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Research Article
Comparative Study on the Influence of Organic Fertilizer and Soil Amendments on Evening Primrose (*Oenothera biennis* L.)

[1]Abdelwahab M. Mahmoud and [2]Amira Sh. Soliman

[1]Division of Plant Physiology, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Natural Resources, Institute of African Research and Studies, Cairo University, Giza, Egypt

Abstract

Background: This study was done in the light of concept safety production of medicinal and aromatic plants. **Materials and Methods:** This study was conducted in open fields for two seasons continuously to compare the effects of organic fertilizer, soil amendments, a mixture of them and chemical NPK fertilizer (control) on growth parameters, floral characteristics and chemical constituents of *Oenothera biennis* L. (evening primrose) plants. **Results:** The outcome data pointed out that mixture of organic fertilizers (cattle manure and humic substances) and soil amendments (zeolite and magnetite) led to significant increment in morphological growth (plant height, number of branches, number of leaves, leaves area, leaves fresh weight and leaves dry weight), floral characteristics (number of flower, number of capsules, seed yield as well as health index) and chemical composition symbolized in plant pigments, total carbohydrates, net photosynthesis, stomatal conductance, water use efficiency, crude protein, total phenolics, N, P, K%, moreover indigenous hormones characterized in Indole Acetic Acid (IAA), gibberellic acid (GA_3), cytokinins (CK) and abscisic acid (ABA), fixed oil content in seeds and oil yield per hectare in comparison with the recommended dose of chemical fertilizer NPK (control) under the same conditions. From an economic view, the feasibility study of experiment clearly indicated that mix of organic and natural fertilizers proven its value and potential to achieve maximum profitable returns. **Conclusion:** These results disclose that mixture organic and natural resources of fertilizers could reduce or replace the addition of chemical fertilizers, accordingly improve the quality and quantity of medicinal and aromatic plants, besides minimizing economic costs and pollution of the agricultural environment.

Key words: Evening primrose, soil amendments, organic fertilizer, growth parameters, chemical composition, economical evaluation

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Corresponding Author: Amira Sh. Soliman, Department of Natural Resources, Institute of African Research and Studies, Cairo University, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Since the dawn of history, medicinal and aromatic plants were used for healing different diseases in all civilizations around the world. The commercial production of therapeutic and remedial plants has two major ways; chemical or/and organic. Hence, the industrialized agro-ecosystem term emerged, which represents one of the main human agricultural practices with the highest environmental emission of pollutants to the atmosphere, soil and water. On the side, organic way in contrast to synthetic materials means to enhance agroecosystem by application and use where possible natural resources to fulfill or improve crop production[1]. Application of manures, composts, humic substances, biofertilizers, zeolites, magnetite feldspar and rock phosphate ores and other natural materials or beneficial microorganisms represent an organic way[2,3]. So many researchers were reported the importance of manures, humic substances, natural zeolites and magnetite feldspar in organic production of crops represented in ameliorate chemical and physical properties of soil, high available water-holding capacity and high adsorption capacities, decreased soil pH, high cation exchange capacities, also produce long-term soil improvements as well as slow-release fertilizer, increased soil organic matter which enhances absorption of available nutrients and maintained nutrients from leaching or loss[4-8].

The genus *Oenothera* consists of 145 species subdivided into 18 sections[9]. *Oenothera biennis* or evening primrose is a biennial plant of the Onagraceae family found in North America and parts of Asia Europe and Africa. It is grown commercially on a wide scale, later years; it has made the transition from being an undomesticated flower and backyard plant to an established agricultural crop[10]. The plant contains a range of different essential fatty acids including, palmitic acid, linoleic acid, oleic acid, γ-stearic acid and linolenic acid. The plant has been used to treat a variety of diseases including, atopic eczema, multiple sclerosis, coronary heart disease, psoriasis, diabetic neuropathy, autoimmune conditions, some types of cancer and gastrointestinal symptoms[11].

Since there is not needed of much water during the period of vegetation[12]. Thus, it could be valuable alternative pharmaceutical purposes and oil crop in the new area (sandy soil) which have limited water resource in Egypt. So, the cultivation of evening primrose requires a proper fertilizer in a sandy soil, which have not; any impact on the environment and is considered an excellent product and to avoid any chemical residues.

Therefore, the key objectives of present study is to evaluate productivity, components and economically feasibility study of *O. biennis* plants yield when cultivated and grown in new reclaimed sandy soils under safety conditions [organic fertilizers (cattle manure and humic substances), soil amendments (zeolite and magnetite) and mixtures of them] comparing with commercial synthetic fertilizers symbolized in NPK.

MATERIALS AND METHODS

The present study was conducted at the experimental farm of Wadi El-Natron, Beheira Governorate at a private farm (newly reclaimed land of the desert) longitude 28°54' E, latitude 28°20' N and altitude 130 m in Egypt, during two consecutive seasons 2014 and 2015. Some physical and chemical analysis of the experimental farm soil (Table 1) was carried out as described by Page *et al.*[13] in both seasons before planting. *Oenothera biennis* L. (Onagraceae) seeds were obtained from the experimental farm of Faculty of Pharmacy, Cairo University and sown in pots 7.5 (26×20 cm) filled with sand and peat (2:1) as a seed bed, at the end of October and harvested in May in both seasons, after 45 days (15-17 cm height) transplanted to open field with a distance of 60 cm between rows and 50 cm spacing between plants in plots with 6×7 m^2. Natural zeolite loaded with micronutrients as granules used in this study was obtained from Prima Company, Indonesia as shown in Table 2. The cattle manure was acquired from the Animals Production Department, Faculty of Agriculture, Cairo University (Table 3). While natural humic substances (Table 4) obtained from Soil and Water and Environment Research Institute, Agriculture Research Center (ARC). As for magnetite (Table 5) was provided by El-Ahram Company for Mining-Egypt. The experimental design was Randomized Complete Blocks Design (RCBD) with ten replicates.

Land preparation: Before planting, the soil was first mechanically plowed and propelled twice till the soil surface has been settled.

Fertilizers added and their combinations

Chemical fertilizers: Chemical fertilizers as recommended dose according to the Ministry of Agriculture and Land Reclamation were added at the rate of 474 kg ha^{-1} as ammonium nitrate (33%) divided into two doses, the first was added after 2 weeks from planting, while the second was added 4 weeks later, both calcium superphosphate

Table 1: Chemical characteristics of the organic fertilizers cattle manure (CM) applied to *O. biennis* L.

Organic characteristics	Moisture content (%)	Organic matter (%)	Organic carbon (%)	N (%)	C:N ratio	NO_3-N (ppm)	NH_3-N (ppm)	P (%)	K (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Seasons													
1st	9.40	62.98	31.68	1.88	17.4:1	173.90	49.40	0.29	1.18	1865.50	192.60	44.30	90.80
2nd	9.10	62.24	36.10	1.83	19.7:1	206.80	63.60	0.60	1.12	2690.90	179.80	43.20	159.20

Table 2: Chemical composition and physical properties of zeolite

CC (%)	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	MnO	MgO	CaO	Na_2O	K_2O	SrO	P_2O_3	LOI
	45.50	2.81	13.30	5.40	8.31	0.51	6.30	9.52	2.83	0.87	0.22	0.67	3.76
TE (ppm)	Ba	Co	Cr	Se	Cu	Zn	Zr	Nb	Ni	Rb	Y		
	10	1.2	35	0.8	19	64	257	13	55	15	22		

Physical properties			
Particle specific gravity (whole rock)	Ambient moisture	Hardness (mohs scale)	Cation Exchange Capacity (CEC) meq/100 g ANZ38 power
2.3 T m^{-3}	Nominal <5%	5.0	147 average

CC: Chemical composition (%), TE: Trace elements, LOI: Loss on ignition

Table 3: Some chemical composition of humic substances

Chemical composition							
Total nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sulfur (S)	Iron (Fe)	Boron (B)
1.40%	0.76%	2.80%	1.00%	0.50%	1.30%	1.39%	0.07%

Table 4: Physical properties and chemical composition of magnetite

Physical properties	
Color	Black to silvery gray
Streak	Black
Luster	Metallic to sub-metallic
Diaphaneity	Opaque
Mohs hardness	5-6.5
Specific gravity	5.2
Diagnostic properties	Strongly magnetic, color, streak, octahedral crystal habit
Tenacity	Brittle
Fracture	Subcon choidal to uneven
Transparency	Opaque
Crystal system	Isometric
Uses	The most important ore of iron
Chemical formula	Chemical composition
Fe_3O_4	Fe^{++}, Fe^{+++}
	Mn, Zn, Mg (trace)

(15.5%) at the rate of 474 kg ha^{-1} and potassium sulfate (48%) at the rate of 118.5 kg fed^{-1} were added 1 day before planting.

Cattle manure and zeolite: Both were added to the soil manually, where cattle manure at (47.4 m^3 ha^{-1}) as well as zeolite (497.7 kg ha^{-1}), both were added 15 days before planting.

Magnetite: Added to the soil (355.5 kg ha^{-1}), added 1 week before planting.

Humic substances: Applied as solution 2.37 L ha^{-1} at transplanting date, then 30 and 60 days, respectively per season. All agricultural practices were followed as recommended during both seasons.

Treatments: The treatments used in this study were as follows:

- NPK fertilizers as the control
- Cattle manure
- Zeolite
- Humic substances
- Magnetite
- Mixture (Cattle manure+Zeolite+Humic substances+ Magnetite)

Data recorded

Growth parameters: Plant height (cm), the number of branches per plant, the number of leaves per plant, leaves area centimeter square per plant, leaves fresh weight (g) and leaves dry weight (g) were recorded.

Floral characteristics: A number of flowers per plant at 50% from opening, the number of capsules per plant, seed yield per hectare as well as health index were recorded.

The health index was determined using the following equation[14]:

$$\text{Health index} = \frac{\text{Stem diameter} \times \text{dry weight}}{\text{Stem height}}$$

Chemical analysis

Total chlorophylls and carotenoid: Total chlorophylls and carotenoid contents (mg g^{-1} fresh weight) were measured by the spectrophotometer and calculated according to the equation described by Moran[15].

Total carbohydrates (%): In plant leaves and seeds were determined by the phosphomolybdic acid method as reported by AOAC[16].

Net photosynthesis, stomatal conductance and water use efficiency: Measurements of net photosynthesis on an area basis (µmol CO_2 m^{-2} sec^{-1}), leaf stomatal conductance (mol H_2O m^{-2} sec^{-1}) and water use efficiency of five different leaves per treatment was monitored using a LICOR 6400 (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set at 1500 µmol $m^{-2}sec^{-1}$, using a Li-6400-02B LED light source (LI-COR). The CO_2 flow into the chamber was maintained at a concentration of 400 µmol mol^{-1} using an LI-6400-01 CO_2 mixer (LI-COR).

Nitrogen and crude protein: The total nitrogen content of the dried material (leaves and seeds) was determined by using the modified-micro-Kjeldahl method as described by AOAC[16]. The nitrogen percentage was multiplied by 6.25 to estimate the crude protein percentages in leaves and seeds.

Phosphorus: Phosphorus was determined calorimetrically by using the chlorostannate molybdophosphoric blue color method in sulfuric acid according to Jackson[17].

Potassium: Potassium concentrations were determined by using the flame photometer apparatus (CORNING M 410, Germany)[18].

Total phenolics content in seed: Soluble phenolics were extracted 5 times from defatted ground seed into aqueous 80% (v/v) methanol (at a ratio of 1:1, w/v) at room temperature for 1 h using an orbital shaker at 250 rpm. The mixture was centrifuged at 1750×g for 10 min and the supernatants were collected, combined, evaporated to near dryness under vacuum at $\leq$40°C and lyophilized. Phenolic acid present in the crude extract was fractionated into free and bound forms according to the procedure described by Kozlowska *et al.*[19] and Zadernowski[20].

Endogenous phytohormones: Freeze-dried plant herbs (equivalent 6 g fresh weight) were ground to a fine powder in a mortar and pestle. The powdered material was extracted 3 times (1×3 and 2×1 h) with methanol (80% v/v, 15 mL g^{-1} fresh weight), supplemented with butylated hydroxytoluene (2, 6-Di-tert-butyl-p-cresol) as an antioxidant at 4°C in darkness. The extract was centrifuged at 4000 rpm. The supernatant was transferred into flasks wrapped with aluminium foil and the residue was twice extracted again. The supernatants were combined and the volume was reduced to 10 mL at 35°C under vacuum. The aqueous extract was adjusted to pH 8.6 and extracted three times with an equal volume of pure ethyl acetate. The combined alkaline ethyl acetate extract was dehydrated over anhydrous sodium sulfate then filtered. The filtrate was evaporated to dryness under vacuum at 35°C and redissolved in 1 mL absolute methanol. The methanol extract was used after methylation for the determination of cytokinins (CK)[21]. The remaining aqueous extract was acidified to pH 2.6 and extracted as previously described by ethyl acetate. The methanol extract was used after methylation according to Fales *et al.*[22] for determination of gibberellic acid (GA), abscisic acid (ABA) and indole-acetic acid (IAA). The quantification of the endogenous phytohormones was carried out with anti-unicum gas-liquid chromatography, 610 series, equipped with flame ionization detector according to the method described by Vogel[23]. The fractionation of phytohormones was conducted using a coiled glass column (1.5 m×4 mm) packed with 1% OV-17. Gasses flow rates were 30, 30 and 330 mL min^{-1}, for nitrogen, hydrogen and air, respectively. The peaks identification and quantification of phytohormones were performed by using external authentic hormones and a Microsoft program to calculate the concentrations of the identified peaks.

Fixed oil content (Percentage of the seeds): Fixed oil extracted from seeds by using a Soxhlet apparatus. The oil percentage was determined according to the methylation (change fixed oil into fatty acid) and GLC analysis was also recorded by G.C. mass in Medicinal and Aromatic Plant Laboratory Dokki (ARC) according to Kinsella[24] then oil yield per hectare was estimated in a hectare.

Methylation of fatty acid: Gas-liquid chromatographic analysis of fatty acid was done on methyl ester which was prepared and purified by the method of Kinsella[24] with some modifications. The methyl ester was prepared by refluxing the liberated fatty acids of *Oenothera* seeds with sulfuric acid (5 mL 1% v/v) in dried methanol for 30 min at 55°C. The fatty acid methyl esters were extracted several times with ether. The combined ether extracts were dried over anhydrous sodium sulfate, filtered and concentrated at 55°C.

GLC of fatty acid methyl esters: Separation of fatty acid methyl esters was carried out using capillary column, which contained 15% diethyl glycol succinate DEGS. The injector port and flame ionization detector were set at 240°C. The flow rate of carrier gas, nitrogen was 10 mL min^{-1}. The gas chromatograph (Perkin-Elmar model 8310) had a temperature program from 100-190°C with interment rate of 7°C min^{-1}. The initial and final time were identified according to their retention time compared to those of authentic samples.

Statistical analysis: The experimental design was Randomized Complete Blocks Design (RCBD) with 10 replicates. The data were analyzed using ANOVA at 5% significance level, the difference between treatments, then analyzed using Duncan Multiple Range Test (DMRT)[25] at 5%.

Economic evaluation: The yield components were calculated and economic analysis was performed using the following equations proposed by FAO[26] and Mubashir *et al.*[27]:

- Gross income = Yield × price
- Profitable Return (PR) = Gross income-Total production cost
- PR% over control = PR-control treatments
- Benefit Cost Ratio (BCR) = PR over control/Total production cost
- Investment Factor (IF) = Gross income/Total production cost
- (IF) must equal or more than 3

RESULTS AND DISCUSSION

Growth parameters: Growth parameters of *O. biennis* [plant height, number of branches, number of leaves, leave area, leaves fresh and dry weight per plant (g)] and floral characteristics (number of flowers, number of capsules, seed yield as well as health index) represented in Table 6 showed significant variation with mixture application treatment (cattle manure+zeolite+humic substances+magnetite). In particular, mixture application treatment was significantly increased the number of branches (83%), the number of leaves (91%) and leaves dry weight per plant (83%) than the control, in both seasons. These results are in accordance with the findings of Ahmed *et al.*[28] on roselle plants, Ramadan[2] on cabbage and Salama[6] on berseem clover and annual rye-grass.

This could be attributed to the positive effects of mixture application treatment (zeolite, magnetite, cattle manure and humic acid) on the improve physical and chemical properties of soil, high available water-holding and high adsorption capacities, decreased soil pH, higher CEC, increased soil organic matter which enhance absorption of available nutrients, in turn, enhanced plant growth and production, may affect phytohormone production leading to improve cell activity and plant growth[2,29-34].

Chemical composition

Total carbohydrates and photosynthetic pigments in leaves of *Oenothera biennis*: Values of total carbohydrates and photosynthetic pigments (carotenoids content and total chlorophyll) in leaves of *O. biennis* showed significant differences in comparison with the control treatment, whereas only in some treatments the contents of few minerals were similar or lower to control (Table 7). The most effective treatment in this respect was the mixture application treatment as plants for this treatment contained 22.55% total carbohydrates, 0.80 and 2.76 mg g^{-1} carotenoids content and total chlorophyll, while, control plant gave 16.78%, 0.57 and 1 mg g^{-1}, in both successive seasons, respectively.

Increased total carbohydrates and photosynthetic pigments in mixture application treatment as compared to other treatments may be due to many biotic and abiotic factors are influencing the production of isoprenoid substrates for carotenogenesis[35]. In addition, mixture treatment caused a considerable change in the soil pH that would have a significant increase in macronutrients availability (N, K, Mg, P and Ca) to plant roots, increased their uptake which increased

Table 5: Some physical and chemical properties of experimental farm

Physical properties										
Sand (%)		Silt (%)		Clay (%)		Texture grade		pH (soil paste)		EC (dS m^{-1} at 25°C)
90.8		7.21		1.99		Sand		7.8		0.38
Chemical properties										
Cations (meq L^{-1})				Anion (meq L^{-1})						
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3	HCO_3^-	HCO_3^-	Cl^{-1}	SO_4	Total-N (%)	Organic-C (%)
0.72	0.33	0.52	0.22	0	1.22	1.22	0.33	0.45	0.02	0.15

Table 6: Effect of different fertilizers and soil amendments on growth parameters and floral characteristics of *O. biennis* during two seasons 2014 (1st) and 2015 (2nd)

	Plant height (cm)		No. of branches per plant		No. of leaves per plant (cm^2)		Leave area per plant (g)		Leaves fresh weight per		Leaves dry weight per plant plant (g)		No. of flowers per plant		No. of capsules per plant	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK control	116.8^{b}	123.3^{b}	9.0^{a}	9.5^{a}	132.5^{b}	136.0^{b}	532.5^{b}	538.8^{b}	143.7^{b}	145.5^{b}	30.7^{b}	31.8^{b}	217^{b}	235^{b}	245^{b}	268^{b}
Cattle manure	113.5^{c}	118.4^{b}	8.6^{a}	8.8^{a}	126.6^{b}	131.9^{b}	510.4^{c}	540.7^{b}	134.4^{c}	138.6^{b}	29.1^{b}	30.3^{b}	190^{c}	228^{b}	236^{b}	257^{b}
Zeolite	110.1^{c}	116.5^{c}	7.4^{b}	7.5^{b}	122.5^{c}	125.6^{c}	498.6^{c}	529.2^{c}	132.3^{c}	135.5^{c}	27.5^{c}	27.9^{c}	101^{d}	155^{c}	191^{c}	216^{c}
Humic substances	115.6^{b}	120.9^{b}	9.2^{a}	9.6^{a}	129.3^{b}	135.6^{b}	530.2^{b}	541.1^{b}	140.4^{b}	143.0^{b}	30.1^{b}	30.8^{b}	209^{b}	230^{b}	233^{b}	261^{b}
Magnetite	112.2^{c}	115.7^{c}	7.0^{b}	7.4^{b}	119.2^{c}	124.8^{c}	500.8^{c}	519.3^{c}	129.5^{d}	131.8^{c}	25.5^{c}	25.8^{c}	89^{d}	142^{c}	188^{c}	211^{c}
Mix	126.3^{a}	129.5^{a}	10.5^{a}	11.7^{a}	145.5^{a}	151.0^{a}	763.6^{a}	781.0^{a}	156.5^{a}	160.2^{a}	37.2^{a}	38.5^{a}	260^{a}	288^{a}	428^{a}	489^{a}

Means with the same letter in a column are not significantly different by p = 5%

the number of chloroplast per cell as well as photosynthetic efficiency and increased sugar content in plants[36,37]. Also, the positive influences of HA on chlorophylls and sugar contents could be mainly due to hormone-like activities of the humic acids through their involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis and various enzymatic reactions[38,39]. There are many reports which are in agreement with the present findings indicating that increased nitrogen supply elevated chlorophyll contents in *Clematis vitalba* leaves[40]. Also, Al-Sahaf[3] reported that chicken manure increased pigment in four *Brassica* vegetables. Moreover, Farouk *et al.*[41] found that cucumber accumulated the highest carbohydrate content due to treatment with chitosan.

Net photosynthesis, stomatal conductance and water use efficiency: The highest significant values of net photosynthesis, stomatal conductance and water use efficiency (18.77 µmol CO_2 m^{-2} sec^{-1}, 1.65 mol H_2O m^{-2} sec^{-1} and 16.65 µmol $mmol^{-1}$) were obtained from treatment of mixture application comparison with control plants (14.91 µmol CO_2 m^{-2} sec^{-1}, 1.45 mol H_2O m^{-2} sec^{-1}, 13.15 µmol $mmol^{-1}$) (Fig. 1) in both seasons.

Bittelli *et al.*[42] concluded that the application of chelators increased significantly root growth as root length, which increased the plant's absorption ability. Moreover, application of chitosan assisted in conserving water the plants by closing the stomata and decreasing transpiration hence increasing relative water content in the leaves. Similar results were reported by Soliman and Mahmoud[43] on *Adansonia digitata.*

Indigenous hormones at stem elongation and flowering stage of *Oenothera biennis*: The obtained data in this concern clearly revealed that the highest values of IAA, GA_3 and CK hormones were recorded with mixture treatment in comparison with both control and all other treatments. Vice versa in the case of ABA hormone which records the lowest value with mixture treatment comparing with all other treatments (Table 8).

The increase in hormones by application of mixture treatment could be due to increasing the microbial populations resulting from adding cattle manure and humic acid in soils that led to the production of plant growth regulators by microorganisms[44,45] or due to the effects of humates[46]. In contrast, Mato *et al.*[47] concluded that the application of HA inhibits indole acetic acid (IAA) oxidase,

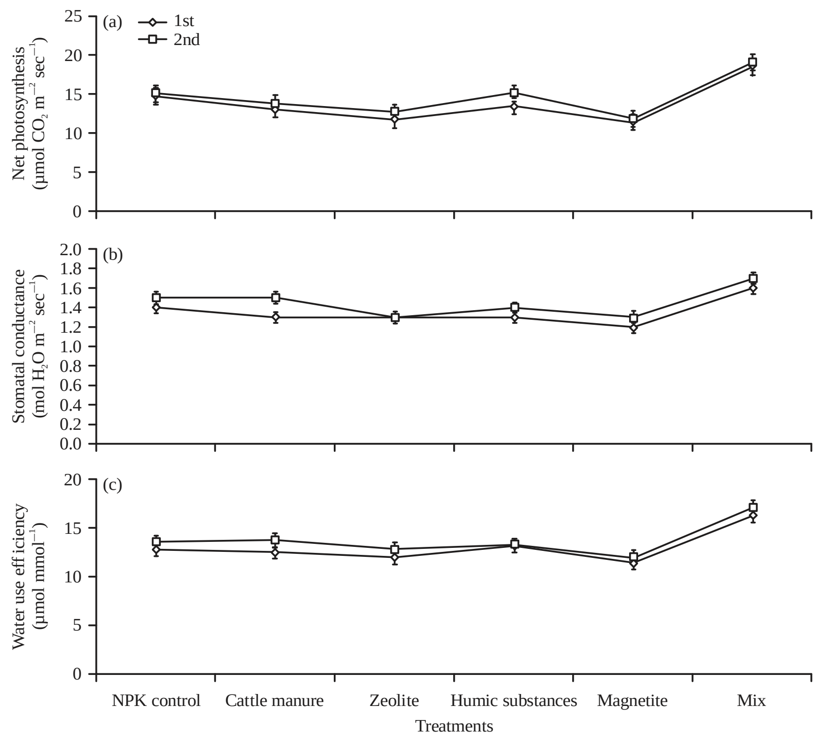

Fig. 1(a-c): Effect of different fertilizers and soil amendments on (a) Net photosynthesis, (b) Stomatal conductance and (c) Water use efficiency of *O. biennis* during both seasons 2014 (1st) and 2015 (2^{nd})

Table 7: Effect of different fertilizers and soil amendments on total carbohydrates and photosynthetic pigments of *O. biennis* during two seasons 2014 (1st) and 2015 (2nd)

	Total carbohydrates in leaves (%)		Carotenoids content (mg g^{-1} fresh weight)		Total Chlorophyll (mg g^{-1} fresh weight)	
Treatments	1st	2nd	1st	2nd	1st	2nd
NPK control	8.23^{b}	8.55^{b}	0.25^{c}	0.32^{b}	0.47^{b}	0.53^{b}
Cattle manure	7.63^{b}	8.48^{b}	0.23^{c}	0.31^{b}	0.38^{c}	0.53^{b}
Zeolite	6.76^{c}	7.69^{b}	0.19^{d}	0.28^{c}	0.33^{c}	0.37^{c}
Humic substances	8.13^{b}	8.45^{b}	0.30^{b}	0.35^{b}	0.44^{b}	0.55^{b}
Magnetite	6.78^{c}	7.72^{b}	0.20^{c}	0.25^{c}	0.33^{c}	0.35^{c}
Mix	10.24^{a}	12.31^{a}	0.39^{a}	0.41^{a}	1.35^{a}	1.41^{a}

Means with the same letter in a column are not significantly different by p = 5%

Table 8: Effect different fertilizers and soil amendments on hormones at stem elongation and flower of *O. biennis* during two seasons 2014 (1st) and 2015 (2nd)

	GA3 (µg g^{-1} fresh weight)		IAA (µg g^{-1} fresh weight)		CK (µg g^{-1} fresh weight)		ABA (µg g^{-1} fresh weight)	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK control	13.27^{b}	14.82^{b}	10.09^{b}	11.14^{b}	9.12^{b}	9.62^{b}	7.24^{b}	6.22^{b}
Cattle manure	13.08^{b}	14.11^{b}	9.58^{c}	11.25^{b}	9.05^{b}	9.68^{b}	7.98^{b}	6.41^{b}
Zeolite	10.24^{c}	11.18^{c}	9.26^{c}	9.71^{c}	8.69^{c}	8.94^{c}	9.06^{a}	7.63^{a}
Humic substances	13.25^{b}	14.55^{b}	10.55^{b}	11.78^{b}	9.11^{b}	9.60^{b}	7.09^{b}	6.45^{b}
Magnetite	9.85^{c}	11.71^{c}	9.13^{c}	9.42^{c}	8.57^{c}	8.85^{c}	9.17^{a}	7.07^{a}
Mix	16.71^{a}	16.89^{a}	13.26^{a}	13.67^{a}	11.81^{a}	12.21^{a}	5.47^{c}	4.38^{c}

Means with the same letter in a column are not significantly different by p = 5%

thereby hindering the destruction of this plant growth hormone. In addition, Maheshwari[48] reported that magnetic treatments also affected by phytohormone production.

Total carbohydrates, protein, total phenolics content and some macro-nutrients in seed: Application of mixture treatment increased significantly total carbohydrates, protein and total phenolics content in seed as compared to control plants in the both seasons (Table 9). Similar results were obtained by Khalil and El-Aref[49] and El-Shayeb[50].

Data in the Table 9 clearly indicate that mixture application treatment provided higher N, P and K% in seeds relative to control. The increased nutrient uptake due to the mixture treatment would be attributed to increasing root volume and surface area together would have led to the more nutrient uptake by providing better means for greater absorption as well as enhancement of microbial activity and reduced nutrient losses in the soil. In addition, the prevention of nutrient fixation in the soil, which led to increased availability of nutrient to the plants[51]. Similarly, several studies reported that humic acid, manure or zeolite applications increased, most macro-nutrient concentrations in many plants[52-55].

Seed yield, health index, oil percentage and oil yield: The mixture application treatment produced the highest significant seed yield, health index, oil percentage and oil yield values were 1291.06 kg ha^{-1}, 1.66, 23.80% and 265.32 L ha^{-1}, respectively, in both seasons (Table 10, Fig. 2). While, on the contrary, the control treatment produced the lowest significant seed yield, health index, oil percentage and oil yield values were 1111.17 kg ha^{-1}, 1.39, 20.24% and 145.95 L ha^{-1}, respectively, in both seasons.

The increase in seed yield, health index, oil percentage and oil yield of *O. biennis* plants in this study could be explained by increase the microbial populations resulting from adding cattle manure and humic acid in soils. These microorganisms can produce materials that may affect plant growth, such as substances acting as plant hormone analogs or growth regulators[44,45]. Also, Mansour[56] and Mosa *et al.*[57] reported that this increase might be attributed to stimulating effect of magnetite on plant growth and the absorption of N, P, K and Ca. The previous result was supported by Habashy and Laila[58] on wheat crop with humic acid; Shehata *et al.*[59] on strawberries with compost, amino and

Table 9: Effect of different fertilizers and soil amendments on total carbohydrates, protein, total phenolic content, macro-nutrients in seeds of *O. biennis* during two seasons 2014 (1st) and 2015 (2nd)

	Total carbohydrates in seeds (g/100 g)		Protein in seeds (%)		Total phenolics content in seeds (mg/100 g dry matter)		N% in seeds		P% in seeds		K% in seeds	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK control	14.27b	15.34b	13.41b	14.22b	203.4b	215.8b	2.18b	2.14b	2.12a	2.27a	1.89b	2.55a
Cattle manure	12.11c	13.79c	12.15c	13.26c	191.6b	200.5b	1.95c	2.10b	1.91c	2.21a	1.77b	2.34a
Zeolite	11.73d	12.96d	10.68d	11.31d	177.3c	180.5c	1.07c	1.20c	2.10a	2.25a	2.12a	2.73a
Humic substances	14.70b	15.11b	13.35b	14.05b	197.8b	217.2b	2.09b	2.17b	1.97c	2.18a	1.95b	2.40a
Magnetite	11.39d	12.62d	10.51d	11.08d	154.7d	161.3d	1.02c	1.17c	2.06b	2.11a	1.68b	2.41a
Mix	17.18a	18.47a	17.32a	17.69a	288.7a	309.5a	3.07a	3.15a	2.79a	2.91a	2.94a	2.97a

Means with the same letter in a column are not significantly different by p = 5%

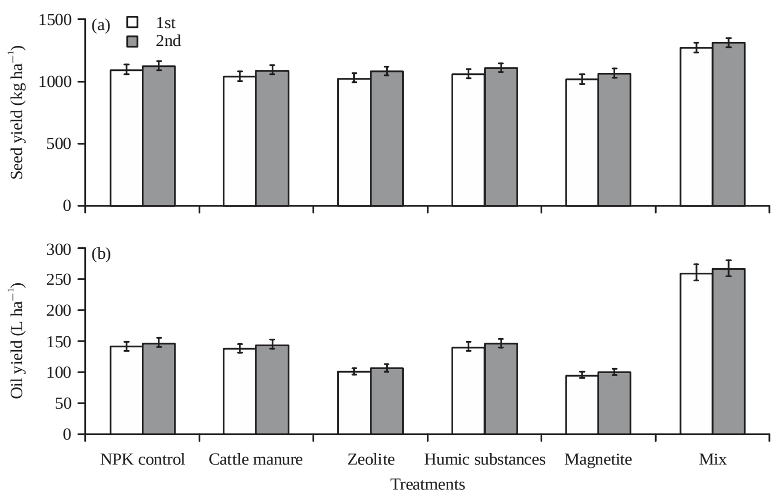

Fig. 2(a-b): Effect of different fertilizers and soil amendments on the (a) Seed yield and (b) Oil yield of *O. biennis* during both seasons 2014 (1st) and 2015 (2nd)

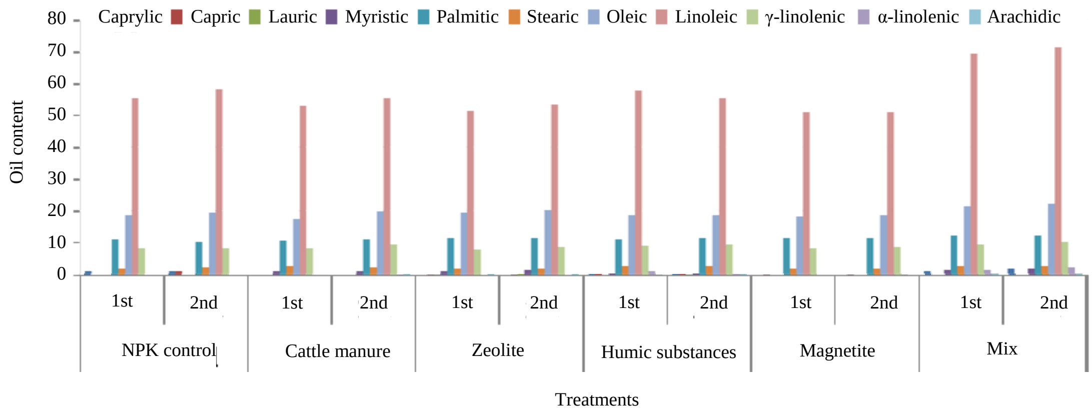

Fig. 3: Effect of different fertilizers and soil amendments on the major compounds of oil of *O. biennis* during both seasons 2014 (1st) and 2015 (2nd)

Table 10: Effect of different fertilizers and soil amendments on the chemical composition of *O. biennis* during two seasons 2014 (1st) and 2015 (2nd)

	Health index		Oil seeds (%)	
Treatments	1st	2nd	1st	2nd
NPK control	1.18	1.60	19.85[b]	20.62[b]
Cattle manure	1.10	1.10	18.36[c]	19.95[b]
Zeolite	0.99	0.95	17.75[d]	18.59[c]
Humic substances	1.17	1.14	19.42[b]	20.88[b]
Magnetite	0.88	0.86	17.22[d]	18.34[c]
Mix	1.56	1.57	22.68[a]	24.91[a]

Means with the same letter in a column are not significantly different by p = 5%

Table 11: Effect of economical evaluation of different treatments

Treatments	Total yield (L ha^{-1})	Total production cost (US$ ha^{-1})	Gross income (US$ ha^{-1})	Profitable return (PR) (US$ ha^{-1})	Profitable return over control (US$ ha^{-1})	Profitable return increase percentage (US$ ha^{-1})	BCR	IF
NPK control	148.6	463.3	19543	19079.0				42.1
Cattle manure	146.5	443.1	19227	18784.0	295.0	3.64	0.66	43.3
Zeolite	108.0	403.8	14184	13780.6	5299.0	65.60	13.10	35.1
Humic substances	147.2	369.2	19540	19171.4	91.7	1.13	0.24	52.7
Magnetite	101.8	294.3	13238	12944.4	6135.2	76.00	20.80	44.9
Mix	268.4	497.7	35618	43653.0	24573.3	305.00	49.30	71.5

humic acids and Mohamed *et al.*[60] on orange trees with magnetite and some biofertilizers application.

Oil components: Concerning the effect of different treatments on fatty acids resulted from chromatographic analysis of oil components (Fig. 3). It could be concluded that the main fatty acids represented in caprylic, myristic, palmitic, stearic, oleic, linoleic, γ-linolenic and α-Linolenic gave the utmost amount comparing with either control plants or all other treatments in both first and second season. Contrary to that, the highest amount of capric acid (0.24-1.08) in both seasons was obtained from control plants. With reference to lauric acid, which recorded the highest amount (0.38-0.47) with zeolite treatment in comparison with both control and all other treatments.

The increment of major oil content with mixture treatment could be explained on the basis of available elements, vitamins, gibberellins, cytokines, hormone-like substances, amino acids and sugars that lead to an increase in biochemical processes within the plant (the luxury of metabolism) consequently an increase in fatty acid content. These results concurred with those of Azim[61] on *Oenothera biennis*, who stated that, application of NPK caused variation in fatty acid content, Abd El-Latif[62] on caraway plants, reported that organic manure affected oil content and Mahmoud[63] on yarrow plants who declared that zeolite and organic fertilizers greatly influenced essential oil content.

Economic evaluation: The data in Table 11 clearly revealed that mixture treatment realized a maximum production cost of 210 US$ ha^{-1}, while the minimum production cost resulted from magnetite treatment. Generally, from data analysis, the cost of commercial production of *O. biennis* differed with variable types of treatments, meanwhile, all treatments fulfilled reasonable profitability because of their (IF)[26] more than 3. The maximum profitable return of 18419 was attained when mix treatment applied. Concerning Benefit Cost Ratio (BCR), the value of BCR was economical with the application of mix since it recorded a value of 49.3. Regarding, Investment Factor (IF), data showed that the highest IF of 71.5 was recorded when mix treatment applied and gross income 15029 US as well.

CONCLUSION

The addition of mixture application (cattle manure+zeolite+humic substances+magnetite) to the soil and plant improved growth parameters and chemical compositions of *O. biennis* plants that led to decrease the addition of chemical fertilizer rate to the level that could replace it with mentioned mix treatment and reduce the production cost. Further developments in using such safety and natural products in this sector could have large-scale economic implications and multiple benefits for consumers, producers, farmers and the ecosystem. Therefore, a mentioned mixture application can be considered as an economical fertilization for *O. biennis* plants.

SIGNIFICANCE STATEMENTS

Best results with a mixture of organic fertilizers and soil amendments which increased:

- Growth parameters (Plant height, the number of branches, number of leaves per plant, leaves an area, leaves fresh and dry weight)
- Floral characteristics (Number of flowers per plant, the number of capsules per plant, seed yield per hectare as well as health index)
- Chemical compositions (total chlorophylls and carotenoid, total carbohydrates, net photosynthesis, stomatal conductance and water use efficiency, N, P, K%, crude protein, total phenolic content in seed and endogenous phytohormones)
- The increment of seed yield, health index, oil percentage and oil yield
- The oil content of seeds in a mixture of organic fertilizers and soil amendments which gave the utmost amount comparing with either control plants or all other treatments especially γ-linolenic acid content

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