INFLUENCE OF SOME PHOSPHORUS SOURCES AND BIOFERTILIZERS (EM AND PHOSPHOREIN) ON VEGETATIVE GROWTH, FIXED OIL PRODUCTIVITY AND CHEMICAL CONSTITUENTS OF *OENOTHERA BIENNIS* L. PLANT

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ABSTRACT: Evening primrose is a beautiful ornamental plant, but at the same time it has medicinal properties that make this plant of great value and importance. In this context, the present research aims to investigate the effect of phosphorus sources and biofertilizers (EM and phosphorein) on growth, flowering, seeds yield, chemical constituents, of *Oenothera biennis* L. plant. This study was conducted at the Experimental Farm and in the Laboratory of Horticulture Department, Faculty of Agric. at Moshtohor, Benha Univ., Egypt during 2018/2019 and 2019/2020 seasons. The results indicated that, plants which were applied with phosphorus sources, biofertilizers as well as their combination treatments scored highly significant increases in all of studied characteristics of vegetative, flowering, seeds yield, chemical composition, fixed oil percentage, and fatty acids determination of the plant. The maximum values of most parameters mentioned above were gained by the combined treatment of monopotassium phosphate with EM in 1st and 2nd seasons. Additionally, the highest values of flowering parameters and P % were recorded by MAP treatment combined with phosphorein in both seasons. Furthermore, the highest seeds oil percentage (27.22 and 28.37) were recorded by the combined treatment between monopotassium phosphate and phosphorein, in the first and second seasons, respectively. The gained fixed oil composition four components were identified, i.e. palmitic acid, oleic acid, linoleic acid and α-linolenic acid. It can be concluded that the monoamonium phosphate (MAP) or monopotassium phosphate with EM or phosphorein were the best for improving growth, seeds yield, fixed oil productivity, chemical constituents and fixed oil components of *Oenothera biennis* L. plant.

Key words: Evening primrose, *Oenothera biennis* L., phosphorus sources, bio-fertilization, fixed oil productivity, chemical composition, GLC.

INTRODUCTION

Oenothera biennis, L., as it is commonly known to call it the evening primrose, which belongs to the Onagraceae (Oenotheraceae) Family, and it is spread in North America and is cultivated well. Evening primrose is a beautiful ornamental plant, meantime it has medicinal properties that make this plant of

great value and importance (Hall *et al.,* 1988). This plant is a beautiful plant in shape used to beautify and decorate the gardens, it is a biennial plant with fluffy surface, and its height reaches from 0.5 to 1.5 m with yellow leaves in a reciprocal position with serrated edges and capsule fruits containing from 200 to about 650 seeds. For this plant to contain its seeds on the quality of oils with medicinal

properties due to the presence of gamma linoleic acid, as an active ingredient for this oil, which is an important component due to the medicinal properties (Bordonaba and Terry, 2008; Ghasemnezhad, 2007; Gholinezhad *et al.,* 2008; Ide, 1988). This fatty acid (gamma-linoleic), is very important, as it is considered an important mediator in human metabolism and it also helps in the work of synthesis of prostaglandins. Evening primrose has beautiful flowers color, as the common color is yellow in most species and the number is few in it white, purple, pink and red, it contains four petals and flowers bloom in the evening and from here it was called evening primrose and is grown in late summer and early of autumn. Primrose is of a great importance as it has a role in regulating hormonal systems. Also, *Oenothera biennis* plant is very important as it has good properties in the form of flowers and the shape of the plant, and therefore its cultivation is spread in the gardens to beautify it. On the other hand, the plant has many medicinal uses, the plant's herb is used in the eczema, acne, rheumatoid arthritis and coronary artery disease. The oil extracted from the seeds of the plant is used to soften and regenerate the skin as well as facial scrubs, as it is combined with vitamin E to prevent oxidation, and the oil is added to skin and cosmetic preparations (Bown, 1996 and Liu *et al.,* 2003). In the same context, we can say that evening primrose oil is an important source of gamma-linolenic acid (GLA, C18:3Δ6, 9, 12), which is an unsaturated fatty acid and is required for its nutritional and pharmaceutical application. It turns out that the average oil content in seeds ranges from 7.3% to 21.7%, in different types of *Oenothera biennis*, and that the average of GLA levels reaches from 0.0 to 10.1%. (Balch *et al.,* 2003). Also, evening primrose oil (GLA, LA, EPO) lowered total cholesterol concentrations (Fukushima *et al.,* 2001).

Hulan *et al.* (1987) showed that the evening primrose seeds contain many important compounds, as they contain high amounts of mineral salts such as calcium, phosphorous, potassium, iron, manganese and zinc, as they contain lipids in a ratio of 21-34%, Petru *et al.* (1993) demonstrated that 24% of oil content for *Oenothera biennis* seeds including two major fatty acids (linolenic and gamma-linolenic acid).

Phosphorus is one of the important and necessary elements of the plant, as it comes second after N in terms of importance for the plant. Phosphorus is well present in the soil, but it is not very easy to absorption. Whereas, phosphorous forms with cations insoluble complexes and limits their availability, as these assemblies are combined with organic matter by microbes and ultimately lead to restriction of the phosphorous component and its unavailability. (Bieleski, 1973; Vance *et al.,* 2000). From here we can say that phosphorus is a nutritional and necessary element as it is one of the specific and controlling basic elements in determining the growth, development and productivity of plants. (Raghothama and Karthikeyan, 2005 and Malhotra *et al.,* 2018).

Available and low levels of phosphorous in acid soils are one of the main constraints that prevent crop production (Wang *et al.,* 2010). On the other hand, it is clear that P fertilization is necessary and important to maintain the productivity of the crop, but the efficiency of P fertilization reaches only 20% and accumulates in large quantities in the soil and results in possible environmental pollution, and this reduces the economic use of it in developing and developed countries. (Ju *et al.,* 2007). Therefore, we should improve and manage P fertilization and work to increase the efficiency of phosphorous and make it easier for plants and add it in the appropriate image preferred by the plant and thus reduce its presence and accumulation in the soil (Conde *et al.,* 2014).

Some studies have indicated that to the different sources of phosphorous and their effect on different plants as Kiliç *et al.* (2012) on *Thymus vulgaris* L., Soliman *et al.* (2016). The results indicated that the use of

monoammonium phosphate (MAP) led to a significant increase in the results of all traits studied compared to control on *Adansonia digitata* L. Azman *et al.* (2018) demonstrated that using MAP maximized vegetative, flowering growth and chemical constituents compared with untreated plants (control) on *Centella asiatica*. In this concern, Moghith (2019) showed that when using treatments from different sources of phosphorus, led to obtain the best results from vegetative growth, seeds yield and chemical compositions,especially when using the monoammonium phosphate treatment in both seasons compared to the control (no phosphorus) in the two seasons for *Salvia hispanica* L. plant.

Although, chemical fertilization is necessary to increase the growth and productivity of medicinal and aromatic plants. However, it is considered a high cost, as it causes environmental pollution and reduces the chances and ability of plants to accept for export. (Sherif and El-Naggar, 2005). So, it is preferable to use biofertilizers as a complete or partial alternative to chemical fertilizers, because it provides an economical aspect for farmers, in addition to being safe and environmentally friendly.

Biofertilizers increase plant growth in all its stages as they increase the availability of nutrients and supply to the host plants, it is a material containing microorganisms added to the plant or treated with seeds or added to the soil in order to affect the growth of plants and enhance it (Vessey, 2003). Recently, as a result of the ability of bio-fertilizers to increase plant growth and they are environmentally and economically friendly and because their continued use has led to an improvement in soil properties and knowledge of many microorganisms that promote plant growth. Also, for a broad knowledge of rhizospheric biology,bio-fertilizers have been increasingly applied in modern agriculture (Mahdi *et al*., 2010). Its effect is attributed to the fact that it causes many elements to dissolve and facilitate the plant, such as insoluble phosphates. It also produces substances that increase plant growth, soil fertility and it also works to fixation of atmospheric N (Mazid and Khan, 2015). In the same time, El-shayeb (2009) on *Oenothera biennis*, L., Dadkhah (2014) on fennel plants, Moghith (2016) on *Origanum vulgare* L., Badran *et al.* (2017) on fennel, Gomaa *et al*. (2018) stated that using NPK as recommended dose or compost at 30 m^3/fed + biofertilizer treatments encouraged the best growth, seeds yield and chemical composition on roselle plants. Also, Mady (2020) on Dutch fennel (*Foeniculum vulgare* Mill) declared that, the treatment with T_2 (75% of the NPK recommended dose plus bio-fertilizers) increased vegetative growth measurements yield and the oil characters compared to other treatments, while, fertilizing with T_5 (bio-fertilizers only) recorded the lowest values compared with the other treatments in the two seasons.

Effective microorganisms known as (E.M.) contain more than 60 types of microorganisms, including (*Lactobacillus plantary*, *lactobacillus casei* and *Streptocous lactis*, photosynthesis bacteria, yeast and algae). Also, it produces lactic acids (Formowitz *et al*., 2007). The results indicate to some studies that the use of technology has led to increase and improve plant growth and its chemical components of ornamental plants, which reflected on increasing the quality and productive capacities of this (Javaid, 2006 and Singh, 2007). In this context, Mohamed and Ghatas (2016) declared that using EM at 30 ml/plant + NPK at 75% or 100% of NPK maximized growth and volatile oil composition in addition to yield of concrete for leaves and flowers of violet. Thereupon, this investigation will study the effect of some phosphorus sources in the presence of biofertilizers (EM and phosphorein) on growth, seeds yield, chemical constituents, oil productivity and fixed oil constituents of *Oenothera biennis*, L. plant.

MATERIALS AND METHODS

Field consecutive experiments were planted at the Experimental Farm and in the Laboratories of Horticulture Department, Faculty of Agric. at Moshtohor, Benha Univ., Egypt during 2018/2019 and 2019/2020 seasons. The investigate aim was to the effect some sources of phosphorus i.e. calcium super phosphate, phosphoric acid, monopotassium phosphate and monoammonium phosphate (MAP) and biofertilizers (EM and phosphorein) as well as their combinations on vegetative growth, flowering, fixed oil productivity and some chemical constituents of *Oenothera biennis*, L. plant.

Evening primrose seeds were obtained from Floriculture Farm, Horticulture Department, Faculty of Agriculture, Benha Univ. Evening primrose seeds were sown in clayey loam soils on October 10th in both seasons in plots (1×1 m) containing two rows (50 cm in between) every row has two hills (50 cm apart) and six weeks later, the plants were thinned, leaving only two seedling/hill.

Physical and chemical analyses of the experimental soil were determined according to Jackson (1973) and Black *et al.* (1982), respectively. The obtained results of soil analysis are presented in Table (1).

The layout of the experiment was a complete randomized block design with two factors with three replicates. The first factor involving five phosphorus sources treatments, whereas the second factor was devoted to three biofertilizers (control, EM and phosphorein). Therefore, the experiment included 15 treatments with three replicates, each replicate contained 20 plants i.e. 60 plants in each treatment.

Phosphorous sources:

All plants received a constant rate of phosphorous sources (35 units P_2O_5/feddan) added for treatments at the time of soil preparation except for control treatment (without phosphorous fertilizer). The phosphorous sources were calculated as follows:

1. Control (without phosphorous fertilizer).

2. Calcium super phosphate (15% P_2O_5) obtained from Abou Zaabal for Fertilizers and Chemical Substances Co. The addition was about (55.5 g/m^2).

3. Phosphoric acid (80% P_2O_5) obtained from Abou Zaabal for Fertilizers and Chemical Substances Co. The addition was about (10.4 g/m^2).

4. Monoamounium phosphate (MAP) containing (61% P_2O_5 and 12% N) from Technogene Co., Dokki, Cario, Egypt), The addition was about (13.6 g/m^2).

5. Monopotassium phosphate (potassium dihydrogen phosphate, KH_2PO_4 M.W. 136.09) (52% P_2O_5 and 34% K_2O) from Technogene Co., Dokki, Cario, Egypt), the addition was about (16.0 g/m^2).

Table 1. Mechanical and chemical analysis of the experimental soil.

Mechanical properties			Chemical analysis		
Parameters	Values		Parameters	Values	
	2018-2019	2019-2020		2018-2019	2019-2020
Coarse sand	5.33 %	6.77 %	**Organic matter**	1.72%	1.82 %
Fine sand	14.26 %	15.44%	**CaCO₃**	0.94 %	1.10 %
Silt	25.96 %	24.12 %	**Available nitrogen**	0.89 %	0.98%
Clay	54.45%	53.67%	**Available phosphorus**	0.49 %	0.58 %
Textural class	Clayey loam	Clayey loam	**Available potassium**	0.60%	0.69%
			pH	7.77	7.87
			EC (dS/m)	0.73	0.85

Bio fertilizers treatments:

The biofertilizer (phosphorein): phosphorus solubilizing bacteria; (*Bacillus megatherium*) phosphorein. Which supplied by the Department of Microbiology, Agric. Res. Center, Giza, Egypt and was used in this study as biological activator. The strains were characterized by a good ability to infect its specific host plant and by its high efficiency in phosphate solubilizing. The seeds of evening primrose were washed with water, thereafter the seeds were soaked in cell suspension of the phosphorein (1 ml contains 108 viable cells) for 30 min. Gum Arabic (16%) was added as an adhesive agent prior to soaking the seeds. The inoculated seeds were air dried at room temperature for one hour before planting. Another two applications were applied (1 kg/fed) as an aqueous solution, the first one was applied just before irrigation after one month from planting date, whereas the second one was done after 45 days from planting date.

Effective microorganisms (EM):

EM (each ml contains 0.6×10^7 microorganisms) was applied either separately or in a mixture in three equal doses to the soil around each plant as 30 ml/plant. The first dose was added after 3 weeks from planting date, while the others at three weeks interval in both seasons and then plants were irrigated immediately.

Harvesting:

The plants were harvested on May 1st in the two seasons.

Data measurements and recorded:

The vegetative and yield parameters were measured and recorded at harvesting time in the May 1st 2019 and 2020 as follows: the vegetative parts were cut about 1 cm above the soil surface. Measurements of the following traits were collected:

Vegetative characteristics:

Plant height (cm), number of branches/plant, leaves fresh and dry weights (g)/plant, stems fresh and dry weights (g)/plant.

Flowering characteristics:

Number of flowers/plants, flowers fresh and dry weights (g/plant).

Seeds yield parameters:

Number of capsules/plants, capsules fresh and dry weights (g/plant), seeds yield (g)/plant and weight of 1000 seed (g).

Chemical constituents :

1. Photosynthetic pigments: chlorophyll a, b and carotenoids were colorimetrically determined in leaves of evening primrose according to the method described by Inskeep and Bloom (1985) and calculated as mg/g fresh weight.

2. Nitrogen, phosphorus, potassium and total carbohydrates (%) were determined in dried evening primrose leaves according to the methods described by Horneck and Miller (1998), Hucker and Catroux (1980), Horneck and Hanson (1998) and Chaplin and Kennedy (1994), respectively.

3. Fixed oil productivity: the clean air dried seeds of evening primrose were separately crushed in a Willey mill, then extracted in Soxhlet apparatus, samples of 10 g of seeds were moved into Soxhlet apparatus in 100 ml of N-hexane and the extraction period extended to three hours (30-36 syphon cycle approx.). The N-hexane extract was dried over anhydrous sodium sulfate, then filtered and the oil was obtained by distillation under vacuum. The percent of fixed oil was calculated as weight/weight using the following equation:
   Fixed oil percentage = Extracted fixed oil weight/seeds sample weight × 100.

4. Fatty acids determination: The methyl esters of fatty acids were prepared by using benzene : methanol : concentrated sulfuric acid (10:86:4) and methylation was carried out for one hour at 80-90 °C, according to Stahl (1967). The residues represented the methylated fatty acids were analyzed by G.L.C. method.

Statistical analysis:

Data from the studied factors were subjected to analyses of variance (ANOVA) as factorial experiments in a complete randomized block design). The differences between the mean values of various treatments were compared by using the least significant differences (L.S.D.) at 5%, by Snedecor and Cochran (1989) using MSTAT-C statistical software package.

RESULTS AND DISCUSSION

Effect of phosphorus sources, biofertilizers (EM and phosphorein) and their interaction treatments on:

Vegetative growth measurements:

Data presented in Tables (2, 3 and 4) suggested that, the addition of various sources of phosphorus i.e. calcium super phosphate, phosphoric acid, monopotassium phosphate and monoammonium phosphate (MAP) resulted in significant increases for all vegetative traits, which are as follows (plant height, branches number, leaves fresh and dry weights and stems fresh and dry weights of evening primrose *Oenothera biennis*, L. in the first and second seasons. The heaviest leaves fresh and dry weights, stems fresh and dry weights and the maximum branches number of this plant were registered by monopotassium phosphate treatment followed the treatment of monoammonium phosphate (MAP) as compared to control (no phosphorus) of both seasons. However, monoammonium phosphate (MAP) addition gave the tallest evening primrose (145.53 and 147.37) in the first and second seasons, respectively. In this context. phosphoric acid addition occupied the third values of all aforementioned vegetative parameters.

Referring to biofertilizers treatments (EM and phosphorein), data showed that all vegetative growth measurements mentioned above were greatly affected by all biofertilizers treatments as compared to control (without any addition). Meanwhile, in 1st and 2nd seasons, the heaviest fresh and dry weights of (leaves and stems), the tallest plants and the largest No. of branches were statistically induced by those plants by treated effective microorganisms (EM) followed by phosphorein.

Additionally, the interaction effect between phosphorus sources and biofertilizers treatments data in the same Tables declared that all combinations between phosphorus sources and biofertilizers treatments increased theses parameters over control. However, the highest values of parameters mentioned afore were gained by using the combined treatment between monopotassium phosphate and EM, with the exception of plant height in the two seasons. Whereas, the tallest plant was recorded by the combined treatment between monoammonium phosphate (MAP) with EM. Irrespective plant height, the combined treatment between monopotassium phosphate with phosphorein or the combined treatment between monoammonium phosphate (MAP) with EM ranked the second and the third values, respectively with non-significant differences between them in some cases. In contrast, the minimum values of these parameters were obtained by control (without any addition) in the both seasons.

The P fertilization is necessary and important to maintain the productivity of the crop, but the efficiency of P fertilization reaches only 20% and accumulates in large quantities in the soil and results in possible environmental pollution, and this reduces the economic use of it in developing and developed countries (Ju *et al.,* 2007). Therefore, we should improve and manage P fertilization and work to increase the efficiency of phosphorous and make it easier for plants and add it in the appropriate image preferred by the plant and thus reduce its presence and accumulation in the soil, which reduces growth (Conde *et al.,* 2014).

The results of different sources of phosphorous on vegetative growth are in close agreement with those reported by Dadkhah (2012) on fennel, Kiliç *et al.*

Table 2. Effect of some phosphorus sources, biofertilizers and their combination treatments on plant height(cm) and number of branches/plant of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Phosphorus sources (A)	Biofertilizers (B)							
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
	Plant height (cm)				Number of branches/plant			
1st season								
Control	116.12	120.41	118.96	118.49	5.33	6.33	7.67	6.44
Calcium super phosphate	120.33	123.51	122.28	122.04	6.67	7.33	6.67	6.89
Phosphoric acid	124.58	129.54	126.20	126.77	7.67	8.00	7.68	7.78
Monopotassium phosphate	133.93	143.33	138.48	138.57	8.33	13.33	11.67	11.11
Monoammonium	139.26	149.43	145.53	144.74	8.67	10.00	9.33	9.33
Mean	126.84	133.25	130.29		7.33	9.00	8.60	
L.S.D at 0.05 for	A=1.044	B= 0.809	A×B= 1.808		A= 0.692	B= 0.536	A×B= 1.198	
2nd season								
Control	117.64	122.15	121.31	120.37	5.67	6.67	6.67	6.33
Calcium super phosphate	122.87	128.32	126.12	125.77	6.67	7.67	7.00	7.11
Phosphoric acid	124.06	132.46	130.68	129.07	8.67	8.67	8.33	8.56
Monopotassium phosphate	135.37	145.48	141.63	140.82	8.67	13.33	12.00	11.33
Monoammonium	139.55	152.42	147.37	146.45	8.33	10.67	9.67	9.56
Mean	127.90	136.17	133.42		7.60	9.40	8.73	
L.S.D at 0.05 for	A=1.148	B= 0.890	A×B= 1.988		A= 0.648	B= 0.502	A×B= 1.123	

EM = Effective microorganisms
Phosph. = Phosphorein

Table 3. Effect of some phosphorus sources, biofertilizers and their combination treatments on leaves fresh and dry weights (g/plant) of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Phosphorus sources (A)	Biofertilizers (B)							
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
	Leaves fresh weight (g/plant)				Leaves dry weight (g/plant)			
1st season								
Control	109.79	134.41	131.18	125.12	16.86	18.77	18.11	17.91
Calcium super phosphate	141.14	175.50	172.35	163.00	20.99	30.22	29.09	26.77
Phosphoric acid	150.33	184.81	181.11	172.08	24.04	33.85	31.03	29.64
Monopotassium phosphate	168.26	199.11	194.29	187.22	31.26	40.37	38.86	36.83
Monoammonium	162.51	190.22	188.91	180.55	29.09	38.26	37.33	34.89
Mean	146.41	176.81	173.57		24.45	32.29	30.88	
L.S.D at 0.05 for	A= 0.834	B= 0.646	A×B= 1.444		A= 0.682	B= 0.528	A×B=1.181	
2nd season								
Control	113.74	137.05	134.22	128.34	17.92	19.40	19.14	18.82
Calcium super phosphate	140.43	178.14	175.29	164.62	21.74	32.32	29.44	27.83
Phosphoric acid	153.51	190.44	186.59	176.85	25.24	34.24	33.01	30.83
Monopotassium phosphate	172.22	202.58	197.46	190.75	32.14	41.92	39.85	37.97
Monoammonium	170.22	195.63	191.03	185.63	31.09	39.37	38.70	36.38
Mean	150.02	180.77	176.92		25.63	33.45	32.03	
L.S.D at 0.05 for	A= 0.732	B= 0.567	A×B= 1.267	A= 0.689	B= 0.534	A×B= 1.193		

EM = Effective microorganisms
Phosph. = Phosphorein

Table 4. Effect of some phosphorus sources, biofertilizers and their combination treatments on stems fresh and dry weights (g/plant) of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Phosphorus sources (A)	Biofertilizers (B)							
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
	Stem fresh weight (g/plant)				Stem dry weight (g/plant)			
	1st season							
Control	104.51	135.37	129.29	123.06	17.75	21.29	24.22	21.09
Calcium super phosphate	131.40	188.51	183.72	167.88	22.19	30.29	28.12	26.87
Phosphoric acid	139.35	197.37	190.07	175.60	27.14	33.20	32.05	30.80
Monopotassium phosphate	160.37	221.99	218.45	200.27	34.42	43.85	40.99	39.75
Monoammonium	158.03	218.29	213.96	196.76	32.11	38.31	36.55	35.66
Mean	138.73	192.31	187.09		26.72	33.39	32.39	
L.S.D at 0.05 for	A= 0.980		B= 0.759	A×B= 1.697	A= 0.648		B= 0.502	A×B= 1.122
	2nd season							
Control	109.42	137.53	131.59	126.18	19.11	22.18	24.54	21.94
Calcium super phosphate	130.74	190.11	185.99	168.95	24.29	31.62	30.11	28.67
Phosphoric acid	140.92	199.66	193.64	178.07	28.66	35.22	32.48	32.12
Monopotassium phosphate	162.33	225.18	220.29	202.60	35.96	44.48	39.37	39.93
Monoammonium	160.29	220.37	217.61	199.42	32.47	38.55	36.37	35.80
Mean	140.74	194.57	189.83		28.10	34.41	32.57	
L.S.D at 0.05 for	A= 0.868	B= 0.672	A×B= 1.502		A=0.777	B= 0.602	A×B= 1.345	

EM = Effective microorganisms
Phosph. = Phosphorein

(2012) on *Thymus vulgaris* L., Rahimi *et al.* (2013) on two basil varieties, Ackerman *et al.* (2013) on canola, Ahmed *et al.* (2014) on damsisa, Soliman *et al.* (2016) on *Adansonia digitata* L., Azman *et al.* (2018) of *Centella asiatica*, Mary *et al.* (2018) on chia (*Salvia hispanica* L.) and Moghith (2019) who suggested that when using treatments from different sources of phosphorus, led to obtain the best results from vegetative growth, especially when using the monoammonium phosphate treatment in both seasons compared to the control (no phosphorus) in the two seasons for *Salvia hispanica* L. plant. Biofertilizers increase plant growth in all its stages as they increase the availability of nutrients and supply to the host plants,they are a material containing microorganisms added to the plant or treated with seeds or added to the soil in order to affect the growth of plants and enhance it (Vessey, 2003). Its effect is attributed to the fact that it causes many elements to dissolve and facilitate the plant, such as insoluble phosphates. It also produces substances that increase plant growth, soil fertility and it also works to fixation of atmospheric N (Mazid and Khan, 2015).

In this concern, other studies strongly confirmed results of our study. Of these studies are El-Shayeb (2009) on *Oenothera biennis*, L., declared that, the combined treatment of biofertilizers at 50 g/pot with garlic extract at 75% improved the vegetative growth of this plant. Dadkhah (2014) on fennel plants, Mohamed *et al.* (2015) on *Ocimum basilicum,* L. cv. Genovese as well as by Shekofteh *et al.* (2015) on *Plantago ovata* plants, Moghith (2016) on *Origanum vulgare* L., Badran *et al.* (2017) and Gomaa *et al.* (2018) on roselle plants. Mady (2020) on Dutch fennel (*Foeniculum vulgare* Mill) declared that, the treatment with T_2 (75% of the NPK recommended dose plus bio-fertilizers) increased vegetative growth measurements yield and the oil characters compared to other treatments, while, fertilizing with T_5 (bio-fertilizers only) recorded the lowest

values compared with the other treatments in the two seasons

Additionally, the use of effective microorganisms (E.M.) technology has led to increase and improve plant growth and its chemical components of ornamental plants, which reflected on increasing the quality and productive capacities of plants (Javaid, 2006 and Singh, 2007). In this context, Mohamed and Ghatas (2016) declared that using EM at 30 ml/plant + NPK at 75% or 100% of NPK maximized growth and volatile oil composition in addition to yield of concrete for leaves and flowers of violet.

Flower growth parameters:

In 1st and 2nd seasons, No. of flowers, flower fresh and dry weights of *Oenothera biennis*, L. presented in Table (5) demonstrated that, applying the plant with phosphorus sources significantly increased all flower growth parameters describe above when compared to the untreated (without phosphorus) in the two seasons. Besides, in both seasons of this study the highest values of these parameters were registered from the monoammonium phosphate (MAP), followed by monopotassium phosphate treatment. On the other hand, the phosphorein treatment produced the highest values of these parameters as compared with other one (EM) and control. Furthermore, presented data in Table (5) illustrated that all the interactions between various phosphorus sources and biofertilizers treatments statistically improved the No. of flowers, flower fresh and dry weights of evening primrose plant, with superior for, the combined treatment between monoammonium phosphate (MAP) with phosphorein which significantly scored the highest values of these parameters. Also, the combined treatment between monopotassium phosphate with phosphorein ranked the second values in this respect.

The results of different sources of phosphorous on flowering growth are in close agreement with those reported by Azman *et al.* (2018) of *Centella asiatica.*,

Mary *et al.* (2018) on chia (*Salvia hispanica* L.) and Moghith (2019) of *Salvia hispanica* L. plant.

Also, El-Shayeb (2009) on *Oenothera biennis*, L. declared that, the combined treatment of biofertilizers at 50 g/pot with garlic extract at 75% improved the vegetative and flowering growth of this plant.

Yield parameters:

Data presented in Tables (6 and 7) showed that the highest number of capsules/plant, the heaviest capsules fresh and dry weights, weight of seeds (g)/plant and weight of 1000 seeds (g) were gained by monopotassium phosphate treatment, followed by monoammonium phosphate (MAP) treatment in the first and second seasons. Hence, the yield parameters mentioned afore were greatly affected by applying evening primrose plants with biofertilizers treatments, particularly the EM as compared with untreated plants. As for the interaction effect between P sources and biofertilizers treatments, data in the same Tables mentioned that, all resulted combinations increased yield parameters in the 1st and 2nd seasons. However, the highest values were listed by the combined treatments between monopotassium phosphate treatment with EM followed descendingly by MAP and applying the plant with EM.

Additionally, the third values in this respect were obtained by monopotassium phosphate treatment combined with phosphorein in both seasons. On the opposite, control gave the minimum values of these yield parameters.

The results of different sources of phosphorous on yield by Dadkhah (2012) on fennel, Kiliç *et al.* (2012) on *Thymus vulgaris* L., Rahimi *et al.* (2013) on two basil varieties, Ackerman *et al.* (2013) on canola, Soliman *et al.* (2016) on *Adansonia digitata* L., Mary *et al.* (2018) on chia (*Salvia hispanica* L.) and Moghith (2019) on *Salvia hispanica* L. plant.

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Table 5. Effect of some phosphorus sources, biofertilizers and their combination treatments on number of flowers/plants, flower fresh and dry weights (g)/plant of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

1st season

Phosphorus sources (A)	Number of flowers/plants				Flower fresh weight (g/plant)				Flower dry weight (g/plant)			
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
Control	123.00	139.33	145.33	135.89	13.14	28.85	31.00	24.33	1.72	2.46	2.77	2.32
Calcium super phosphate	186.67	212.67	217.00	205.44	18.99	33.85	35.85	29.57	3.60	6.42	6.83	5.62
Phosphoric acid	190.00	217.00	217.00	208.22	23.87	38.66	40.26	34.26	4.07	6.85	7.78	6.23
Monopotassium phosphate	197.67	228.00	235.00	220.22	33.12	43.70	47.26	41.36	6.78	7.38	9.17	7.78
Monoammonium phosphate	200.67	229.00	240.00	223.22	36.78	46.07	51.59	44.81	6.83	8.68	9.64	8.38
Mean	179.60	205.20	211.00		25.81	38.23	41.19		4.60	6.36	7.24	
L.S.D at 0.05 for	A= 1.060	B= 0.821	A×B= 1.836		A= 0.640	B= 0.496	A×B= 1.108		A= 0.110	B= 0.085	A×B= 0.191	

2nd season

Phosphorus sources (A)	Number of flowers/plants				Flower fresh weight (g/plant)				Flower dry weight (g/plant)			
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
Control	127.67	144.67	150.00	140.78	14.05	28.73	31.01	24.60	1.99	3.03	3.29	2.77
Calcium super phosphate	191.00	215.00	217.33	204.78	21.17	35.14	36.77	31.03	4.05	6.85	7.25	6.05
Phosphoric acid	193.00	217.00	219.33	209.78	25.63	40.03	42.22	35.96	5.29	7.29	7.35	6.64
Monopotassium phosphate	200.67	230.67	242.33	224.56	36.77	46.63	48.40	43.93	7.15	8.71	9.39	8.41
Monoammonium phosphate	202.00	239.33	245.33	228.89	39.11	48.29	52.51	46.64	6.77	9.18	10.09	8.68
Mean	182.87	209.33	214.87		27.35	39.77	42.18		5.05	7.01	7.47	
L.S.D at 0.05 for	A= 0.878	B= 0.680	A×B= 1.521		A= 0.817	B= 0.633	A×B= 1.415		A= 0.624	B= 0.484	A×B= 1.081	

EM = Effective microorganisms
Phosph. = Phosphorein

Table 6. Effect of some phosphorus sources, biofertilizers and their combination treatments on number of capsules/plant, capsules fresh and dry weights (g)/plant of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Number of capsules/plant

Phosphorus sources (A)	Biofertilizers (B)			
	Control	EM	Phosph.	Mean
1st season				
Control	211.33	257.67	249.00	239.33
Calcium super phosphate	240.00	347.67	344.00	310.56
Phosphoric acid	269.67	364.00	356.67	330.11
Monopotassium phosphate	312.00	398.00	386.33	365.44
Monoammonium phosphate	302.00	395.00	378.67	358.56
Mean	267.00	352.47	342.93	
L.S.D at 0.05 for	A= 1.580	B= 1.224	A×B= 2.736	
2nd season				
Control	213.67	271.33	264.00	249.67
Calcium super phosphate	253.00	361.67	351.00	321.89
Phosphoric acid	282.67	378.67	364.00	341.78
Monopotassium phosphate	319.00	411.67	394.33	375.00
Monoammonium phosphate	310.00	399.67	389.00	366.22
Mean	275.67	364.00	352.47	
L.S.D at 0.05 for	A= 1.523	B= 1.180	A×B= 2.639	

Capsules fresh weight (g)/plant

Phosphorus sources (A)	Biofertilizers (B)			
	Control	EM	Phosph.	Mean
1st season				
Control	71.39	91.07	85.44	82.65
Calcium super phosphate	79.40	120.63	114.81	104.95
Phosphoric acid	80.48	121.99	118.00	106.83
Monopotassium phosphate	107.07	150.29	138.66	132.01
Monoammonium phosphate	102.22	143.58	133.88	126.56
Mean	88.11	125.52	118.16	
L.S.D at 0.05 for	A= 1.074	B= 0.832	A×B= 1.860	
2nd season				
Control	75.33	97.29	89.70	87.44
Calcium super phosphate	83.44	127.99	119.29	110.24
Phosphoric acid	89.44	130.29	123.37	114.37
Monopotassium phosphate	111.29	159.33	147.44	139.35
Monoammonium phosphate	102.81	153.40	141.26	132.49
Mean	92.46	133.66	124.21	
L.S.D at 0.05 for	A= 1.025	B= 0.794	A×B= 1.775	

Capsules dry weight (g)/plant

Phosphorus sources (A)	Biofertilizers (B)			
	Control	EM	Phosph.	Mean
1st season				
Control	23.11	30.37	29.22	27.56
Calcium super phosphate	31.66	44.07	41.55	39.09
Phosphoric acid	37.92	46.14	40.29	41.45
Monopotassium phosphate	45.33	62.33	55.40	54.35
Monoammonium phosphate	40.77	59.11	51.41	50.43
Mean	35.76	48.40	43.78	
L.S.D at 0.05 for	A= 0.469	B= 0.363	A×B= 0.813	
2nd season				
Control	24.72	32.98	29.40	29.03
Calcium super phosphate	32.33	43.26	40.18	38.59
Phosphoric acid	39.25	49.33	41.96	43.51
Monopotassium phosphate	47.63	65.63	58.26	57.17
Monoammonium phosphate	43.63	62.22	55.43	53.76
Mean	37.51	50.68	45.05	
L.S.D at 0.05 for	A= 0.914	B= 0.708	A×B= 1.583	

EM = Effective microorganisms
Phosph. = Phosphorein

Table 7. Effect of some phosphorus sources, biofertilizers and their combination treatments on weight of seeds (g)/plant and weight of 1000 seeds (g) of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Phosphorus sources (A)	Biofertilizers (B)							
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
	Weight of seeds (g)/plant				Weight of 1000 seeds (g)			
1st season								
Control	18.71	23.43	21.25	21.13	0.567	0.650	0.717	0.644
Calcium super phosphate	21.06	31.88	29.29	27.41	0.620	0.740	0.710	0.690
Phosphoric acid	25.96	34.01	33.14	31.04	0.663	0.727	0.700	0.697
Monopotassium phosphate	30.07	44.96	39.66	38.23	0.783	0.913	0.817	0.838
Monoammonium	27.29	41.96	36.86	35.37	0.750	0.860	0.783	0.798
Mean	24.61	35.25	32.04		0.677	0.778	0.745	
L.S.D at 0.05 for	A= 0.751 B= 0.584 A×B= 1.301				A= 0.0021 B= 0.0016 A×B= 0.004			
2nd season								
Control	20.06	24.85	22.32	22.41	0.597	0.727	0.747	0.690
Calcium super phosphate	21.35	32.27	29.15	27.59	0.627	0.723	0.717	0.689
Phosphoric acid	27.43	35.04	32.97	31.82	0.670	0.743	0.727	0.713
Monopotassium phosphate	32.50	47.81	41.33	40.55	0.773	0.957	0.807	0.846
Monoammonium	29.44	46.29	41.33	39.02	0.763	0.897	0.827	0.829
Mean	26.16	37.25	33.42		0.686	0.809	0.765	
L.S.D at 0.05 for	A= 0.801 B= 0.620 A×B= 1.387				A= 0.0019 B= 0.0015 A×B= 0.0033			

EM = Effective microorganisms
Phosph. = Phosphorein

Hence, other studies strongly confirmed results of our study. Of these studies are, El-Shayeb (2009) on *Oenothera biennis*, L., Dadkhah (2014) on fennel plants, Mohamed *et al.* (2015) on *Ocimum basilicum,* L. cv. Genovese as well as by Shekofteh *et al.* (2015) on *Plantago ovata* plants, Moghith (2016) on *Origanum vulgare* L., Badran *et al.* (2017) and Gomaa *et al.* (2018) they stated that using NPK as recommended dose or compost at 30 m^3/fed + biofertilizer treatments encouraged the best seeds yield on roselle plants. Mady (2020) on Dutch fennel (*Foeniculum vulgare* Mill) declared that, the treatment with T$_2$ (75% of the NPK recommended dose plus bio-fertilizers) increased yield characters compared to other treatments, while, fertilizing with T$_5$ (bio-fertilizers only) recorded the lowest values compared with the other treatments in the two seasons

In this context, Mohamed and Ghatas (2016) declared that using EM at 30 ml/plant + NPK at 75% or 100% of NPK maximized volatile oil composition in addition to yield of concrete for leaves and flowers of violet.

Chemical composition determinations:

Chlorophyll a, b and carotenoids (mg/g fresh weight), N, P, K and total carbohydrates (%):

Data presented in Tables (8, 9 and 10) declare that chemical composition i.e. (chlorophyll a, b, carotenoids, N, P, K % and total carbohydrates %) were affected by applying the different sources of phosphorus when compared to untreated (no phosphorus) in both seasons. However, the maximum chlorophyll a, b, carotenoids, K % and total carbohydrates % were registered by monopotassium phosphate treatment in the two seasons. Moreover, in both seasons, MAP treatment scores the richest percentage of N and P. Regarding biofertilizers treatments, data in Tables (8, 9 and 10) suggested that all biofertilizer treatments significantly increased these chemical parameters describe above of evening

Table 8. Effect of some phosphorus sources, biofertilizers and their combination treatments on chlorophyll a, b and carotenoids (mg/g f.w.) of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Phosphorus sources (A)	Chlorophyll a (mg/g f.w.)				Chlorophyll b (mg/g f.w.)				Carotenoids (mg/g f.w.)			
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
1st season												
Control	0.798	0.825	0.814	0.812	0.399	0.435	0.417	0.417	0.195	0.235	0.229	0.220
Calcium super phosphate	0.838	0.890	0.874	0.867	0.413	0.528	0.518	0.487	0.202	0.295	0.278	0.258
Phosphoric acid	0.865	0.955	0.942	0.921	0.415	0.547	0.538	0.500	0.212	0.301	0.291	0.268
Monopotassium phosphate	0.981	1.123	0.998	1.034	0.465	0.588	0.572	0.542	0.285	0.342	0.335	0.320
Monoammonium phosphate	0.975	1.037	0.990	1.001	0.474	0.580	0.567	0.540	0.270	0.338	0.318	0.309
Mean	0.891	0.966	0.923		0.433	0.536	0.522		0.233	0.302	0.290	
L.S.D at 0.05 for	A=0.0027	B=0.0021	A×B=0.0047		A=0.0027	B=0.0021	A×B=0.0047		A=0.0030	B=0.0022	A×B=0.0050	
2nd season												
Control	0.812	0.838	0.834	0.828	0.418	0.445	0.434	0.433	0.198	0.237	0.229	0.221
Calcium super phosphate	0.855	0.901	0.890	0.882	0.425	0.541	0.528	0.498	0.210	0.289	0.282	0.260
Phosphoric acid	0.865	0.962	0.945	0.927	0.429	0.553	0.545	0.509	0.220	0.298	0.283	0.267
Monopotassium phosphate	0.962	1.177	0.998	1.046	0.493	0.607	0.585	0.562	0.285	0.358	0.343	0.329
Monoammonium phosphate	0.980	1.081	0.989	1.017	0.484	0.588	0.581	0.551	0.278	0.347	0.332	0.319
Mean	0.895	0.992	0.931		0.450	0.547	0.535		0.238	0.306	0.294	
L.S.D at 0.05 for	A= 0.0024	B= 0.0018	A×B= 0.0041		A= 0.0019	B= 0.0015	A×B= 0.0033		A= 0.0017	B= 0.0013	A×B= 0.0029	

EM = Effective microorganisms
Phosph. = Phosphorein

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Table 9. Effect of some phosphorus sources, biofertilizers and their combination treatments on N, P and K (%) of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

1st season

Phosphorus sources (A)	N (%)				P (%)				K (%)			
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
Control	2.12	2.38	2.27	2.25	0.208	0.248	0.275	0.244	1.40	1.55	1.44	1.46
Calcium super phosphate	2.29	2.88	2.73	2.63	0.225	0.305	0.330	0.287	1.68	1.81	1.75	1.75
Phosphoric acid	2.38	3.15	3.03	2.85	0.238	0.360	0.375	0.325	1.65	2.05	1.98	1.89
Monopotassium phosphate	2.82	3.28	3.22	3.11	0.286	0.369	0.382	0.346	1.93	2.44	2.45	2.28
Monoammonium phosphate	2.58	3.58	3.41	3.19	0.296	0.375	0.391	0.354	1.75	2.37	2.10	2.07
Mean	2.44	3.05	2.93		0.251	0.332	0.351		1.68	2.05	1.94	
L.S.D at 0.05 for	A=0.0027	B=0.0021	A×B=0.0047		A=0.0017	B=0.0013	A×B=0.0029		A= 0.061	B=0.047	A×B=0.106	

2nd season

Phosphorus sources (A)	N (%)				P (%)				K (%)			
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
Control	2.18	2.41	2.36	2.32	0.210	0.258	0.289	0.252	1.54	1.65	1.65	1.62
Calcium super phosphate	2.27	2.99	2.80	2.69	0.235	0.307	0.338	0.293	1.78	1.90	1.82	1.83
Phosphoric acid	2.40	3.05	2.95	2.80	0.240	0.365	0.378	0.328	1.76	2.15	2.03	1.98
Monopotassium phosphate	2.88	3.23	3.32	3.14	0.299	0.377	0.388	0.355	2.00	2.67	2.61	2.43
Monoammonium phosphate	2.76	3.60	3.49	3.28	0.301	0.381	0.406	0.363	1.88	2.48	2.39	2.25
Mean	2.50	3.06	2.99		0.257	0.338	0.360		1.79	2.17	2.10	
L.S.D at 0.05 for	A=0.0030	B=0.0022	A×B=0.0050		A=0.0027	B=0.0021	A×B=0.0047		A= 0.053	B=0.041	A×B= 0.092	

EM = Effective microorganisms
Phosph. = Phosphorein

Table 10. Effect of some phosphorus sources, biofertilizers and their combination treatments on total carbohydrates (%) and fixed oil (%) of *Oenothera biennis* L. plant, during 2018/2019 and 2019/2020 seasons.

Phosphorus sources (A)	Biofertilizers (B)							
	Control	EM	Phosph.	Mean	Control	EM	Phosph.	Mean
	Total carbohydrates (%)				Fixed oil (%)			
1st season								
Control	12.89	15.47	14.44	14.26	14.75	16.55	17.77	16.36
Calcium super phosphate	15.23	15.72	15.42	15.46	16.73	19.58	23.68	19.99
Phosphoric acid	15.24	16.89	14.78	15.64	17.15	21.27	24.43	20.95
Monopotassium phosphate	16.65	21.65	20.22	19.51	19.94	23.25	27.22	23.47
Monoammonium	15.86	18.90	17.26	17.34	18.94	21.94	25.40	21.89
Mean	15.17	17.73	16.42		17.38	20.52	23.70	
L.S.D at 0.05 for	A= 0.769	B= 0.596	A×B= 1.332		A= 0.608	B= 0.471	A×B= 1.052	
2nd season								
Control	13.24	14.50	15.58	14.44	14.95	16.37	18.06	16.46
Calcium super phosphate	16.05	15.92	16.51	16.16	17.51	18.92	24.99	20.47
Phosphoric acid	15.44	17.75	16.47	16.55	18.99	22.38	26.04	22.47
Monopotassium phosphate	17.33	22.30	19.91	19.84	21.26	25.40	28.37	25.01
Monoammonium	16.3	19.13	17.54	17.66	18.88	22.75	27.24	22.96
Mean	15.67	17.92	17.20		18.32	21.16	24.94	
L.S.D at 0.05 for	A= 0.708	B= 0.549	A×B= 1.227		A= 0.878	B= 680	A×B= 1.521	

EM = Effective microorganisms
Phosph. = Phosphorein

primrose *Oenothera biennis*, L. plant when compared to control. In this concern, EM gave higher values of all aforementioned chemical parameters, with the exception of P % in 1st and 2nd seasons. Whereas, in both seasons the maximum values of P% was listed by phosphorein treatment.

Meanwhile, the highest values of chlorophyll a, b, carotenoids, K % and total carbohydrates % were recorded by using the combined treatment between monopotassium phosphate treatment and EM. While, MAP treatment combined with EM showed to be the most effective of N %. Additionally, the highest values of P % was gained by MAP treatment combined with phosphorein in both seasons.

In this concern, other studies strongly of biofertilizers on chemical composition are in close agreement with those reported, El-Shayeb (2009) on *Oenothera biennis*, L., Dadkhah (2014) on fennel plants, Mohamed *et al.* (2015) on *Ocimum basilicum,* L. cv. Genovese as well as by Shekofteh *et al.*

(2015) on *Plantago ovata* plants, Moghith (2016) on *Origanum vulgare* L.

Badran *et al.* (2017) and Gomaa *et al.* (2018) they stated that using NPK as recommended dose or compost at 30 m^3 /fed + biofertilizer treatments encouraged the best growth, seeds yield and chemical composition on roselle plants. Mady (2020) on Dutch fennel (*Foeniculum vulgare* Mill declared that, the treatment with T$_2$ (75% of the NPK recommended dose plus bio-fertilizers) increased chemical composition as compared to other treatments. While, fertilizing with T$_5$ (bio-fertilizers only) recorded the lowest values compared with the other treatments.

Additionally, the use of effective microorganisms (E.M.) technology has led to increased and improved plant growth and its chemical components of ornamental plants, which reflected on increasing the quality and productive capacities of this (Javaid 2006 and Singh, 2007). In this context, Mohamed and Ghatas (2016) declared that using EM at

30 ml/plant + NPK at 75% or 100% of NPK maximized chemical composition in addition to yield of concrete for leaves and flowers of violet.

Fixed oil percentage:

According to data listed in Table (10) illustrated that, fixed oil percentage, of evening primrose was more affected by using applied treatments of P sources and biofertilizers as well as their combinations as compared to control plants in the first and second seasons. In this context, in both seasons, monopotassium phosphate or phosphorein significantly gained the maximum values of fixed oil percentage/plant. However, the highest seeds oil percentage (27.22 and 28.37) were recorded by the combined treatment between monopotassium phosphate and phosphorein, followed the combined treatment between MAP and phosphorein (25.40 and 27.24) in the first and second seasons, respectively. Furthermore, using the combined treatment of monopotassium phosphate with EM occupied the third value in this respect as it (23.25 and 25.40). The lowest value of fixed oil percentage of Oenothera *biennis*, L. plant was produced by un-treated control plants in the two seasons (14.75 and 14.95), in 1st and 2nd seasons respectively. In this concern, Hudson (1984) mentioned that, evening primrose seeds *Oenothera biennis*, L.

contain 24% oil with 7% to 14% gamma-linolenic acid of the fatty acid components.

Fixed oil compositions of evening primrose (*Oenothera biennis*, L.) seeds:

Table (11) and Figs. (1-4) showed the data belonging to the effect of different combined treatments of P sources and biofertilizers i.e. control, monoammonium phosphate (MAP) with EM, monopotassium phosphate with phosphorein, monopotassium phosphate with EM on the qualitative of the fixed oil compositions of evening primrose seeds. The fixed oil composition gained four components were identified, i.e. palmitic acid, oleic acid, linoleic acid and α-linolenic acid. Hence, the main component was linoleic acid (55.19 to 64.85%). Meanwhile, the components of fixed oil compositions were ranged a descending order, as follows linoleic acid (55.19 to 64.85%), palmitic acid (8.56 to 14.68%), α-linolenic acid (12.11 to 12.55%) and oleic acid (10.95 to 11.53%). Moreover, the combination treatments of monopotassium phosphate with EM score the richest values of linolenic acid as (64.85%) followed by the combined treatment of monopotassium phosphate with phosphorein as (63.35%) and the combined treatment of monoammonium phosphate (MAP) with EM (57.23%) when compared to control (55.19%).

Table 11. Effect of different treatments on fixed oil composition of *Oenothera biennis* L. plant, during the second season 2019/2020.

Peak No.	Component name	Area %			
		Control (tap water)	Monoammonium phosphate (MAP) with EM	Monopotassium phosphate with phosphorein	Monopotassium phosphate with EM
1	Palmitic acid	12.72	14.68	9.12	8.56
2	Oleic acid	10.95	11.13	11.33	11.53
3	Linoleic acid	55.19	57.23	63.35	64.85
4	α-linolenic acid	12.11	12.33	12.44	12.55
-	Total identified	90.97	95.37	96.24	97.49
*	Unknown	9.03	4.063	3.76	2.51
Total		100.00	100.00	100.00	100.00

Fig. 1. G.L.C. of *Oenothera biennis* oil composition from control treatment.
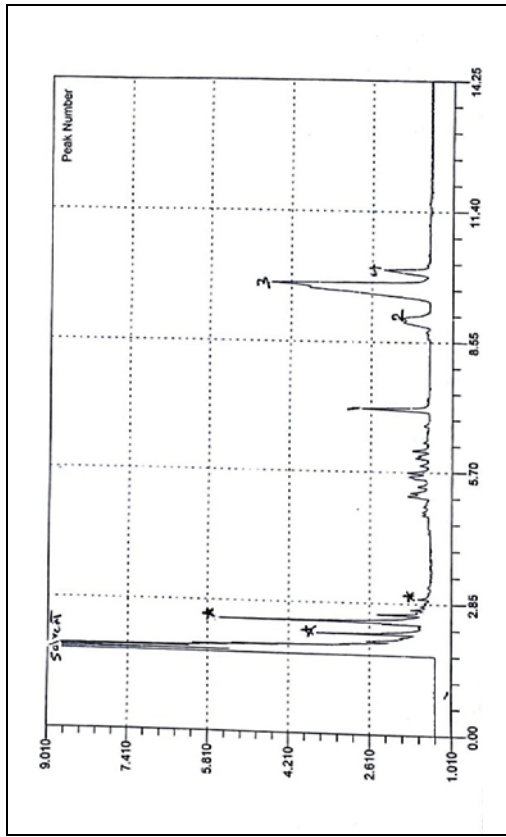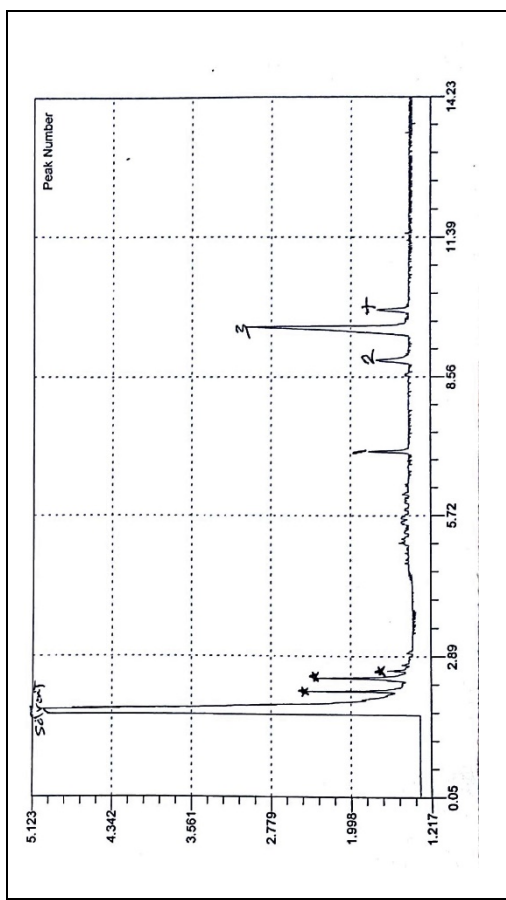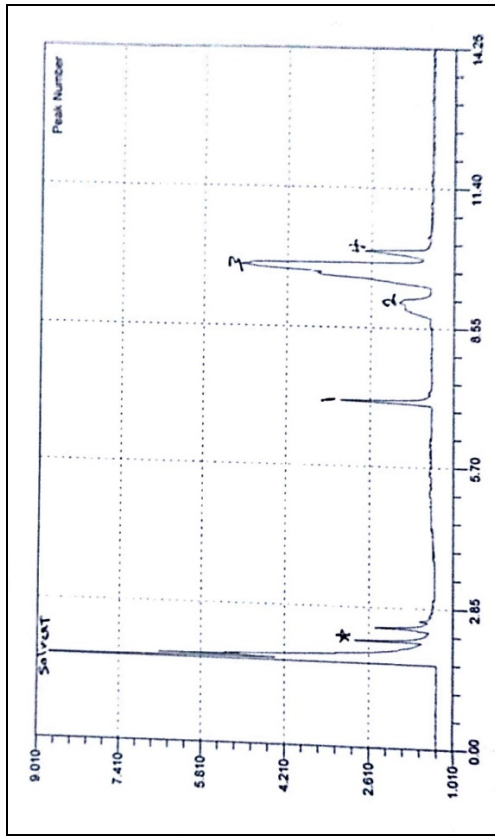

Fig. 2. G.L.C. of *Oenothera biennis* oil composition from monoammonium phosphate (MAP) with EM.
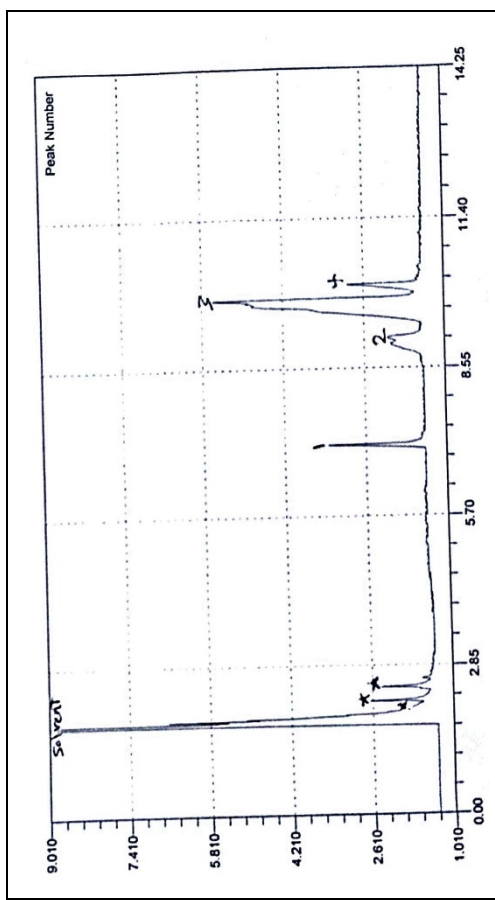

Fig. 3. G.L.C. of *Oenothera biennis* oil composition from monopotassium phosphate with phosphorein treatment.

Fig. 4. G.L.C. of *Oenothera biennis* oil composition from monoammonium phosphate (MAP) with EM.

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Additionally, different combined treatments caused slightly increases in the percentage of α-linoleic acid from 12.11 in control to 12.55% of the combination treatments of monopotassium phosphate with EM. Furthermore, the combined treatment monoammonium phosphate (MAP) with EM produced the maximum values of palmitic acid (14.68%) followed by the control treatment (12.72%). Finally, the combination treatments of monopotassium phosphate with EM register the highest values of oleic acid followed by combined treatment of monopotassium phosphate with phosphorein as it (11.53 and 11.33%), respectively. Hulan *et al.* (1987) showed that the evening primrose seeds contain many important compounds, as they contain high amounts of mineral salts such as calcium, phosphorous, potassium, iron, manganese and zinc, as they contain lipids in a ratio of 21-34%., Hudson (1984) mentioned that, evening primrose seeds (*Oenothera biennis*, L.) contain 24% oil with 7% to 14% gamma-linolenic acid of the fatty acid components. Petru *et al.* (1993) demonstrated that 24% of oil content for *Oenothera biennis* seeds including two major fatty acids (linolenic and gamma-linolenic acid).

Swaefy *et al.* (2008) illustrated that the major saturated fatty acid was palmitic of evening primrose (*Oenothera biennis* L.). which ranged from 4.45% in the plants treated with 2 m³ compost/feddan plus 50% chemical fertilizers to 10.88% with 4 m³ compost/feddan plus 50% chemical fertilizers. Also, El-Shayeb (2009) illustrated that, GLC analysis of methylated fatty acid on *Oenothera biennis*, L., showed that the main components of fixed oil compositions linoleic acid (49.65%), oleic acid (18.65%). palmitic acid (10.21%).

Conclusively, from the obtained results, it could be recommended that the monoamonium phosphate (MAP) or monopotassium phosphate with EM or phosphorein were the best for improving growth, seeds yield, chemical constituents, fixed oil productivity and fixed oil constituents of evening primrose (*Oenothera biennis*, L.) plant.

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تأثير بعض مصادر الفسفور والأسمدة الحيوية (EM و phosphorein) على النمو، إنتاجية الزيت الثابت، المكونات الكيميائية لنبات ربيع الليل (.Oenothera biennis L).

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الاونثرا من نباتات الزينة الجميلة، ولكن في نفس الوقت لها خصائص طبية تجعلها من النباتات ذات قيمة وأهمية كبيرة. في هذا السياق، يهدف البحث الحالي إلى دراسة تأثير مصادر الفوسفور والأسمدة الحيوية (EM والفوسفورين) على النمو، والإزهار، وإنتاج البذور، والمكونات الكيميائية، وإنتاجية الزيت، ومكونات الزيت الثابت لنبات الاونثرا (.Oenothera biennis L). أجريت الدراسة في مزرعه التجارب والمعامل بقسم البساتين بكلية الزراعه بمشتهر جامعه بنها خلال عامي ٢٠١٨/٢٠١٩و٢٠١٩/٢٠٢٠ م. قد أوضحت النتائج أنه عند معاملة النباتات بمصادر الفوسفور والأسمدة

الحيوية بالإضافة إلى معاملات التفاعل بينهم ادت الى زيادة كبيرة في جميع الخصائص المدروسة والصفات الخضرية والزهرية ومحصول البذور والتركيب الكيميائي ونسبة الزيت الثابتة ومكونات الزيت الثابت (الأحماض الدهنية) لنبات الاونثرا. تم الحصول على أعلى القيم لمعظم القراءات سابقة الذكر من خلال معاملة التفاعل بين فوسفات البوتاسيوم الاحادية مع EM في الموسمين الأول والثاني. بالإضافة إلى ذلك، تم تسجيل أعلى القراءات الزهرية والنسبة المئوية للفوسفور من خلال معاملة التفاعل بين فوسفات الامونيوم الاحادية (MAP) مع الفوسفورين في كلا الموسمين. علاوة على ذلك، تم تسجيل أعلى نسبة زيت بذور (٢٧٫٢٢ ــ ٢٨٫٣٧) من خلال معاملة التفاعل بين فوسفات البوتاسيوم الاحادية والفوسفورين فى الموسم الاول والثانى على التوالى. أظهرت البيانات الخاصة بتحليل الزيت الثابت انه يتكون من ٤ أحماض دهنية هي حمض البالميتك و حمض الأوليك و حمض اللينوليك وحمض الألفا-لينولينك. يمكن الاستنتاج أن فوسفات أحادي الأمونيوم (MAP) أو فوسفات البوتاسيوم الأحادي مع EM أو الفوسفوريين كانت الأفضل لتحسين النمو، وإنتاج البذور، وإنتاجية الزيت الثابت، والمكونات الكيميائية ومكونات الزيت الثابت لنبات الاونثرا (*Oenothera* *biennis* L.).