

### Increasing Omega 6, Omega 9 Fatty Acids and Oil Contents in Black Sesame Seed by Biofertilizer and Micronutrients Application

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#### ABSTRACT

A filed experiment was carried out to study the effect of bio fertilizers and micronutrient compounds on oil content and their physicochemical properties as well as fatty acids composition of black sesame oil (BSO). Biofertilizers and micronutrients increased oil content in BS by more than 20% compared with control treatment .Oleic acid (18:1-Omega 9) increased by more than 27%, linoleic acid (Omega 6-18:2) increased by more than 33% compared to control. The EM-FPE application showed the lowest values for total saturated fatty acids content (11.95%) and the highest value of unsaturated fatty acids (87.72%) compared with control treatment. Moreover, EM-FPE application exhibited the highest level of unsaponifiable matter and iodine value of oil. The bio fertilizer application improved seed oil quantity and quality of BS, as well.

Keywords: Black sesame seed oil, EM-FPE, Micronutrients compound, Omega 6, Omega 9, Seed oil content.

#### **INTRODUCTION**

Sesame (*Sesamum indicum* L.) is a member of *Pedaliaceae* family. It is grown for the production of seeds that are rich in oil. It comes in a variety of colors, creamy-white to charcoal-black. In general, Sesame is found in tropical, subtropical, and southern temperate areas of the world, particularly in India, China, South America and Africa having seeds and its edible oil that are highly valued as a traditional healthy food ingredient.<sup>1,2</sup>

Among the bioactive components in sesame seeds are IP-6 (Phytate; one of the most powerful antioxidants yet found), lignans, pinoresinol, tocopherols, lecithin, myristic acid and linoleate have been identified as the major antioxidants which responsible for the resistance of oxidative deterioration of sesame seeds and oil.<sup>3</sup> The potent antioxidant properties of sesame seed extract mainly are attributed to the presence of lignans which are phytoestrogens.<sup>4,5</sup>

Sesame oil is very stable and contains an antioxidant system comprising sesamol and sesamolinol formed from sesamolin, which substantially reduces its oxidation rate.<sup>6</sup> It is also highly nutritious, rich in vitamin A, B and E as well as the minerals iron, calcium, magnesium, copper, phosphorus and silicic acid.<sup>7</sup> Sesame seeds are high in protein, carbohydrates, fiber and some minerals that are widely used in food items. Additionally, fat of sesame seeds contain about 2.25 times as much energy as the equal amount of carbohydrates from feed grains or forages.<sup>8</sup> Oil bearing seeds are much higher in proteins than are the cereals seeds.<sup>9</sup> Sesame seed showed a high content of oil (52%), protein (24%) and ash (5%).<sup>10</sup> Sesame oil comprises approximately 50% of the seed weight, contains large amounts of natural antioxidants, they also contain a good type of monounsaturated and polyunsaturated fatty acids.<sup>8,11,12</sup> Lignans are a large class of secondary metabolites in plants, with numerous biological effects.<sup>13</sup> It has been found that sesame seeds oil contain oleic (Omega 9), linolenic (Omega 3) and arachidonic acids but linoleic acid is predominant and plays an important role in the metabolic system.<sup>14,3</sup>

Recent awareness has been offered to reduce pollution practices in sustainable agriculture. One of the ways to decrease soil pollution is the use of bio-stimulants compounds.<sup>15</sup> Bio fertilizers and humic compounds have beneficial return to increase population of soil microorganisms, especially in the surface layer of root rhizosphere, that create substances which stimulate plant growth.<sup>16</sup> Further, combination between both types is the most imperative factors needed to diminish agricultural chemicals, protect the air, soil and water from pollution as well as acquiring high yield quality.

Therefore, the present investigation was carried out to assess oil productivity and the content of Omega 6 and Omega 9 fatty acids in response to EM-FPE application. Oil seed properties of black sesame oil (BSO) were also investigated.

#### **MATERIALS AND METHODS**

#### Plant material and agriculture conditions

Black sesame (*sesamum indicum* L.) cultivated under field conditions conducted at El-Shohda village, Monfya governorate. Seeds were obtained from Johnw, Seeds Co. USA and were grown in a complete randomized blocks system. Each plot consisted of ten rows with spacing 60 cm between plants. Each experiment included four treatments beside the control treatment as follows:

#### **Treatment I - Control treatment**

Plants sprayed with tap water were used two times, before and after the flowering stage.



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#### Treatment II (MN1)

Plants sprayed with the diluted micronutrients solution of Wuxal suspension fertilizer which contained Zn (4.5 %), Fe (3.0 %) and Mn (1.5 %). It was diluted with 1.0 ml/l of water which and was used two times before the flowering stage (two weeks intervals).

#### Treatment III (MN2)

Plants sprayed with the diluted micronutrients solution of Wuxal suspension fertilizer which containing Zn (4.5 %), Fe (3.0 %) and Mn (1.5 %). It was diluted with 1.5 ml/l of tap water and was used two times before the flowering stage (two weeks intervals).

#### Treatment VI (EM-FPE)

Plants sprayed with EM-FPE as a biofoliar application (1:10) which were used two times, before and after the flowering stage.

At full maturity, harvesting was carried out and plants were dried under field conditions. Then the seeds were transferred to the laboratory for oil extraction.

## Preparation of (EM-FPE) from effective microorganisms (EM) in fermented plant extracts (FPE).

The principal microorganism in EM are photosynthetic bacteria, lactic acid bacteria and yeast and was obtained from the Ministry of Agriculture, Egypt and was fermented with plant extracts (FPE) to produce a mixture of biofertilizer according to.<sup>17</sup>

Evening primrose (EP) seeds were obtained from field experiment growing without adding any chemical fertilizers. Olive oil (OO) was obtained from the local market. These oils were used as a reference for comparison. Hence EPO has Omega 6 and Omega 9 (~ 70% and 22% respectively) and the chemical analysis olive oil has omega 9 and omega 6 (~ 60% and 20% respectively).

#### **Chemical analysis**

#### Extraction of oils

Extraction of oil was performed according to.<sup>18</sup>

#### Physical-chemical analysis

The chemical properties (acid value, free fatty acids, total fatty acids, iodine value, peroxide value, saponification value and unsaponifiable matters), and the physical properties (color, refractive Index and specific gravity) were determined.<sup>18</sup>

#### Separation of fatty acids and unsaponifiable matter

The oil samples were saponified overnight with ethanolic - KOH (20 %) mixture at room temperature. The fatty acids were separated from their potassium salts by acidification with hydrochloric acid (5N), followed by extraction with petroleum ether (40-60°C). The petroleum ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered as was described.  $^{\ensuremath{^{19}}}$ 

#### Preparation of fatty acid methyl esters

The soap solution was acidified with sulfuric acid (5N); the liberated fatty acids were extracted with ether, washed several times.

Methylation was carried out according to previously published literature.<sup>18</sup> and the methyl esters fatty acids were separated and were injected into the Gas Liquid Chromatography apparatus (GLC).

#### Standard fatty acids and other chemicals

A set of standard fatty acids of 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 18:1 *omega 9*, 18:2 *omega 6*, 18:3 *omega 3*, 18:3 *omega 6*, 20:0, 20:1 and 22:0 with a stated purity of 99 % were applied. All chemicals used were of analytical grade provided from Sigma and Merck Co. Germany.

## Identification and determination of fatty acids by Gas liquid chromatography (GLC)

The methyl esters of fatty acids were analyzed with a Pye Unicam series 304 gas chromatography equipped with dual flame ionization detector and dual channel recorder. The separation of fatty acid methyl esters was conducted using a coiled glass column (1.5 m x 4 mm) packed with Diatomite (100 – 120 mesh) and coated with 10 % polyethane glycol adipate (PEGA). The column oven temperature programmed at 8°C / min from 70 °C to 190 °C, then isothermally at 190 °C for 24 min with nitrogen at 30 ml/min.<sup>20</sup>

The fatty acids were identified by comparing their retention times with those of standard fatty acids methyl esters. While, the percentage of each fatty acid was measured from the calculation of peak areas.

#### **RESULTS AND DISCUSSION**

#### Effect of EM-FPE foliar on oil yield of black sesame

Data illustrated in (Table 1) revealed that the Biofertilizers application (EM-FPE) increased oil content of BS seeds by nearly 20% in response to Biofertilizers application. High and low concentration of MN showed slight increases (35.28% and 33%) compared to control 32.59%. In this concern, the oil percentage of BS seed is ranged from 42.5%- 46.2 % due to many factors such as variety (white -black- yellow), fertilizers used, age of plant, growth conditions and possibly with processing conditions.<sup>21,22</sup> In the present study the use of EM-FPE as Biofertilizers (foliar) enhanced the oil content by 20% which may due to the high nutrients contents of Biofertilizers. This was previously suggested by previous studies.<sup>23,24</sup> The author reported that the increase of oil content might attribute to the role of EM-FPE recycling and increased availability of nutrients to plant. The EM-FPE is rich with microorganisms, proteins, organic acids, chelated micronutrients, phosphorus, potassium and secondary



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metabolites (polyphenols and flavonoids). These nutrients might promote the growth and the biosynthesis of oil components and the unsaturated activities that stimulate the synthesis of polyunsaturated fatty acids.<sup>24</sup> Also, it has been found that sesame seed oil increased by 17% using fermented natural substances.<sup>25</sup>

The oil content of evening primrose (EPO) seeds grown without any chemical fertilizers was used for comparison. The seeds contained 19.83% oil (Table 1). It is also clear from the same table that oil content of BSO (35.28% is higher than oil content of EPO (nearly two fold). It has been reported that the oil content in evening primrose seed is varied from 11 to 18.20%.<sup>26</sup> In this concern, the increase of oil in evening primrose seed might be attributed to the application of yeast, biotin and riboflavin as a biofertilizers.<sup>23</sup> Combination of EM, organic materials and mineral NPK increased the efficiency of both organic and mineral nutrient sources. Also, the main effect of EM-FPE is to supply nutrients to crops and to suppress pathogens.<sup>17, 24</sup>

Iron, Zn and Mn, had a key role for stimulating the oil content. The spraying application of plants with compounds containing Zn increased seed oil content, which may attributed to the increase of total photo assimilates activities. The oil content in Egyptian cotton seeds increased by the application of Zn as chelated form.<sup>25, 27</sup> The increase may be due to Zn was found to enhance the activity of tryptophan synthesis, which is involved in the synthesis of the major hormone indole-3 acetic acid. Moreover, the chelated Fe, Mn and Zn are important for the metabolic processes of cotton plant leading to both better production and high quality.<sup>28</sup>

**Table 1:** Influence of foliar application of MN and EM-FPEon oil yield of black sesame seed oils

Plant	Treatments	Seed oil content %	% Oil increase		
	Control	32.594± 4.2	100%		
DSO	MN 1	33.00± 1.8	101.20%		
B30	MN 2	35.284± 2.8	108.30%		
	EM-FPE	39.06± 3.6	120%		
Seeds of Evening Primrose		19.83 ± 2.6			
Olive Oil					

Treatment I - Control treatment: Plant sprayed with tap water

Treatment 4 - EM-FPE: Effective microorganism (EM) in fermented extract (FPE)

Furthermore, the higher fixed oil percentage from *O. bennis* L. was obtained when fertilized with potassium, iron and zinc.<sup>26</sup> In addition<sup>29</sup> observed that the application of nitrogen in combination with Fe-EDTA fertilization can be beneficial to improve early growth and final yield of

soybean in Mediterranean – type soils. Iron plays a key role in proper structure and functioning of desaturases. The denaturises are characterized by the presence of three conserved histamine tracks, which are presumed to compose the Fe-binding active centers of the enzymes.<sup>30</sup> The role of manganese in lipid metabolism is more complex. It increases the content of typical thylakoid membrane constituents such as glycolipids and PUFAs. In addition, it is involved in the photosynthesis (O<sub>2</sub>-evolving system), arginase cofactor and phosphorus transferase.<sup>28,31</sup>

## Influence of EM-FPE and MN foliar application treatments on oils physico-chemical properties

Data presented in (Table 2) showed the influence of micronutrient compound and Biofertilizers foliar treatment on the physicochemical guality parameters of sesame oil. The oil had a clear yellow colour free of haziness. The iodine value obtained in control treatment is high 37.95 % which suggests the presence of unsaturated fatty acid and increased to the maximum 92.43 when EM-PFE was applied. It indicates the degree of unsaturation in the fatty acids of triacyglycerol. This value could be used to quantify the amount of double bonds present in the oil, which signifies the susceptibility of oil to oxidation. The free fatty acid content is 1.557% which is higher than in the range of the reference. The unsaponifiable matter value was 1.622% comparable with the reference value. The oil of control treatment had a peroxide value 109.77 m.eqv.kg and reached the maximum value when PFE was applied (128.31 eqv.kg). This is an indication of the ability of oil to get rancid because of oxygen absorption during storage or processing.

However, the decrease of acid value, free fatty acids and increase peroxide value (Table 2) might be due to the increase of polyunsaturated fatty acids content.<sup>32</sup> They added that sesame seed oil was susceptible to rancidity based on its high content of polyunsaturated fatty acids which reflected its high free acid and peroxide values.

From the present study, the obtained results showed that biofoliar application induced highest significant reduction in acid value from (3.1 to 1.65 mg KOH/g)free fatty acids from 1.557 to 0.829, saponification value from (201.44 to 183.37) mg KOH/g and total phenolic from (3.763 to 1.805) m.eqv.kg compared with control. The Biofertilizers application significantly increased the level of unsaponifiable to 1.944 compared with control treatment 1.622% (Table 2). This observation expressed the increase of the stability and shelf life of oils from rancidity process.<sup>10</sup> Data given in (Table 2) depict that sesame oil can be used as edible oil because it has a low level of acide value as well as low level of free fatty acids. Since this oil has also been implicated with a high value of iodine number, majority of fatty acids are unsaturated and consumption of USFA is very healthier life style.



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Treatment 2 - MN1

Treatment 3 - MN2

Table 2: Influence of foliar application of MN and EM-FPE on physical characteristics of black sesame seed oils

Plant	Treatment	Acid Value (mg KOH/g)	Free Fatty Acid as Oleic (%)	Total Fatty Acid (%)	Unsaponifiable Matter (%)	Saponification Value (mgKOH/g)	Peroxide value (m. eqv. kg)	lodine value (g l₂/100g)	
BSO	Cont	3.1±0.22	1.557± 0.05	89.4± 2.6	1.622± 0.17	201.44± 9.5	109.77± 4.9	37.95±2.6	
	MN 1	2.46± 0.25	1.236± 0.09	90.1± 3.2	1.643± 0.18	196.2±11.5	115.63± 6.2	66.81±5.7	
	MN 2	2.26± 0.14	1.135± 0.11	92.6± 1.9	1.652± 0.11	190.41± 6.5	122.28± 9.5	76.09±7.5	
	EM-FPE	1.65± 0.10	0.829± 0.07	92.7± 3.5	1.944± 0.13	183.37±10.6	128.31± 8.6	92.43± 7.5	
LSD (0.05)		0.81	0.33	2.44	2.44 0.26		0.94	6.5	
Evening Primrose Oil		1.088± 0.09	0.55± 0.07	95.6± 7.5	1.622±0.17	191.07± 8.3	3.94± 0.18	159.92± 7.3	
Olive Oil		$0.83 \pm 0.04$	0.417± 0.06	93.1± 8.3	1.68± 0.10	188.9±7.4	10.66± 1.86	95.855± 8.85	
LSD (0.05)		0.21	0.09	2.20	0.05	0.05 2.94		36.88	

All values are means of three replicates and are significantly different at  $P \ge 0.05$ ,  $\pm$  standard deviation. Where, Cont.: Control group, MN 1: Micronutrient wuxal Zn solution (1 ml/l twice foliar application), MN 2: Micronutrient wuxal Zn solution (1.5 ml/l twice foliar application EM- FPE.: Effective Microorganisms with Fermented Plant Extract

Pla	ant	Caprylic acid 8:0	Capric acid 10:0	Lauric acid 12:0	Myritc acid 14:0	Palmitic acid 16:0	Stearic acid 18:0	Oleic acid 18:1 <i>Omega9</i>	Linoleic acid 18:2 <i>Omega6</i>	α-Lino lenic acid 18:3 <i>Omega 3</i>	γ- Linolenic acid 18:3 <i>Omega 6</i>	TS	TU	TU / TS
BSO	Cont	0.53	0.34	0.425	0.63	8.20	21.1	40.11	27.54	ND	ND	31.30	67.65	2.16
	MN1	0.27	0.19	0.147	0.17	7.83	3.10	45.29	30.74	ND	ND	11.73	76.03	6.48
	MN2	0.18	0.23	0.15	0.20	8.68	4.05	50.94	35.66	ND	ND	13.52	86.61	6.41
	EM- FPE	0.35	0.16	0.27	0.22	10.2	0.70	51.12	36.59	ND	ND	11.95	87.72	7.34
OI	ive	ND	ND	ND	ND	21.2	1.82	55.80	19.21	0.54	ND	23.11	75.55	3.27
ΕF	0	0.145	0.014	0.4	0.13	0.06	7.51	21.3	62.19	ND	8.0	8.36	91.57	10.9

Table 3: Influence of foliar application of MN and EM-FPE on fatty acids composition of black sesame oil

Where, BSO: Black Sesame Oil, EPO: Evening Primrose Oil, TS: Total Saturated Fatty Acids, TU: Total Unsaturated Fatty Acids

Treatment I - Control treatment: Plant sprayed with tap water

Treatment 2 - MN1

Treatment 3 - MN2

Treatment 4 - EM-FPE: Effective microorganism (EM) in fermented extract (FPE)

# Influence of EM-FPE and MN foliar application treatments on fatty acids composition of black sesame oil

Data presented in (Table 3) showed the influence of biofertilizer and two levels of micronutrient compound on the fatty acids composition of BSO. The black sesame oil (BSO) had variations of fatty acids ranging from medium chain of fatty acids (C12- C16) and long chain fatty acids (C18-C24). The oil of BSO contained high proportions of C18 polyunsaturated fatty acids (PUFAs) in particular, *Omega* 6 and *Omega* 9.

The major saturated fatty acids in *Sesamum indicum* L. seed oil were palmitic (8.20%), stearic (21.1%) acids with lower content of caprice acid (0.34%). The main unsaturated fatty acids are linoleic acids. As shown in table (4), total saturated and total unsaturated acids were (31.30% and 67.65%) respectively. *Sesamum indicum* L. oil is predominantly made up of oleic and linoleic acids (40.11 and 27.54%) respectively. The results obtained are in agreement with <sup>(1)</sup> that Palmitic acid is the predominant saturated fatty acid of sesame oil. In addition to, higher

amounts of oleic acid while having low linoleic acid contents. Oleic and linoleic acid balance in the mutants increases the oil stability as found by <sup>(33).</sup> The saturated fatty acids of BSO: stearic 18:0, myristic 14:0, lauric 12:0, capric 10:0, caprylic 8:0 acids were 21.15 %, 0.63 %, 0.42 %, 0.34 % and 0.53 respectively .The EM-FPE application led a reduction of these fatty acids to 0.71 %, 0.22%, 0.27, 0.16 and 0.35% respectively compared to control treatment (Table 3).

The most abundant fatty acids found were oleic acid (18:1 *omega* 9), and linoleic acid (18:2 *omega* 6), which increased by more than 27.4 and 33 % respectively by EM-FPE application compared with control treatment.

The total polyunsaturated fatty acids (oleic acid 18:1, *Omega* 9 and linoleic acid 18:2, *Omega* 6) made up 87.72 % by EM-FPE application, while, saturated fatty acids contributed only 11.95 % compared with control treatment (67.65 % and 31.3 %) respectively. Similar finding have been reported previously.<sup>34</sup>



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The biofertilizer (EM-FPE) application increased the proportion of polyunsaturated and monounsaturated fatty acids, while saturated fatty acids were decreased. When, organic waste compost was applied for sesame plants, the ratios between the total unsaturated fatty acids and the total saturated fatty acids showed minor changes.35 In the present study the oleic acid was increased from 40.11% to 51.12% and of linoleic acid from 27.54% to 36.59 % in sesame seed oil. Other study showed highest level of oleic acid 40 - 42.4 % and lower palmitic acid 9.5-11.6 % content in sesame seeds oil.<sup>36</sup> On the other hand, the black sesame seed sprayed with high dose of MN (Table 2) enhanced the linoleic acid from 27.54 to 35.66 and oleic acid content from 40.11 to 50.94% respectively. A significant decrease in the saturated fatty acids were mainly stearic acid (0.70 %) compared to control treatment (21.15%). The application of low dose of MN shows scrimpy effect on the linoleic acid and oleic acid content (30.74 % and 45.29 % respectively) compared with control treatment (27.54 % and 40.11 % respectively).

The linoleic acid is the most significant polyunsaturated fatty acids in human diet as its ability for prevention heart vascular diseases.<sup>37</sup> Sesamum indicum L. oil is mostly composed of oleic and linoleic acids (37.74% and 45.15%) respectively. The content of myristic and palmitic acids which raise blood cholesterol was appreciably high (48.4%).The long-chain  $\omega$  3  $\omega$  6 and  $\omega$  9 fatty acids are commonly called PUFAs. Long-chain  $\omega$  3 PUFAs cannot be readily synthesized by the human body and are mostly obtained through the diet and  $\omega$  3/ $\omega$  6 ratios are considered to be important as reported by.<sup>38,39</sup>

#### CONCLUSION

The response of black sesame to Biofertilizers (EPM) application in terms of increasing oil content, improved their physiochemical properties and increasing the content of poly unsaturated fatty acids  $\omega$  3,  $\omega$  6 and  $\omega$  9. Therefore, this study confirmed the potential application of (BSO) treated with Biofertilizers as a potent natural source for  $\omega$  6 and  $\omega$  9. These metabolites have high potency as hypolipidemic agent and can be applied as neutraceuticals.

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