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The Effect of Biofertilization in Fatty Acids Content for Many Flax Varieties

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Abstract

Field experiment was carried out in the fields in Al-Mahaweel district of Babil Governorate during the agricultural season 2020-2021 to estimate the effect of biological fertilization on the content of some fatty acids in flax varieties seeds. A factorial experiment was applied according to a Randomized Complete Block Design (R.C.B.D) with three replications. The experiment included two factors, the first included eight varieties of flax (Indian, Giza11, Giza10, Sahka5, Sahka6, Giza8, Syrian Thorshansity72), The second factor, biological fertilization included four treatments: (control, bacterial fertilization, fungal fertilization and a bacterial-fungal mixture). The fatty acids (α -Linolenic acid, Oleic acid, Linoleic acid, Palmitic acid, Stearic acid) in seeds were determined using HPLC High-Performance Liquid Chromatography. The results showed that Sahka6 Variety was significantly superior on other varieties in seed content of (Oleic acid and Linoleic acid) amounted to (29.70, 28.39) %, respectively, while Indian Variety superiority in seed content of Palmitic acid and Stearic acid amounted to (21.28, 25.27)% respectively. Bacterial fertilizer + Mycorrhiza were significantly superior to the other fertilizer treatments in all indicators of study, and the interaction between a variety and biofertilization did not show any significant differences except for the seed content of (α -Linolenic acid Palmitic acid).

Keywords: Flax, Bio-fertilization, Fatty acids, Varieties.

1. Introduction

Flax seed (*Linum usitatissimum* L.) is one of the medicinal and industrial crops that belongs to the Linaceae family and ranks third in the world in the manufacture of fibers and fifth in the world in the manufacture of vegetable oils [1]. Flax seed contains 30-45% oil consisting of monounsaturated fatty acids such as oleic acid, which constitutes (19-20) % of the oil [1]. Flax oil also contains linoleic acid, which makes up (17-19) % of the oil, and it is one of the polyunsaturated fatty acids. Furthermore, flax oil contains linolenic acid, which constitutes (45-60) % of the oil, and it is one of the unsaturated fatty acids [3]. Many studies have shown that flax varieties differ in their fatty acid content according to varieties environmental conditions and crop service operations [4].

Recently reseaches have confirmed the medical importance of flaxseed and its positive effect in treating many different diseases and its use as drugs. It is used in the treatment of cardiovascular diseases and also helps in the treatment of atherosclerosis by reducing the level of cholesterol in the blood and reducing low-density fats and as a result of containing soluble fibers that work to reserve fats and cholesterol in the digestive system and prevent their access to the blood, which lowers the proportion of cholesterol in it [5,6]. As well as regulating blood pressure in people with high blood pressure and is also used in the treatment of nephritis caused by the presence of stones, in addition to many other medical and therapeutic uses [7,8].

Plant bio stimulants contain substance whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality [9,10]. Bio stimulants are natural or synthetic substances that can be applied to seeds plants and soil [11,12]. These substances cause changes in vital and structural processes in order to influence plant growth through improved tolerance to abiotic stresses and increase seed or grain yield and quality [13].

Bio-fertilizers are considered one of the modern technologies that are safe to use depending on what is available in nature and limit or reduce chemical additives [14]. In addition to the production of natural compounds free of pollutants used in various industrial and pharmaceutical fields.. Thus, it takes into account the indicators of sound environmental management that are compatible with the modern world trends and aim to return to clean agriculture [15,16].

In view of the medicinal and industrial value of this crop, the study aimed to estimate the effect of biological fertilization on the content of some fatty acids in flax seeds.



2. Materials and Methods

The field experiment was carried out in the fields of one of the farms in the district of Al-Mahaweel in the Babil Governorate during the agricultural season 2020-2021. A factorial experiment was applied according to a Randomized Complete Block Design (R.C.B.D) with three replications. The experiment included two factors, the first eight varieties of flax (Indian Variety, Giza11, Giza10, Sahka5, Sahka6, Giza8, Syrian Variety, Thorshansity72), The second factor, biological fertilization includes four treatments as following:

- Control
- Bacterial Fertilization, included three types of bacteria:
 - Azotobacter chroococcum,
 - Bacillus mucilaginosus
 - Pseudomonas fluorescens
- Fungal Fertilization (Mycorrhiza)
- Bacterial-Fungal Mixture

The fatty acids (α -Linolenic acid, Oleic acid, Linoleic acid, Palmitic acid, Stearic acid) in seeds were determined using HPLC High-Performance Liquid Chromatography. The mixture of fatty acid was separated on m FLC (Fast Liquid Chromatographic) on reversed phase 3 μ m particle size, (50 x 2.0 mm I.D) C-18DB column, separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by shimadzu SPD 10A vp Detector at 215 nm, the data were recorded on shim pack C-R8A integrator (Shimadzu, koyota, Japan). The optimum separation condition as follow;

Column: Flc (Fast Liquid Chromatographic) Column, 3 mm particle size,

(50 X 2.0 Mm I.D) C- 8db Column

Mobile phase was: Acetonitrile: Tetrahydrofuran (Thf):0.1 % Phosphoric acid

in THF (50.4: 11.6: 38, V/V)

Detection: Uv Set At 215 Nm

Flow rate 1.5 Ml/Min.

Temperture : 40 C.

The sequences of the eluted fatty acids standard were as follow, each standard was 25ug/ml

Table1. Retention time and the area of fatty acids.

Seq	Subjects	Retention time minute	Area	Concentration
1	Palmitic C16:1	2.67	168523	25ug/ml
2	Stearic acid C18:0	3.48	235444	25ug/ml
3	Oleic C18:1	5.74	210588	25ug/ml
4	Linolenic C18:2	6.04	196344	25ug/ml
5	α -Linolenic C18:2	8.0	185612	25ug/ml

The HPLC separation profile revealed the presence of various chromatographic peaks in the studied flax seeds sample extract. The assayed of the separated compounds representing the major detected peaks and summarizing the obtained data for each of the detected chromatographic peak are discussed below. Quantitative determination of fatty acids was done by comparison the peak area of authentic standard with that of sample peaks under the same optimum separation condition, by using the following equation:

$$\text{Concentration of sample } \mu\text{g/ml} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution factor}$$

The results were statistically analyzed using the SAS statistical analysis program, and the averages were compared on the basis of the least significant difference L.S.D at 0.05 [17].

3. Results and Discussion

The results in Table (2) indicated that there are significant differences between flax seed cultivars in the content of their seeds of Oleic acid. Sahka6 variety showed significantly superior on other varieties and it gave the highest mean of (29.70) %, while Thorshansity72 gave the lowest mean of (18.18) %. Bio-fertilization had a significant differences on the seed content of Oleic acid by the superiority of the treatment (Bacterial fertilizer + Mycorrhiza) over other of the treatments and it gave the highest mean of (27.48%) compared to the non-fertilization treatment which gave the lowest mean of (19.22%). Whereas, The interaction between the varieties and the Biofertilization did not show any significant differences.

Table 2. Effect of Bio fertilizer and Variety on Flaxseed Content of Oleic acid (%).

Varieties	Bio-fertilizer				Mean
	Control	Bacterial fertilizer	Mycorrhiza	Bacterial fertilizer+ Mycorrhiza	
Indian Variety	16.62	18.95	21.07	25.44	20.52
Giza 11	18.70	22.89	25.74	28.03	23.84
Giza 10	19.11	20.43	23.72	27.37	22.65
Sahka 5	24.91	26.39	27.95	30.11	27.34
Sahka 6	25.21	29.80	30.77	33.05	29.70
Giza 8	17.32	20.91	22.45	26.87	21.88
Syrian variety	18.03	20.21	25.31	27.08	22.65
Thorshansity72	13.90	16.56	20.31	21.96	18.18
Mean	19.22	22.01	24.66	27.48	
L.S.D _{0.05}	Varieties	Bio-fertilizer	Bacterial fertilizer+ Mycorrhiza		
	1.801	1.274	N.S		

The results in Table (3) show that the varieties showed significant differences in the content of flax seeds from Linolenic acid. Sahka6 variety showed significant differences on other varieties and provided the highest mean of (28.39) %, while the Thorshansity72 variety gave the lowest mean of (15.90)%. The bio-fertilization exhibited significant differences in the characteristics of the seed content of Linolenic acid with the superiority of the treatment (Bacterial fertilizer + Mycorrhiza) over on other treatments and it passed the highest mean of (28.39) %, while control treatment recorded the lowest mean of (19.67) %. The interaction between the varieties and the Biofertilization did not show any significant differences.

Table 3. Effect of Bio fertilizer and Variety on Flaxseed Content of Linolenic acid (%).

Varieties	Bio-fertilizer				Mean
	Control	Bacterial fertilizer	Mycorrhiza	Bacterial fertilizer+ Mycorrhiza	
Indian Variety	17.20	18.90	22.40	25.60	21.02
Giza 11	22.40	24.50	28.10	28.90	25.97
Giza 10	19.70	20.80	22.50	26.70	22.42
Sahka 5	24.10	25.26	29.40	30.20	27.24
Sahka 6	25.80	26.99	28.10	32.70	28.39
Giza 8	17.60	18.90	22.70	25.30	21.12
Syrian variety	18.23	20.30	23.90	26.10	22.13
Thorshansity72	13.10	15.30	16.80	18.40	15.90
Mean	19.76	21.36	24.23	26.73	
L.S.D _{0.05}	Varieties	Bio-fertilizer	Bacterial fertilizer+ Mycorrhiza		
	1.751	1.238	N.S		

The results in Table (4) showed that there were significant differences between flaxseed varieties in the content of their seeds of α -linolenic acid. the Sahka 5 variety presented significant differences on other varieties and gave the highest mean of (26.91) %, while Giza10 provided the lowest mean of (21.74) %. Bio-fertilization had a significant effect on the seed content of α -linolenic acid with the superiority of the treatment (Bacterial fertilizer + Mycorrhiza) on other treatments and it provided the highest mean (47.98) % compared to the non-fertilization treatment which gave the lowest mean (37.37%). The interaction between the cultivar and the biofertilization exhibited significant differences, with the superiority of the combination (Sahka 5 + Bacterial fertilizer + Mycorrhiza) on other combinations and gave the highest mean of (30.09) %, while the combination (Giza 10 + Control) gave the lowest mean of (18.37)%.

Table 4. Effect of Bio fertilizer and Variety on Flaxseed Content of α -linolenic acid (%).

Varieties	Bio-fertilizer				Mean
	Control	Bacterial fertilizer	Mycorrhiza	Bacterial fertilizer+ Mycorrhiza	
Indian Variety	21.30	22.56	24.82	25.55	23.55
Giza11	20.23	23.64	25.76	28.35	24.49
Giza10	18.37	20.89	22.83	24.87	21.74
Sahka5	23.65	25.56	28.35	30.09	26.91
Sahka6	21.14	22.09	24.75	26.54	23.63
Giza8	20.33	23.54	24.99	26.01	23.71
Syrian variety	23.01	24.99	26.32	28.54	25.71
Thorshansity72	20.14	22.07	24.14	25.99	23.08
Mean	37.37	41.18	44.88	47.98	
L.S.D _{0.05}	Varieties 1.88	Bio-fertilizer 1.24	Bacterial fertilizer+ Mycorrhiza 3.57		

The results of Table (5) indicated that the varieties showed significant differences in the content of flaxseed from Palmitic acid. The Indian variety showed significantly differences on other varieties and gave the highest mean of (28.21) %, while Sahka6 provided the lowest mean of (20.01) %. The bio-fertilization showed significant differences in the characteristics of the seed content of Palmitic acid with the superiority of the treatment (Bacterial fertilizer + Mycorrhiza) on other treatments and it gave the highest mean of (27.30) %, while the control treatment gave the lowest mean of (18.12) %. The interaction between the variety and the biofertilization showed significant differences with the superiority of the combination (Indian + Bacterial fertilizer + Mycorrhiza) on other combinations and gave the highest mean of (30.56) %, while the combination (Sahka 5 + Control) gave the lowest mean (14.33) %.

Table 5. Effect of Bio fertilizer and Variety on Flaxseed Content of Palmitic acid (%).

Varieties	Bio-fertilizer				Mean
	Control	Bacterial fertilizer	Mycorrhiza	Bacterial fertilizer+ Mycorrhiza	
Indian variety	25.32	27.34	29.64	30.56	28.21
Giza11	18.54	21.54	24.37	29.66	23.52
Giza10	17.23	21.86	25.43	29.01	23.38
Sahka5	14.33	18.44	22.03	26.33	20.28
Sahka6	15.89	18.32	20.99	24.85	20.01
Giza8	16.45	19.42	22.09	25.99	20.98
Syrian variety	17.03	19.32	21.74	23.54	20.40
Thorshansity72	20.22	24.06	26.38	28.53	24.79
Mean	18.12	21.28	24.08	27.30	
L.S.D _{0.05}	Varieties 1.72	Bio-fertilizer 1.19	Bacterial fertilizer+ Mycorrhiza 3.44		

The results in Table (6) indicated that there were significant differences between flaxseed Varieties in their stearic acid content, as the Indian Variety significantly on other Varieties and gave the highest mean of (25.27) %, while the Sahka 5 variety gave the lowest mean of (20.01) %.

The biofertilization had a significant effect on the seed content of stearic acid by the superiority of the treatment (Bacterial fertilizer + Mycorrhiza) on other treatments and it gave the highest mean of (27.64%) compared to the non-fertilization treatment which gave the lowest mean of (17.45) %.

The interaction between the varieties and the Biofertilization did not show any significant differences.

Table 6. Effect of Bio fertilizer and Variety on Flaxseed Content of Stearic acid (%).

Varieties	Bio-fertilizer				Mean
	Control	Bacterial fertilizer	Mycorrhiza	Bacterial fertilizer+ Mycorrhiza	
Indian variety	22.36	23.57	26.53	28.63	25.27
Giza11	17.45	20.03	24.54	29.59	22.90
Giza10	16.54	19.53	25.58	30.00	22.91
Sahka5	15.45	18.00	21.96	24.65	20.01
Sahka6	16.23	19.03	21.00	23.99	20.06
Giza8	18.43	20.36	23.75	26.64	22.29
Syrian variety	15.11	18.04	22.78	29.01	21.23
Thorshansity72	18.09	19.99	22.76	28.65	22.37
Mean	17.45	19.81	23.61	27.64	
L.S.D _{0.05}	Varieties	Bio-fertilizer	Bacterial fertilizer+ Mycorrhiza		
	1.93	1.40	N.S		

The difference between the flax varieties in the fatty acid content of the seeds may be due to the genetic differences between the varieties and the extent of the genetic ability of the variety and its response to the environmental conditions in the area of its cultivation. This difference agreed with their findings [18-23], who confirmed in their study the difference of varieties in their content of oil content (fatty acids).

The reason for the superiority of the biological mixture (bacterial + fungal) over other biofertilization treatments in increasing the content of flaxseed of fatty acids may be due to its efficiency due to its content of beneficial microorganisms that fix nitrogen and solvent for phosphorus and potassium, and this is reflected positively on the ability of the plant to absorb, transport and accumulate nutrients Inside the plant tissues, thus increasing the yield and increasing the percentage of oil in the seeds, in addition to the increase in the number of microorganisms in the soil increases their ability to secrete growth-stimulating substances such as cytokines and gibberellins, and this is in agreement with what was obtained by [24-26] .

Conclusion

From obtained results, it can be concluded that the variety and biofertilization, especially bacterial and fungal fertilization, have an effect on increasing the flaxseed content of fatty acids with an important medical and therapeutic effect.

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