultivar. It is worthy to mention that Sakha-1 cultivar showed more adaptation to the conditions of sandy soil than the linola and Line-3 cultivars and reflected on the highest significant value of seed yield (619.28 kg/fed).

Variations in vegetative growth, biochemical constituents, and yield of flax under effect of biofertilizers

All applied treatments showed significant variations in most investigated parameters (vegetative growth parameters, photosynthetic pigments, carbohydrate, phenolic content, as well as seed yield, yield components, and nutritive value of the yielded seeds as shown in Tables 5 and 6).

It is obvious that yeast extract (2) treatment showed the highest significant increase in fresh and dry weight of both shoot and root/plant, total carbohydrate, IAA, phenolic content of leaf, capsules yield/plant, straw and seed yield/fed, as well as oil and protein content of the yielded seeds. On the other hand, mycorrhiza treatment showed the lowest significant increase in total carbohydrate; IAA; capsules, biological, and seed yield/plant; straw and seed yield/fed; and nutritive value of the yielded seeds (oil, protein, flavonoid, and phenolic content). It is worthy to mention that both yeast extract (1) and milk whey treatments showed moderate significant effect on flax plant.

Variations in vegetative growth and some biochemical constituents of leaf under the interaction effect between flax cultivars and biofertilizers

Regarding sakha 1 cultivar, it was found that interaction between sakha 1 cultivar and mycorrhiza treatment showed the highest significant increase in fresh and dry weight of both shoot and root/plant. Whereas, the interaction with yeast extract (2) treatment showed the highest significant increase in total carbohydrate, total soluble carbohydrate, IAA, and phenolic content as shown in Tables 7 and 8.

In respect to linola cultivar, Tables 7 and 8 show that the interaction with mycorrhiza treatment significantly increased fresh and dry weight of root and resulted in the lowest significant increase in biochemical constituents of leaf (total carbohydrate, IAA, and phenolic content). On the other hand, the interaction with yeast extract (2) decreased fresh and dry weight of both shoot and root/plant and significantly increased total carbohydrate, IAA, and phenolic content. Interaction between

Table 3 Variations in growth parameters and chemical content between three flax cultivars grown under sandy soil conditions

Characters	Cultivars			L.S.D. 0.05
	Line-3	Linola	Sakha-1	
Shoot height (cm)	66.60	62.80	78.97	2.17
Root length (cm)	11.33	12.17	14.63	1.03
Number of basal branch	4.13	3.47	2.43	0.33
Shoot fresh wt. (g)	28.19	10.77	20.14	3.14
Root fresh wt. (g)	3.52	1.58	2.63	1.05
Shoot dry wt. (g)	7.28	3.19	5.70	1.12
Root dry wt. (g)	0.97	0.37	0.77	0.27
Chlorophyll a (mg/100 g fresh leaf)	1.60	1.32	1.57	0.18
Chlorophyll b (mg/100 g fresh leaf)	0.52	0.45	0.52	0.06
Carotenoids (mg/100 g fresh leaf)	0.43	0.45	0.38	0.01
Total pigments (mg/100 g fresh leaf)	2.55	2.22	2.47	0.11
Total carbohydrate content (%)	19.07	20.79	20.67	0.46
Total soluble carbohydrate (%)	1.77	2.45	1.54	0.17
Polysaccharides (%)	17.31	18.34	19.14	0.43
Indole acetic acid (μg/g fresh leaf)	29.48	46.16	34.66	2.53
Total phenolic content (mg/100 g fresh leaf)	54.34	56.72	45.24	1.33

Table 4 Variations in seed yield, its related characters, and nutritive value between three flax cultivars grown under sandy soil conditions

Characters	Cultivars			L.S.D. 0.05
	Line-3	Linola	Sakha-1	
Plant height (cm)	88.50	75.94	76.07	2.18
Fruiting zone length (cm)	30.33	26.66	33.40	2.05
Technical stem length (cm)	58.17	49.28	42.67	3.22
No. of basle branch/ plant	2.93	3.27	4.60	0.55
Biological yield /plant (g)	14.26	12.33	26.38	1.15
No. of fruiting branches / plant	27.33	21.70	34.40	3.16
No. of capsules/ plant	71.87	79.40	149.80	6.33
Capsules yield /plant (g)	9.82	5.73	13.31	3.12
Seed yield /plant (g)	6.28	4.02	5.40	1.08
1000 seeds weight (g)	3.93	2.78	4.68	0.27
Straw yield (ton/fed)	1.86	2.00	3.03	0.21
Seed yield (kg/fed)	399.92	446.88	619.28	35.18
Oil content (%)	22.52	22.36	21.42	0.37
Protein content (%)	19.72	21.18	22.45	0.27
Total phenolic content (mg/100 g dry seed)	287.00	354.63	222.43	8.99
Total flavonoids (mg/100 g dry seed)	45.13	23.95	19.29	3.30

milk whey and linola cultivar showed significant increase in fresh and dry weight of shoot. Line 3 cultivar interaction with yeast extract (2) showed the highest significant increase in fresh and dry weight of both shoot and root/plant, biochemical constituents of leaf (Tables 7 and 8).

Variations in seed yield and yield components under interaction effect between flax cultivars and biofertilizers

It is clear that sakha-1 cultivar showed the highest seed yield/fed (417.60 kg) followed by linola and Line-3 cultivars under control treatment as shown in Table 9. All

Table 5 Effect of bio-fertilizer treatments on growth parameters and chemical content of flax plants grown under sandy soil conditions

Characters	Treatments					L.S.D. 0.05
	Control	Micro	Milk whey	Yeast extract (1)	Yeast extract (2)	
Shoot height (cm)	63.50	66.72	74.56	73.33	69.17	2.19
Root length (cm)	10.33	14.00	13.61	13.06	12.56	1.32
Number of basal branch	2.78	3.94	3.72	2.67	3.61	0.22
Shoot fresh wt. (g)	9.85	23.18	22.28	17.88	25.31	1.05
Root fresh wt. (g)	1.06	3.17	3.12	1.99	3.53	0.25
Shoot dry wt. (g)	2.78	6.31	6.18	4.84	6.86	0.22
Root dry wt. (g)	0.35	0.98	0.78	0.48	0.92	0.19
Chlorophyll a mg/100 g fresh wt	1.170	1.601	1.769	1.437	1.521	0.01
Chlorophyll b	0.489	0.501	0.522	0.512	0.468	0.10
Carotenoids	0.404	0.438	0.509	0.366	0.389	0.05
Total pigments	2.064	2.540	2.801	2.316	2.377	0.18
Total carbohydrate content (%)	17.82	19.74	20.42	20.77	22.37	0.53
Total soluble carbohydrate (%)	1.79	1.79	1.97	2.01	2.07	0.10
Polysaccharides (%)	16.03	17.95	18.45	18.76	20.30	0.51
IAA (μg/g fresh leaf)	26.02	33.25	37.35	40.64	47.15	3.62
Total phenolic(mg/100 g fresh leaf)	41.13	52.84	46.87	54.44	59.97	1.38

Table 6 Effect of bio-fertilizer treatments on seed yield, its related characters, and nutritive value of flax plants grown under sandy soil conditions

Characters	Treatments					L.S.D. 0.05
	Control	Micro	Milk whey	Yeast extract (1)	Yeast extract (2)	
Plant height (cm)	71.33	78.34	85.67	88.17	77.34	2.13
Fruiting zone length (cm)	24.43	30.22	33.78	32.11	30.11	1.19
Technical stem length (cm)	46.90	48.12	51.89	56.05	47.23	1.77
No. of basal branch/plant	2.67	3.67	3.89	3.67	4.11	0.33
Biological yield/plant (g)	10.97	16.80	20.87	21.75	17.91	1.17
No of fruiting branches/plant	19.05	31.55	34.78	29.11	24.56	2.35
No .of Capsules/plant	64.00	88.78	115.66	122.67	110.67	7.17
Capsules yield/plant (g)	5.01	8.78	10.12	11.79	12.39	2.08
Seed yield/plant (g)	3.44	4.29	4.98	7.36	6.09	0.24
1000 seeds weight (g)	3.46	3.91	3.93	3.87	3.81	0.11
Straw yield (ton/fed)	1.42	2.11	2.66	2.42	2.87	0.21
Seed yield (kg/fed)	284.00	489.73	574.93	526.00	568.80	30.55
Oil content (%)	20.11	21.84	21.67	22.82	23.69	0.43
Protein content (%)	19.18	20.72	20.74	21.89	22.67	0.31
Total phenolic mg/100 g dry seed	228.87	270.79	297.24	326.58	325.83	10.38
Total flavonoids mg/100 g dry seed	24.64	28.38	28.34	31.75	33.06	0.34

applied treatments caused significant increases in seed yield and its components of three flax cultivars under investigation. Sakha-1 cultivar showed the highest significant increase in seed yield/fed due to yeast extract (2) treatment (781.20 kg/fed) followed by milk whey treatment (706.0 kg/fed). Since these treatments caused increases in seed yield by 87.1% and 69.1%, respectively relative to control treatment. Further, mycorrhiza treatment significantly increased seed yield of linola cultivar by 177.6% followed by yeast extract (2) treatment (114.9%) relative to control treatment. Regarding Line-3, the highest significant increase in seed yield /fed was

Table 7 Effect of interaction between flax cultivars and bio-fertilizer treatments

Cultivars	Treatments	Shoot height (cm)	Root length (cm)	No. of branch	Shoot F wt. (g)	Root F wt. (g)	Shoot D wt. (g)	Root D wt. (g)	Chlor a (mg/100g fresh wt)	Chlor b	Carot	Total pigments
Line-3	Control	59.50	7.50	3.00	10.15	0.75	2.60	0.05	1.483	0.552	0.360	2.395
	Micro	62.33	10.00	5.67	29.67	3.35	7.23	1.10	1.596	0.561	0.400	2.557
	Milk whey	75.00	12.50	4.50	31.75	4.10	8.95	1.20	2.234	0.669	0.650	3.553
	Yeast extract (1)	67.67	12.67	3.00	22.97	3.03	6.13	0.80	1.220	0.369	0.370	1.959
	Yeast extract (2)	68.50	14.00	4.50	46.40	6.35	11.50	1.70	1.492	0.472	0.380	2.344
Linola	Control	58.67	9.50	3.67	8.67	1.03	2.53	0.40	0.426	0.426	0.430	1.282
	Micro	59.33	17.50	3.67	12.53	2.07	3.73	0.55	1.849	0.506	0.560	2.915
	Milk whey	62.33	12.67	4.67	15.57	2.83	4.13	0.43	1.480	0.443	0.440	2.363
	Yeast extract (1)	68.00	11.50	3.00	9.80	1.17	3.07	0.30	1.491	0.438	0.420	2.349
	Yeast extract (2)	65.67	9.67	2.33	7.30	0.80	2.50	0.15	1.371	0.418	0.390	2.179
Sakha 1	Control	72.33	14.00	1.67	10.73	1.40	3.20	0.60	1.360	0.436	0.350	2.146
	Micro	78.50	14.50	2.50	27.35	4.10	7.95	1.30	1.594	0.454	0.440	2.488
	Milk whey	86.33	15.67	2.00	19.53	2.43	5.47	0.70	1.601	0.489	0.430	2.52
	Yeast extract (1)	84.33	15.00	2.00	20.87	1.77	5.33	0.35	1.602	0.730	0.310	2.642
	Yeast extract (2)	73.33	14.00	4.00	22.23	3.43	6.57	0.90	1.699	0.513	0.390	2.602
L.S.D. 0.05		2.77	0.57	0.23	1.15	0.35	0.18	0.05	0.105	0.025	0.020	0.056

Table 8 Effect of interaction between flax cultivars and bio-fertilizer treatments on some chemical contents of leaf

Cultivars	Treatment	Total carbohydrate%	TSS%	Polysaccharides%	IAA μg/100 g	Phenolic mg/100 g
Line 3	Control	16.62	1.36	15.27	21.71	44.21
	Micro	18.21	1.58	16.64	28.66	53.77
	Milk whey	21.65	1.87	19.78	34.25	58.35
	Yeast extract (1)	20.22	1.86	18.36	31.64	57.13
	Yeast extract (2)	21.24	2.28	18.97	35.91	62.25
Linola	Control	18.78	2.41	16.37	31.56	42.80
	Micro	20.31	2.62	17.69	39.28	62.42
	Milk whey	20.68	2.54	18.14	46.35	57.35
	Yeast extract (1)	21.33	2.61	18.73	52.14	58.28
	Yeast extract (2)	22.73	2.16	20.57	61.64	63.36
Sakha 1	Control	18.07	1.61	16.46	24.80	36.39
	Micro	20.70	1.18	19.53	31.81	42.34
	Milk whey	20.04	1.47	18.57	35.35	43.75
	Yeast extract (1)	20.78	1.58	19.20	38.14	47.92
	Yeast extract (2)	23.14	1.78	21.37	43.90	54.30
L.S.D. 0.05%		1.08	0.16	1.02	2.26	1.77

Table 9 Effect of interaction between flax cultivars and bio-fertilizer treatments on seed, straw yield, and its related characters of flax cultivars grown under sandy soil conditions

Cultivars	Treatments	Plant height	Fruiting zone length	Technical stem length	No. of Basle branch/ plant	Biol yield/ plant	No of fruiting branches/ plant	No .of capsules/ plant	Capsules yield/ plant (g)	Seed yield/ plant (g)	1000 seeds weight (g)	Straw yield (ton/fed)	Seed yield (kg/fed)
Line-3	Control	75.00	23.67	51.33	2.67	10.30	19.33	57.33	5.03	4.03	3.67	1.07	208.80
	Micro	86.67	30.33	56.34	3.00	11.03	41.33	62.00	8.37	5.37	3.95	1.13	335.20
	Milk whey	105.67	34.33	71.34	3.00	15.73	29.67	72.33	9.83	6.53	4.16	2.56	591.60
	Yeast extract (1)	94.50	33.00	61.50	3.00	21.53	27.67	103.00	11.93	9.43	3.80	1.82	423.60
	Yeast extract (2)	80.67	30.33	50.34	3.00	12.73	18.67	64.67	13.93	6.03	4.05	2.72	440.40
Linola	Control	73.67	24.30	49.37	2.33	10.77	17.50	57.67	3.97	2.87	2.62	1.16	225.60
	Micro	74.67	26.33	48.34	3.67	13.53	23.33	71.67	6.30	4.13	2.87	2.68	626.40
	Milk whey	69.67	31.33	38.34	3.33	10.97	28.00	87.33	4.90	3.63	2.73	1.73	427.20
	Yeast extract (1)	85.00	25.67	59.33	3.00	15.80	20.33	97.33	8.43	5.83	2.86	2.19	470.40
	Yeast extract (2)	76.67	25.67	51.00	4.00	10.57	19.33	83.00	5.03	3.63	2.81	2.23	484.80
Sakha-1	Control	65.33	25.33	40.00	3.00	11.83	20.33	77.00	6.03	3.43	4.08	2.04	417.60
	Micro	73.67	34.00	39.67	4.33	25.83	30.00	132.67	11.67	3.37	4.91	2.51	507.60
	Milk whey	81.67	35.67	46.00	5.33	35.90	46.67	187.33	15.63	4.77	4.90	3.69	706.00
	Yeast extract (1)	85.00	37.67	47.33	5.00	27.93	39.33	167.67	15.00	6.83	4.94	3.26	684.00
	Yeast extract (2)	74.67	34.33	40.34	5.33	30.43	35.67	184.33	18.20	8.60	4.57	3.66	781.20
LSD 0.05		2.05	1.13	2.33	0.36	1.27	2.04	7.09	0.57	0.21	0.11	0.23	25.77

183.3% and 110.9% due to milk whey and yeast extract (2) treatment respectively.

Variations in nutritive value of seed under interaction effect between flax cultivars and biofertilizers

Data in Table 10 showed that Line-3 and linola cultivars have approximately the same oil percentage (20.69 and 20.77%) under control treatment. The sakha-1 cultivar has the lowest oil percentage (18.88%) and highest protein percentage (20.67%). Line-3 cultivar has the highest values of secondary metabolites (flavonoid and phenolic content) followed by linola and sakha-1 cultivars under control. It is important to mention that the yeast extract (2) treatment is the most promising treatment that showed the highest significant increase in nutritive value of the yielded seeds of the three flax cultivars under investigation.

Variations in fatty acid composition of the yielded oil under interaction effect between flax cultivars and biofertilizers

The main fatty acids of the fixed oil as determined with GLC are shown in Table 11. The data indicate that palmitic acid is the predominant saturated fatty acid in three flax cultivars. Oleic acid is the predominant unsaturated fatty acid in Line-3 and linola cultivars followed by linoleic acid. Whereas, oil of sakha-1 cultivar characterized by high percentage of linoleic acid (40.65%) followed by oleic acid (22.74%) and linolenic acid (10.85%) under control treatment.

All applied treatments caused marked increases in total unsaturated fatty acid of three flax cultivars except mycorrhiza and milk whey treatments showed flight decreases in total unsaturated fatty acid of lenola cultivar. Regarding saturated fatty acid, it was decreased in lenola and sakha-1 cultivars by all treatments. Yeast extract (1) treatment only caused a marked decrease in Line-3 cultivar. It is worthy to mention that the most promising treatment that caused the highest increase in total unsaturated fatty acid accompanied by the lowest decrease in total saturated fatty acid of three flax cultivars was yeast extract (1) treatment.

Discussion

The significant variations between three flax cultivars under investigation are shown in Tables 3 and 4. These results were confirmed recently by Bakry et al. (2013, 2014). The significant variations between three flax cultivars may be due to the differences of these cultivars in genetic constituent, origin, and growth habit.

This increase in growth parameters in response to the application of active dry yeast may be attributed to its contents of different macro- and micronutrients, growth regulators, proteins, and vitamins that stimulate plant to build up dry matters (Mirabal-Alonso et al. 2008). It is also a natural source of cytokinins that stimulates cell proliferation and differentiation, controlling shoot and root morphogenesis and chloroplast maturation, as well as the synthesis of protein and nucleic acid (Amer 2004).

Regarding mycorrhiza, a symbiotic association between beneficial soil fungi and plant roots are characterized by a bi-directional movement of nutrients, where carbon flows to the fungus and inorganic nutrients move to the plants (Abdel-Fattah 2001) leading to improve

Table 10 Effect of interaction between flax cultivars and bio-fertilizer treatments on nutritive value of the yielded seeds

Cultivars	Treatments	Oil%	Protein %	Phenolic content mg/100 g	Flavenoids mg/100 g
Line 3	Control	20.69	17.43	274.67	40.73
	Micro	22.80	19.32	239.81	44.04
	Milk whey	23.54	19.87	287.65	46.98
	Yeast extract (1)	22.95	20.78	307.20	47.66
	Yeast extract (2)	23.85	21.35	326.30	48.10
Linola	Control	20.77	19.43	229.63	19.21
	Micro	21.98	20.53	363.18	24.75
	Milk whey	22.57	20.14	347.52	25.87
	Yeast extract (1)	22.98	22.07	430.44	24.55
	Yeast extract (2)	23.70	22.70	395.25	27.29
Sakha 1	Control	18.88	20.67	182.30	13.98
	Micro	20.75	22.32	209.38	16.35
	Milk whey	21.54	22.79	238.94	23.41
	Yeast extract (1)	22.52	22.83	242.11	23.03
	Yeast extract (2)	23.53	23.97	255.93	23.80
L.S.D. 0.05%		0.33	0.64	21.22	0.69

Table 11 Effect of interaction between flax cultivars and bio-fertilizer treatments on fatty acid composition of the yielded oil

Fatty acid %	Cultivar/Treatment														
	Line 3					Lenola					Sakha 1				
	C	M	S	Y1	Y2	C	M	S	Y1	Y2	C	M	S	Y1	Y2
Myristic (C14:0)	0.31	0.33	0.34	0.41	0.47	0.54	0.35	0.65	0.75	0.57	0.74	0.25	0.44	0	0.15
Palmitic (C16:0)	9.97	7.78	8.68	8.84	7.20	11.90	10.03	10.75	9.36	10.91	10.16	10.91	10.25	9.17	10.75
Stearic (C18:0)	2.14	4.65	3.58	3.24	4.65	6.36	8.55	6.01	6.10	6.84	8.33	7.49	8.02	8.15	8.935
Oleic (C18:1)	59.06	56.32	58.68	57.34	57.69	61.17	61.45	62.02	62.59	65.44	22.74	24.65	24.68	22.70	22.25
Linoleic (C18:2)	18.88	23.68	22.14	22.49	22.87	16.35	14.67	14.32	17.35	13.29	40.65	41.96	42.35	47.32	43.65
Linolenic (C18:3)	3.69	3.65	3.54	3.65	2.47	2.24	2.27	2.35	2.45	2.54	10.85	12.04	12.52	11.66	11.07
Beheric (C22:0)	0.21	0.245	0.21	0.37	0.41	0.33	0.05	0.05	0.43	0.26	0.25	0.25	0.29	0.35	0.046
Lignocenic (C24:0)	0.01	0.246	0.05	0.15	0.17	0.25	0.48	0.46	0.01	0.05	0.05	0.15	0.18	0.32	0.05
Total unsaturated	81.63	83.65	84.36	83.48	83.03	79.76	78.39	78.69	82.39	81.27	74.25	78.65	79.55	81.68	76.97
Total saturated	12.65	13.25	12.86	13.00	12.90	19.38	19.46	17.92	16.64	18.62	19.52	19.04	19.16	17.99	19.92
Total identified	94.28	96.90	97.23	96.48	95.93	99.14	97.84	96.61	99.04	99.89	93.77	97.69	98.71	99.68	96.89
T Uns./TS	6.45	6.31	6.56	6.42	6.44	4.12	4.03	4.39	4.95	4.36	3.80	4.13	4.15	4.54	3.86

plant growth and reproduction (Cekic et al. 2012). Mycorrhiza enhances growth of the plants by increased absorption of water and nutrients from soil due to increase in root surface area (Khan et al. 2000). Bass and Kuiper (1989) stated that AM fungal inoculation increased the cytokinins content of shoots which in turn promote the cell division and cell expansion and play major role in shoot morphology. AM fungi are associated with improved growth of many plant species due to increased nutrients uptake, production of growth-promoting substances, delays senescence, increases leaf area, and modifies root architecture (Cekic et al. 2012). Other factors that could be associated with mycorrhiza colonization are improved leaf water, turgor potentials, maintenance of stomatal opening and transpiration, and increased rooting length and depth. Mycorrhization is beneficial to the host plant as it stimulates the growth of the seedlings (Machineski et al. 2009) and accumulates nutrients in the aerial parts (Ngwene et al. 2010), besides protecting the plant from pathogens (Elsen et al. 2008).

The use of whey as biofertilizer is being important for plant nutrition and growth (Sensoy et al. 2013). The main constituents of milk whey are N, P, K, S, Ca, Na, Mg, lactose, and proteins (Morris 1985). Demir et al. (2015) reported that single, dual, and triple applications of whey (50 mL kg^{-1}) improved the morphological growth and nutritional status of all three host species (tomato, pepper, and eggplant). Demir and Ozrenk (2009) and Erman et al. (2011) reported that a combined application of whey and AMF significantly increased macro- and micronutrient contents of chickpeas and lentils grown in pots and in field conditions. Haroun and Ibrahim (2003) found that whey treatment at 50% level induced a marked increase in shoot length, shoot fresh and dry masses, and total leaf area of wheat plants.

The improvement of photosynthetic pigments in response to the foliar application of active dry yeast may be attributed to its bio-regulator role which affects the balance between photosynthesis and photorespiration in plants (Olaiya 2010) and delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis (Shalaby and El-Nady 2008).

Regarding mycorrhiza, improvement photosynthesis in plants through mycorrhiza symbiosis is mainly due to the increase in transporting of inorganic elements from soil to plants. Enhanced mineral nutrition helps in increased chlorophyll content thus helping in higher photosynthetic rate (Feng et al. 2002). Zhu et al. (2010) reported that inoculated maize plants with mycorrhiza increased chlorophyll content. Similar results were reported by Abdallah et al. (2013) on sunflower plant and Abdallah et al. (2015) on wheat plant.

Regarding milk whey, Haroun and Ibrahim (2003) found that 50% whey level improved photosynthetic pigments and increased total ^{14}C photoassimilates.

Yeast is rich in tryptophan which is considered as a precursor of indole acetic acid (IAA) which stimulates cell division and elongation (Warring and Phillips 1973).

Addition of mycorrhiza to soil caused significant increases in IAA contents and these results are in agreement with those obtained by Abdallah et al. (2013) on sunflower plant and Abdallah et al. (2015) on wheat plant.

Moreover, all applied treatments may prevent the oxidative degeneration of IAA and consequently increased the level of IAA in plants.

Yeast extract triggered the production of endogenous jasmonic acid and/or methyl jasmonate, which influence the production of secondary metabolites as reported by Sanchez-Sampedro et al. (2005). Abraham et al. (2011) indicated that yeast extract did not perform as nutrient supplement for the growth of in vitro plantlets of *Curcuma mangga* but with optimum amount it could be used for the enhancement of phenolics production.

Regarding mycorrhiza, AMF infection induces a chemical defense of host plants as a result of changes in their metabolism (Pozo and Azco'n-Aguilar 2007). Symbiosis with AMF created significant changes in the enzymatic activities (Marin et al. 2002) and physiological mechanisms, which lead to the accumulation of secondary metabolites such as carotenoids and polyphenols in host plants (Schliemann et al. 2008). Dixon et al. (1994) mentioned that phenolics are the main compounds in pathogenic interactions between plants and fungi. In this respect, Ceccarelli et al. (2010) showed an increase in the content of phenolic compounds in inoculated Artichoke plants with fungi in comparison to non-mycorrhizal plants. Volpin et al. (1994) showed that inoculation with mycorrhizal fungi leads to increasing in PAL enzyme activity and flavonoids accumulation in the roots of mycorrhizal alfalfa plants compared to non-mycorrhizal plants.

Yeast exerts significant role in increasing the release of carbon dioxide through fermentation process leading to increase in photosynthetic pigments and effectively activates the photosynthesis process or may be due to its enhancing role on cell division, cell elongation producing more leaf area (Hayat 2007; Stino et al. 2009), and consequently accelerates the biosynthesis of carbohydrates (Kurtzman and Fell 2005). Yeast extract was suggested to participate in a beneficial role during vegetative and reproductive growths through improving flower formation and their set in some plants due to its high auxin and cytokinins content and enhancement of carbohydrates accumulation (Barnett et al. 1990).

Regarding mycorrhizal treatment, our obtained data are in harmony with those of Abdallah et al. (2013) on *Helianthus annuus L.* who reported that the increase in total carbohydrates are positively correlated with AM of the host plant. Porcel and Ruiz-Lozano (2004) reported that the positive correlation between sugar content and AM is due to the sink effect of the fungus demanding sugars from the shoot tissues (Augé 2001). In addition, MF have significant role in increasing the contents of chlorophylls and rate of photosynthesis thus increased carbohydrate synthesis (Swaefy et al. 2007).

Regarding milk whey, Haroun and Ibrahim (2003) found that whey treatment at 50% level increased total ^{14}C photoassimilates and consequently soluble and insoluble carbohydrate fractions of wheat plants as compared with control plants. Furthermore, this treatment induced a noticeable increase in total carbohydrates and total nitrogen content of wheat seedlings.

Yeast extract has a beneficial role during vegetative and reproductive growth through improving flower formation and their set of some plants due to its high auxin and cytokinin contents and enhancement of carbohydrate accumulation (Barnett et al. 1990). The high content of dry yeast from vitamin B_5 and minerals might play a considerable role in orientation and translocation of metabolites from leaves (source) into the seeds (sink) (Mohamed et al. 1999). Mekki and Ahmed (2005) reported that application of yeast increased yield and yield attributes of soybean plants. Moreover, khalil and Ismael (2010) indicated that the highest growth parameters were observed when *Lupinus termis* plants treated with yeast by different ways resulting in an increase in yield and yield attributes. In addition, foliar application with yeast gave the highest significant values of nitrogen, protein, and carbohydrate percentages.

Regarding AMF, the increase in yield and yield components mainly attributed to the significant effect of mycorrhiza fungus on absorbing various nutrients such as nitrogen, calcium, potassium, copper, zinc, sulfur, and especially phosphorous (Sharifi et al. 2007). Since, MF inoculation leads to lower pH of the soil and favorable air water balance, which shows positive impacts on the yield Habashy et al. 2008). Moreover, mycorrhizal inoculation have a positive effect on plant growth by stimulating the production of growth regulating substances, improving the rate of photosynthesis and transpiration (Cho et al. 2009), enhancing the concentration of different organic compounds in root (Selvaraj et al. 1995), inducting of IAA (Kavitha and Nelson 2014), and increasing resistance to pests and soil borne diseases (Al-Karaki 2006).

The oil biosynthesis in plant is the integration of several metabolic pathways which require linking of several steps such as continuous production of precursors, their transport, and translocation to the active site of synthesis. It depends finally upon normal functioning of associated metabolic pathways such as carbon fixation, respiration, and isoprenoid pathway (El-Sherbeny et al. 2007). Robinson (1973) revealed the fact that yeast contains vitamins recognized as coenzymes involved in specific biochemical reaction in the plant such as oxidative and non-oxidative carboxylation process. He added that the biochemical active pyrophosphates are the units that constitute the terpene. Moreover, Ezz et al. (2012) mentioned that the promotive effect of yeast foliar fertilizers on oil percentage and oil yield of rue plants may be due to its high content of some organic substances that improve the growth and flower setting which is reflected on oil yield. In this connection, Emam (2012) revealed

that yeast significantly increased the oil yield of flax plants.

Despite numerous reports on mycorrhizal fungi symbiosis with different plants, this fungus affects on gene expression of terpenoids and increased of essential oil content in medicinal plants (Heydarizadeh et al. 2013). Inoculated plants with mycorrhiza lead to changes in hormonal profiles and increased levels of auxin, cytokinin, and gibberellin in plant (Torelli et al. 2000), which eventually lead to the increase of the oil content. The fungus efficiently acquires P, which is required in large amounts for the biosynthesis of primary and secondary compounds, since P has essential functions in the energy metabolism of the cells and as constituent of nucleic acids and phospholipids (Marschner 2002). The fungus acquires carbon as hexose within the root (Solaiman and Saito 1997), but it is stored primarily as triacylglycerol (Gaspar et al. 1994), but also as glycogen (Bago et al. 2003).

The increase in the total soluble proteins content could be attributed to the growth hormones produced by yeast (Khalil and Ismael 2010), direct stimulation of the synthesis of protein (Stino et al. 2009), providing plants with essential nutrient elements required for protein formation (Hayat 2007).

It has been also suggested that mycorrhizal plants can derive nitrogen from organic sources that are less available to non-mycorrhizal plants (Ibijbijen et al. 1996). AM fungi have been reported to proliferate in organic matter and scavenge the mineral N released from soil organic particles (Hamel 2004). The hyphae of AM fungi can also take up amino acids (Govindarajulu et al. 2005). Arbuscular mycorrhizal fungi (AMF) usually enhance nodulation and nitrogen fixation in legumes, but the extent of these effects depends on AMF species (Valdenegro et al. 2001). Inorganic [i.e., nitrate (NO_3^-) and ammonium (NH_4^+)] and organic N (i.e., amino acids) sources from soil can be effectively taken by AM fungi and translocate to the host plant representing a significant route for N uptake by the plant (Jin et al. 2012). Arbuscular mycorrhizal fungi also produce enzymes such as glutamine synthase that are required for the assimilation of nitrate into amino acids (Tian et al. 2009).

It is possible to alter the percentage of various fatty acids by physiological and/or agronomic methods (Scheiner and Lavado 1999). Darzi et al. (2009) stated that using organic and biofertilizers lead to a change in the composition of essential oil in the different plant species. Linoleic acid and linolenic acid are essential for humans because our bodies cannot manufacture them and we must consume them in our diets (Morris 2003). The oil quality is usually valued according to the content of essential fatty acids (Johnson et al. 2008). In addition, Emam (2012) revealed that yeast treatment markedly decreased the saturated fatty acids [palmitic (C 16:0) and stearic acid (C 18:0)] contents in seed oil of flax plant. On the other hand, yeast application stimulated linolenic acid (18:3, ω-3) production at the expense of linoleic acid (18:2, ω-6) and oleic acid (18:1, ω-9) contents. The increase in total unsaturated fatty acid accompanied by decrease in total saturated fatty acid of three flax cultivars by yeast extract treatment was confirmed by Dawood et al. (2013) on soybean. Unfortunately, there is no clear work and explanation on the physiological role of both mycorrhiza and milk whey treatments on fatty acid composition.

Conclusion

We could conclude that different biofertilizers treatments on flax cultivars increased significantly different studied parameters.

Abbreviation

AMF: *Arbuscular mycorrhizal* fungi; IAA: Indole acetic acid; PUFAs: Polyunsaturated fatty acids

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Authors' contributions

Mona G Dawood and Mervat Sh Sadak designed and performed the experiment, responsible of all the physiological and biochemical analysis, and also wrote and reviewed the manuscript. Bakry A Bakry designed and farming plants and statistical analysis. Osama M. Darwish was responsible for fatty acid analysis. All authors read and approved the final manuscript.

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