Effect of Arbuscular Mycorrhizal Fungi And Some Phosphorus Sources on Growth, Seeds Yield, Chemical Compositions, Oil Productivity and Fixed Oil Constituents of Chia (*Salvia hispanica* L.) Plant

Moghith, W. M. A.[2]; Youssef, A. S. M.[1]; Abd El-Wahab, M. A.[2]; Mohamed, Y. F. Y.[1] and Abou El-Ghait, Eman M.[1]

1. Horticulture Department, Faculty of Agriculture, Benha University, Egypt.
2. Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt.

Corresponding author: walidmoghith@gmail.com

Abstract

This study was conducted at the Experimental farm of the Heliopils University, El Sharqea, Egypt during the two successive seasons of 2016/2017 and 2017/2018. The aim of this study was to investigate the possibility of improving growth, seed yield, oil productivity and fixed oil constituents of chia (*Salvia hispanica* L.) plants grown in sandy soil by using arbuscular mycorrhizal fungi (AMF) and some phosphorus sources i.e., phosphate rock - super phosphate - monoamonium phosphate (MAP) and phosphoric acid (30 units P_2O_5).

Seeds of chia (*Salvia hispanica* L.) were inoculated with mixed spores of arbuscular mycorrhizal fungi (AMF) from genera *Glomus*, *mosseae* and *fasciculatum* [275 Spores/g oven dry basses in addition to the colonization root pieces (the infectivity 104 propagola) sterilized peatmoss: vermicolite: perlite as a carries] at the rate of 20g /5g of seeds and after 10 days by injection into the soil at 3.5 g/hill.

The obtained results indicated that, plants receiving mycorrhizal fungi inoculum gave the significantly highest mean values for most of studied characteristics (i.e., plant heigt, number of branches per plant, plant fresh and dry weights, seeds yield/plant and/or feddan, weight of 1000 seeds, chlorophyll "a, b,", carotenoids, N, P, K, total carbohydrate, fixed oil percentage, fixed oil yield /plant and/or feddan and fatty acids constituents of chia (*Salvia hispanica* L.) plants, while uninoculated plants (control) gave the lowest values. Whereas different sources of phosphorous fertilizers statistically affected most of the mentioned parameters, especially monoaminum phosphate treatment (MAP) in both seasons. Moreover, all the combination treatments between inoculated with AMF, uninoculated and phosphorous sources improved all the studied parameters. The recorded results indicated that, the combined treatments between mycorrhizal fungi inoculated and MAP gave the highest values for most recorded parameters, i.e., chlorophyll a, b, total carotenoids, N, P, K, total carbohydrate contents, fixed oil percentage, fixed oil yield/plant and/or feddan as compared with the other combined treatments both seasons. Meanwhile, the combined treatments of inoculation with the mixed spores of arbuscular mycorrhizal fungi and phosphoric acid induced highly significant increments in this concern in both seasons. Chromatography analyses of chia fixed oil revealed the identification of 23 components, the main component was α-linolenic acid (54.96 to 63.23%). The major components were α-linolenic acid, linoleic acid, oleic acid, and palmitic acid. It can be concluded that the inoculation with the mixed spores of arbuscular mycorrhizal fungi and monoamonium phosphate (MAP) was the best for improving growth, seeds yield, fixed oil productivity, chemical compositions and fixed oil constituents of chia (*Salvia hispanica* L.) plant.

Keywords: *Salvia hispanica*, Chia, Fixed oil, Omega-3, Phosphorus sources, Mycorrhizal, GLC.

Introduction

The chia (*Salvia hispanica* L.) is an annual plant belonging to family Lamiaceae or Labiate native to Mexico and Guatemala (**Ixtaina *et al.*, 2008**). In pre-Columbian times, chia is one of the basic foods of Central American civilizations (**Ayerza and Coates 2005**). Owing to the fact that it can grow in arid environments, it has been highly recommended as an alternative crop for the field crop industry **(Peiretti and Gai 2009).** The cultivation of chia is gaining popularity in Africa because it is considered as a healthy food and good nutrition (**Ayerza and Coates 2000**).

Salvia hispanica L. is the richest botanical oil source of α-linolenic acid (omega-3) Known **(Ayerza 2013).** Chia is an oilseed crop with potential use as human food **(Zanqui *et al.*, 2015 and Coorey *et al.*, 2012).**

The seed contains from 25% to 40% oil with 60% of its comprising α-linolenic acid (omega 3) and 20% of linoleic acid (omega 6). Chia can grow up to 1 m tall and has opposite arranged leaves. Chia flowers are small flower (3-4 mm) with small corollas and fused flower parts that contribute to a high self-pollination rate. The seed color varies from black, gray, and black spotted to white, and the shape is oval with size ranging from 1 to 2 mm **(Ali *et al.*, 2012; Bresson *et al.*, 2009; Peiretti and Meineri 2008; Reyes-Caudillo *et al.*, 2008; Cahill and Provance 2002).**

Chia seed is composed of protein (15-25%), fats (30-33%), carbohydrates (26-41%), high dietary fiber (18-30%), ash (4-5%), minerals, vitamins, and dry matter (90-93%). It also contains a high amount of antioxidants **(Ixtaina *et al.*, 2008).**

Mycorrhizal fungi are widespread in agricultural systems and are especially relevant to organic

agriculture because they can act as natural fertilizers, enhancing plant yield, Mycorrhizal fungi forms extensive hyphal networks in the soil and provide plants with nutrients in return for assimilates **(Van der Heijden *et al.*, 2008).**

Arbuscular mycorrhizal fungi (AMF) increases the plant uptake of immobile phosphate ions from the soil as well as N, P, K, Mg and some micronutrients leading to stimulating growth **(Smith *et al.*, 2011 and Veresoglou *et al.*, 2011).** It regulates the synthesis and distribution of plant hormones **(Barker and Tagu 2000).** Some agriculture practices such as inoculation with arbuscular mycorrhizal fungi (AMF) as a natural biofertilizer are obligate stability by using their thick extraradical hyphal network and secret glycoproteins (glomalin and glomail related proteins) to compact the soil particles **(Bedini *et al.*, 2009)**.

The most important biotic factor affecting the P status of plants is AMF significantly to the mineral nutrition of the host plant, especially in terms of P uptake **(Santos *et al.*, 2010).** Phosphorus is an essential macronutrient for plant growth and an essential element determining plant growth and productivity **(Raghothama and Karthikeyan 2005 and Malhotra *et al.*, 2018)**.

Low P availability is a major limiting constraint for crop production on acid soils **(Wang *et al.*, 2010).** In spite of application of P fertilizer is essential to maintain crop yield, applied P fertilization efficiency is usually low (only 20%) and readily, leading to P accumulation in soils and resulting in potentially environmental pollution, and thus not economical for agricultural production in developing countries or even in developed countries **(Ju *et al.*, 2007).** Furthermore, P fertilizers are produced from phosphate rock which is a non-renewable resource, and will be fully consumed within next few decades **(Stewart *et al.*, 2005)**. Therefore, to improve P fertilizer management as well as to enhance P efficiency in crops is absolutely necessary for environment-friendly agriculture **(Conde *et al.*, 2014).**

Some of the studies indicate to the effect of phosphorous sources on different plants, **Dadkhah (2012)** showed that inoculation AMF of bio-fertilizers applied with 50% recommended dosage of NP (super phosphate), increased vegetative growth of fennel plants compared to chemical fertilizer treatments only. **Kilic *et al.*, (2012)** on *Thymus vulgaris* L. mentioned that using phosphoric acid in as a source of phosphor significantly increased green herb yield (kg ha^{-1}) and N, P, k content, thymol, paracymen and carvacrol. **Awad Alla *et al.*, (2013)** showed that coriander plants were significantly responded to Egyptian phosphate rock increased the vegetative growth and fruit yield. **Soliman *et al.*, (2016)** on baobab (*Adansonia digitata* L.). The results indicate that monoammonium phosphate MAP increased significantly all studied traits compared to control. **Azman *et al.*, (2018)** on *Centella asiatica* stated that, MAP monoammonium phosphate enhanced N, P and K content, no. of leaves, no. of branches no. of flowers, total fresh biomass and total dry biomass compared with untreated plants (control).

Hassan *et al.*, (2018) on caraway (*Carum carvi* L.) plants showed that, using different sources of rock phosphate at all treatments led to a significant increase in plant height, total herb dry weight, fruit yield $plant^{-1}$**,** fruit yield $feddan^{-1}$, the essential oil yield $plant^{-1}$, essential oil $feddan^{-1}$ and phosphorus percentage compared with control in both seasons**. Pedone-Bonfim *et al.*, (2018)** reported that, the plant growth is severely restricted at low P levels, but the addition of AMF appears to remove this limiting factor. Although *M. tenuiflora* responds to levels of phosphate fertilization, it responds well to mycorrhizal inoculation, which promotes benefits for secondary metabolite content in this plant.

Of greater importance of chia plant and its recent entry into Egyptian agriculture, this investigation will study the effect of some phosphorus sources in presence of arbuscular mycorrhizal fungi on growth, seeds yield, chemical compositions, oil productivity and fixed oil constituents of chia (*Salvia hispanica* L.) plant.

II. Material and Methods

The study was carried out in open field at Experimental Farm of Heliopils University, El Sharqea Governorat, Egypt. (30° 22' 49.1" N Latitude 31° 39' 37.8" E Longitude) during the two successive seasons of 2016/2017 and 2017/2018. The aim of this study was to investigate the role of arbuscular mycorrhizal fungi and different sources of phosphorus in improving vegetative growth, seed yield, fixed oil productivity, component and chemical compositions of chia (*Salvia hispanica* L.) plant.

Seeds were obtained from Sekem Company, Egypt. On 5th October 2016 and 7th October 2017 in the first and second seasons, respectively, seeds of chia (1000 seed weight was 1.25 g) were sown directly in the hills at a rate of 10 seeds /hill. The distance between rows was 70 cm and 30 cm between hills on both sides of each row. After three weeks from sowing, the seedlings were thinned out to two plants /hill and after one week from the first thinning the plants were thinned out to one plant/hill (about 40000 plants/feddan approx.). Drip irrigation system was used with drippers (2.0 liter/hour/hill) 35 cm distance in two lines for each row, drippers were setup at 2.0 liter/hour/plant for only two hours every 2-3 days in the whole period of both seasons.

Randomized soil sample representing the experimental area was taken at 0-30 cm depth before beginning any treatments. The soil samples were examined for its physical and chemical

characteristics (as illustrated in Table A) at a laboratory in the Desert Research Center. Physical analyses of the soil used during the two seasons were evaluated according to **(Jackson 1973)**, whereas chemical analyses was estimated according to **(Black *et al.*, 1982).** The water analyses (as illustrated in Table B) have taken from the irrigation water used during the two seasons were analyzed at the same laboratory according to **(Rainwater and Thatcher 1960).**

Table (A): Physical and chemical analyses of the soil used during the two seasons.

Chemical analyses	pH	E. C. (dS/cm)	O.M. (%)	Cations (meq/l)			Anions (meq/l)				TDS (mg/l)	N (mg/l)	P (mg/l)	K (mg/l)
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCo_3^{-}	$So4^{--}$	Cl^{-}				
	7.6	0.93	1.7	3.10	1.01	4.65	0.57	2.50	3.21	3.60	584.9	71.2	5.12	65.4

Physical analyses	Very coarse sand (%)	Coarse sand (%)	Medium sand (%)	Fine sand (%)	Very fine sand (%)	Silt and clay (%)	Soil texture
	11.25	20.50	33.20	25.10	6.94	2.89	Fine sandy

The chemical properties of soil in ppm (water extract 1:2:5 v/v)

Table (B): Water analyses of the irrigation water**.**

pH	E. C. (dS/cm)	Soluble cations (mg/l.)				Soluble anions (mg/l.)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3^{--}	HCo_3^{-}	So_4^{--}	Cl^{-}
7.3	3.81	8.2	9.0	19.0	2.5	7.0	9.6	10.5	13.5

Arbuscular mycorrhizal fungi inoculum consisted of roots, hyphae, spores and growth media from a pot culture of onion plants colonization with *Glomus mosseae* NRC31 and *G*lomus *fasciculatum* NRC15 originally isolated from Egyptian soils and multiply on sterilized peat: vermicolite: perlit **(Badr El-Din *et al.*, 1999).** Arbuscular mycorrhizal fungi obtained from Agricultural Microbiology Department, National Research Center. Inoculum material contained 275 spores g^{-1} oven dry bases in addition to the colonization roots pieces (the infectivity 104 propagola). Mycorrhizal fungi inoculation was done by mixed 20 g of it with 5 g of chia seed before cultivation and then after 10 days by injection into the soil in roots area of seedlings from four sides at 3.5 g/hill of inoculum material.

Compost was added at the time of soil preparation and mixed thoroughly with the soil of the experimental area at the rate of 10 m^3/feddan. All plants received a constant rate of phosphorous sources (30 units P_2O_5/feddan) were added for treatments at the time of soil preparation except control treatment (without phosphorous fertilizer). The phosphorous sources were calculated as follows:

1. Monoamounium phosphate (MAP) which containing (61% P_2O_5 and 12% N) obtained from Everfert Co., the applied amount was 12.5 g/m^2. The same amount of nitrogen in MAP was supplied to another four treatments.
2. Calcium super phosphate (15% P_2O_5) obtained from Abou Zaabal for Fertilizers and Chemical substances Co. the applied amount was 48 g/m^2.
3. Phosphate rock (30% P_2O_5) was obtained from Alahram Mining Co. the applied amount was 22 g/m^2.
4. Phosphoric acid (80% P_2O_5) obtained from Abou Zaabal for Fertilizers and Chemical substances Co. the applied amount was 8.5 g/m^2.

The layout of the experiment was completely randomized design with two factors. The first factor was arbuscular mycorrhizal fungi (uninoculum and inoculum), the second factor was phosphorous sources (0.00 phosphorous, phosphate rock, super phosphate, monoamonium phosphate and phosphoric acid).

The experiment included 10 treatments with three replicates, each replicates consisted of 10 plants, i.e. 30 plants in each treatment. The plants were harvested on on 17th and 18th February in the first and second season, respectively. The vegetative parts were cut about 1.00 cm above the soil surface. Measurements of the following traits were collected:

Vegetative growth measurements at the beginning of flowering

Plant height [(cm), length of the main stem from soil surface to the plant apex using measuring tape], number of branches (No.), fresh weight $plant^{-1}$ (g) and dry weight $plant^{-1}$ (g).

Seeds yield parameters

After harvesting time plants were removed to station for sampling and other measurements the following measurements were taken:

Weight of 1000 seeds (g), seeds yield $plant^{-1}$ (g), and seeds yield $feddan^{-1}$ (kg).

Chemical compositions

Chemical analyses were determined for internal plant compositions as follows:

1. Photosynthetic pigments:

Chlorophyll a, b and total carotenoids contents (mg/g) were determined in fresh leaves samples according to **Saric *et al*, (1967).**

2. N, P, and K elements % determination in dry leaves:

The chemical analyses were carried out on dried leaves samples obtained from the different

treatments. The dry leaves were ground to a fine powder for the determination of N, P, and K elements.

N, P, and K, elements were determined in the acid digested solution, which was prepared according to **Hach *et al.*, (1987)** using a mixture of sulfuric acid and hydrogen peroxide (10:1). Elements estimated were made with 0.2 g of the dried samples.

Nitrogen content was determined by modified micro Kjeldahle method as described by **(A. O. A. C. 1970)**. Phosphorus was colorimetrically determined using the method described by **Murphy and Riley (1962)** using spectrophotometer at 882 µv. As for potassium, and calcium it was estimated using flame photometry according to **(Cottenie *et al.*, 1982)**.

1. Total carbohydrate:

Total carbohydrate percentages in the dried leaves were determined according to **(Chaplin and Kennedy 1994)**.

2. Fixed oil productivity:

The clean air-dried seeds of chia 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.

$$\text{Fixed oil percentage} = \frac{\text{Extracted fixed oil weight}}{\text{Seeds sample weight}} \times 100$$

The fixed oil percentage was used to calculate fixed oil yield/plant as well as fixed oil yield feddan^{-1} using the following equations:

$$\text{Fixed oil yield per plant (g)} = \frac{\text{Fixed oil percentage} \times \text{seeds dry weight per plant}}{100}$$

$$\text{Fixed oil yield per feddan (kg)} = \frac{\text{Fixed oil yield per plant (g)} \times \text{Number of plants per feddan}}{1000}$$

Oil samples were kept in a sealed dark glass tube in the dark at 1-5^{o} until G.L.C. analyses were conducted.

3. Determination of fatty acids:

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^{o} C. The acidified solution was extracted three times with ether. The ether extract was washed by a distilled water (many times) till neutral condition was noticed with phenolphethaline indicator. The ether extract was dried over anhydrous sodium sulfate, filtered and finally evaporated under vacuum according to **(Stahl 1967).** The residues represented the methylated fatty acids were analyzed by G.L.C. method.

Statistical analysis

The means of all data obtained 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 0.05%, as given by (**Snedecor and Cochran 1989**) using MSTAT-C statistical software package.

III. Results

Effect of mycorrhizal fungi, phosphorus sources and their interaction treatments on:

III.1. Vegetative growth measurements:

III.1.1. Fresh and dry weight (g), plant height (cm) and number of branches

Data presented in Tables (1 and 2) illustrated that, vegetative growth measurements i.e. fresh weight, dry weight, plant height and number of branches of chia increased by using arbuscular mycorrhizal fungi (AMF) when compared to untreated (uninoculum mycorrhizal fungi)

Referring to, phosphorus sources treatments, data showed that all above mentioned vegetative growth measurements were greatly affected by all phosphorus sources treatments as compared to control (no phosphorus) in both seasons. Hence, in the two seasons, the heaviest fresh and dry weights, the tallest plants and the largest number of branches were statistically induced by those chia (*Salvia hispanica* L.) plants by monoamounium phosphate (MAP) in the first and second seasons. Whereas, the lowest values of these parameters were obtained from untreated plants in both seasons.

Furthermore, the interaction effect between mycorrhizal fungi and phosphorus sources treatments data in Tables (1 and 2) revealed that all combinations between mycorrhizal fungi and phosphorus sources increased plant height, fresh weight and dry weights of chia (*Salvia hispanica* L.) plants over control.

This trend was true during both seasons of this study. However, the highest values of these parameters were recorded by using the combined treatment between mycorrhizal fungi (uninoculum and inoculum) and MAP, followed descendingly by the combined treatment between mycorrhizal fungi and phosphoric acid in the two seasons. On the reverse, the lowest values of these parametrs scored by control (uninoculum mycorrhizal fungi and no phosphorus) in the both seasons.

Table 1. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on fresh weight plant $^{-1}$ (g) and dry weight plant $^{-1}$ (g) of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

AMF P. Sources	Fresh weight plant $^{-1}$ (g)						Dry weight plant $^{-1}$ (g)					
	First season		Mean	Second season		Mean	First season		Mean	Second season		Mean
	Uninoculum	Inoculum		Uninoculum	Inoculum		Uninoculum	Inoculum		Uninoculum	Inoculum	
Without phosphorus	52.60	57.70	55.15	47.00	58.33	52.67	5.94	7.85	6.90	5.31	7.93	6.62
Phosphate rock	65.29	77.33	71.31	57.33	60.33	58.82	7.36	10.52	8.94	6.48	8.21	7.35
Super phosphate	76.40	80.33	78.36	75.67	78.00	76.83	8.63	10.92	9.78	8.55	10.61	9.58
MAP	96.31	101.30	98.81	85.67	93.67	89.67	10.88	13.78	12.33	9.68	12.74	11.21
Phosphoric acid	79.50	90.20	84.85	75.00	78.67	76.83	8.98	12.27	10.62	8.81	10.70	9.76
Mean	74.02	81.37		68.13	73.80		8.37	11.07		7.77	10.04	
L.S.D at 0.05 %												
AMF	4.23			10.99			0.64			0.44		
P. Sources	6.69			17.38			1.01			0.70		
AMF * P. Sources	9.46			24.58			1.43			0.98		

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

Table 2. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on plant height (cm) and number of branches plant $^{-1}$ (No.) of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

AMF	Plant height (cm)						Number of branches plant^{-1} (No.)					
	First season		Mean	Second season		Mean	First season		Mean	Second season		Mean
	Uninoculum	Inoculum		Uninoculum	Inoculum		Uninoculum	Inoculum		Uninoculum	Inoculum	
Without phosphorus	95.96	98.54	97.25	98.48	102.2	98.85	19.22	20.75	19.99	20.34	23.00	21.67
Phosphate rock	101.5	104.3	102.9	102.7	105.8	104.2	21.00	23.00	22.00	25.89	27.45	26.67
Super phosphate	104.7	107.2	105.9	104.2	107.1	105.6	21.22	28.11	24.67	26.11	29.44	27.78
MAP	111.9	117.5	114.7	109.3	113.8	111.6	31.44	36.55	34.00	33.00	35.00	34.00
Phosphoric acid	105.9	109.8	107.8	105.6	107.3	106.4	23.67	33.22	28.44	30.33	32.33	31.33
Mean	104.00	107.5		103.4	107.2		23.31	28.33		27.13	29.44	
L.S.D at 0.05 %												
AMF		3.08			2.81			4.32			2.92	
P. Sources		4.88			4.44			6.83			4.62	
AMF * P. Sources		6.894			6.28			9.66			6.53	

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

III. 2. Seeds yield parameters:

III. 2.1. Weight of 1000 seeds, seeds yield plant^{-1} and seeds yield feddan^{-1}.

According to data presented in Table (3) inoculated the plant with mycorrhizal fungi significantly increased the seeds yield parameters, i.e. weight of 1000 seeds, seeds yield plant^{-1} and seeds yield feddan^{-1} when compared to the untreated (uninoculum mycorrhizal fungi) in the two seasons.

On the other side, all the four used phosphorus sources treatments progressively increased the aforementioned parameters of *Salvia hispanica* L. as compared with control in both seasons. Hence, in both seasons of this study the highest values of these parameters were obtained from the MAP in the first and second seasons, followed by phosphoric acid treatment which scored the second highest values of these parameters with non-significant differences between them on seeds yield feddan^{-1} during the two seasons. On the contrary, the lowest values of aforementioned parameters were scored by using control (no phosphorus) in the two seasons.

Additionally, data in Table (3) recorded that all the interactions between arbuscular mycorrhizal fungi (AMF) and phosphorus sources treatments statistically improved the weight of 1000 seeds, seeds yield plant^{-1} and seeds yield feddan^{-1} of chia plants when compared to control in both seasons especially, the combination between arbuscular mycorrhizal fungi (AMF) and MAP significantly produced the highest values of these parameters in the two seasons.

Also, the combination between uninoculum mycorrhizal fungi and MAP or inoculum mycorrhizal fungi and phosphoric acid recorded highly increments of these parameters in both seasons. The lowest values of above-mentioned parameters were obtained by uninoculum mycorrhizal fungi and unfertilized plants (no phosphorus) in the both seasons.

III. 3. Chemical composition determinations:

III. 3. 1. Chlorophyll a, b and total carotenoids contents in the fresh leaves

Data in Table (4) indicate that chlorophyll "a, b" and total carotenoids contents in the fresh leaves increased by inoculated arbuscular mycorrhizal fungi (AMF) when compared to untreated (uninoculum mycorrhizal fungi) in most cases in both seasons. Regarding phosphorus sources treatments, data showed that all phosphorus sources treatments significantly increased the chlorophyll "a, b" and total carotenoids contents in the fresh leaves of chia (*Salvia hispanica* L.) when compared to control in both seasons. In this concern, Monoamounium phosphate (MAP) gave higher values of these parameters as compared to control in the two seasons. Also, phosphoric acid produced the second highest values in both seasons.

Moreover, all combinations between arbuscular mycorrhizal fungi (AMF) and phosphorus sources treatments statistically increased chlorophyll "a, b" and total carotenoids contents of chia (*Salvia hispanica* L.) when compared to control in both seasons.

However, the highest values of these parameters were recorded by using the combined treatment between AMF and MAP, followed descendingly by the combined treatment between AMF and phosphoric acid in 1st and 2nd seasons. On the opposite, the lowest values chlorophyll "a, b" and total carotenoids contents were scored by control (uninoculum mycorrhizal fungi and no phosphorus) during the both seasons.

III. 3. 2. N, P, K and total carbohydrate contents

According to data presented in Tables (5 and 6) it could be concluded that inoculated the plant with arbuscular mycorrhizal fungi (AMF) significantly enhanced the percentage of N, P, K and total carbohydrate when compared to the untreated (uninoculum mycorrhizal fungi) in both seasons. On the other hand, all the four used phosphorus sources treatments progressively increased the percentage of N, P, K and total carbohydrate of chia as compared with control in both seasons. Hence, in both seasons of this study the highest values of these parameters were obtained from the monoamounium phosphate (MAP) in the first and second seasons. Also, in this concern phosphoric acid produced the second highest values of these parameters in both seasons. On the contrary, the lowest values of the aforementioned parameters of chia (*Salvia hispanica* L.) plants were obtained by using control (without any addition) in the first and second seasons.

Moreover, data in Tables (5 and 6) show that all the interactions between AMF and phosphorus sources treatments statistically increased the percentage of N, P, K and total carbohydrate of chia (*Salvia hispanica* L.) plants when compared to control in both seasons especially, the combined treatment between inoculum mycorrhizal fungi and MAP significantly produced the highest values of these parameters in 1st and 2nd seasons. Also, the combined treatment between uninoculum AMF and MAP or inoculum AMF and phosphoric acid or MAP or inoculum AMF and super phosphate recorded highly increments of abovementioned parameters in both seasons. The lowest values of these parameters were produced by uninoculum AMF and unfertilized plants (no phosphorus) during the two seasons.

Table 3. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on weight of 1000 seeds (g), seeds yield plant $^{-1}$ (g) and seeds yield feddan^{-1} (kg) of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

AMF / Phosphorus	Weight of 1000 seeds (g)						Seeds yield plant $^{-1}$ (g)						Seeds yield feddan $^{-1}$ (kg)					
	First Season			Second season			First Season			Second season			First season			Second season		
	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean
Without phosphorus	1.04	1.21	1.12	1.25	1.28	1.27	2.57	3.71	3.14	3.13	3.65	3.39	102.7	148.4	125.5	125.4	145.9	135.6
Phosphate rock	1.07	1.23	1.15	1.13	1.34	1.33	3.11	3.87	3.49	4.02	4.17	4.10	124.4	154.7	139.6	160.8	166.6	163.7
Super phosphate	1.07	1.25	1.16	1.35	1.44	1.40	3.75	6.22	4.99	4.89	5.60	5.25	150.2	248.8	199.5	195.6	224.1	209.9
MAP	1.23	1.32	1.28	1.40	1.53	1.47	6.40	8.78	7.59	6.79	8.13	7.55	256.2	351.4	303.8	271.5	332.5	302.0
Phosphoric acid	1.12	1.29	1.21	1.35	1.47	1.42	5.93	7.99	6.96	6.24	7.50	6.87	237.1	319.7	278.4	249.6	300.1	274.9
Mean	1.10	1.26		1.33	1.42		4.35	6.11		5.02	5.85		174.1	244.6		200.6	233.9	
L.S.D at 0.05 %																		
AMF	0.05			0.07			1.23			0.76			49.10			30.48		
P. Sources	0.09			0.11			1.94			1.20			77.64			48.19		
AMF * P. Sources	0.12			0.15			2.75			1.70			109.8			68.15		

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

Table 4. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on chlorophyll a (mg/g F.W.), chlorophyll b (mg/g F.W.) and total carotenoids contents (mg/g F.W.) in fresh leaves of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

	Chlorophyll a (mg/g F.W.)						Chlorophyll b (mg/g F.W.)						Carotenoids (mg/g F.W.)					
	First Season			Second season			First Season			Second season			First season			Second season		
AMF / P. Sources	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean
Without phosphorus	0.181	1.149	0.984	0.852	0.807	0.830	0.255	0.321	0.288	0.328	0.258	0.293	0.120	0.120	0.120	0.112	0.126	0.119
Phosphate rock	0.907	1.160	1.033	0.899	0.814	0.857	0.361	0.342	0.352	0.333	0.312	0.323	0.131	0.158	0.145	0.133	0.145	0.139
Super phosphate	0.911	1.315	1.113	0.923	1.019	0.971	0.332	0.421	0.377	0.388	0.438	0.413	0.140	0.390	0.265	0.169	0.359	0.264
MAP	1.139	1.323	1.231	1.145	1.122	1.134	0.411	0.478	0.445	0.392	0.601	0.497	0.280	0.403	0.342	0.199	0.560	0.380
Phosphoric acid	0.916	1.315	1.116	1.025	1.067	1.046	0.313	0.456	0.385	0.321	0.570	0.446	0.198	0.394	0.296	0.190	0.490	0.341
Mean	0.938	1.252		0.969	0.966		0.335	0.404		0.353	0.436		0.174	0.293		0.160	0.336	
L.S.D at 0.05 %																		
AMF		0.087			0.077			0.024			0.024			0.001			0.001	
P. Sources		0.138			0.121			0.038			0.038			0.001			0.001	
AMF * P. Sources		0.196			0.172			0.054			0.054			0.002			0.002	

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

Table 5. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on nitrogen (%) and phosphorous (%) in the dry leaves of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

	Nitrogen (%)						Phosphorous (%)					
	First season			Second season			First season			Second season		
AMF / **P. Sources**	**Uninoculum**	**Inoculum**	**Mean**	**Uninoculum**	**Inoculum**	**Mean**	**Uninoculum**	**Inoculum**	**Mean**	**Uninoculum**	**Inoculum**	**Mean**
Without phosphorus	1.96	1.96	1.96	1.94	1.98	1.96	0.173	0.180	0.177	0.193	0.200	0.197
Phosphate rock	2.03	2.10	2.07	2.12	2.17	2.15	0.223	0.243	0.233	0.203	0.263	0.233
Super phosphate	2.17	2.17	2.17	2.17	2.15	2.16	0.237	0.290	0.263	0.2167	0.283	0.250
MAP	2.73	2.94	2.84	2.38	2.82	2.60	0.283	0.303	0.293	0.307	0.323	0.315
Phosphoric acid	2.21	2.87	2.54	2.24	2.13	2.28	0.247	0.293	0.270	0.250	0.287	0.268
Mean	2.22	2.41		2.17	2.29		0.234	0.262		0.234	0.271	
L.S.D at 0.05 %												
AMF	0.17			0.12			0.024			0.024		
P. Sources	0.26			0.19			0.038			0.038		
AMF * P. sources	0.37			0.27			0.054			0.054		

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

Table 6. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on potassium (%) and total carbohydrate (%) in the dry leaves of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

	Potassium (%)						Total carbohydrate (%)					
	First season			Second season			First season			Second season		
AMF / P. Sources	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean
Without phosphorus	1.24	1.37	1.31	1.05	1.02	1.03	10.07	10.34	10.21	9.66	9.99	9.82
Phosphate rock	1.60	1.71	1.65	1.37	1.62	1.50	10.15	11.11	10.64	11.23	12.61	11.92
Super phosphate	1.71	1.73	1.72	1.69	1.89	1.79	10.79	11.64	11.21	11.42	13.79	12.61
MAP	2.18	2.26	2.22	2.06	2.23	2.14	17.02	18.26	17.64	14.43	15.76	15.10
Phosphoric acid	1.79	1.98	1.86	1.79	2.09	1.94	13.71	14.23	14.23	11.81	15.16	13.49
Mean	1.70	1.81		1.59	1.77		12.35	13.23		11.72	13.46	
L.S.D at 0.05 %												
AMF	0.14			0.14			0.48			0.73		
P. Sources	0.21			0.22			0.76			1.16		
AMF * P. Sources	0.30			0.31			1.07			1.65		

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

III. 3. 3. Oil percentage, oil yield / plant and oil yield feddan^{-1}

Data presented in Table (7) indicate that, inoculated the plant with arbuscular mycorrhizal fungi (AMF) significantly increased the oil percentage, oil yield plant^{-1} and oil yield feddan^{-1}, when, compared to the untreated (uninoculum mycorrhizal) in the two seasons.

On the other side, the oil percentage, oil yield plant^{-1} and oil yield feddan^{-1} of chia (*Salvia hispanica* L.) seeds were more affected by using all phosphorus sources treatments as compared to control (no phosphorus) in both seasons. However, the highest values of these parameters were scored by MAP treated plants, in the first and second seasons.

Additionally, the combined treatment between (AMF) with MAP recorded the highest values of these parameters as compared to control and the other one in the first and second seasons, followed in descending order by using, the combined treatment between inoculum mycorrhizal fungi and phosphoric acid in both seasons. On the contrary, the lowest values of these parameters were scored by control (uninoculum mycorrhizal fungi and no phosphorus) during the both seasons.

III. 3. 4. Fixed oil constituents of chia (*Salvia hispanica* L.) seeds.

Table (8) and Figs. (From 1-10) show the data belonging to the effect of different treatments of AMF (uninoculum or inoculum) and phosphorous sources, i.e. (0.00 - phosphate rock - super phosphate - MAP - phosphoric acid) on the qualitative of the fixed oil constituents of chia (*Salvia hispanica* L.) seeds. The fixed oil composition of chia produced 23 compounds were identified, i.e. butaric, caporic, caprilic, capric, undecylic acid, lauric acid, myristic acid, pentadecanoic, palmitic acid, palmitoleic, margaric acid, heptadecaenoic, stearic acid, oleic acid, linoleic acid, α-linolenic acid, γ-linolenic acid, arachidic acid, gadoleic, arachidonic, mead acid, behenic and lignoceric.

The main component was α-linolenic acid (54.96 to 63.23%). The major components were α-linolenic acid (54.96 to 63.23%), linoleic acid (15.82 to 21.36%), oleic acid (6.19 to 15.86%) and palmitic acid (6.30 to 8.15%). Moreover, the combination treatments of AMF uninoculum and super phosphate gave the maximum values of α-linolenic acid as (63.23%) followed by the combined treatment of AMF uninoculum and phosphoric acid as (63.70%) and the combined treatment of AMF uninoculum and phosphate rock (62.39%) as when compared to as (54.96%) the combined treatment of AMF inoculum and phosphoric acid. On the other hand, different treatments caused decreases in the percentage of linoleic acid from 19.85 in control to 16.50 and 15.82%, with the exception of the combined treatment of AMF uninoculum and super phosphate fungi and AMF uninoculum and phosphoric acid surpassed control to 21.36 and 20.57%, respectively.

Furthermore, the combined treatment AMF inoculum and MAP gave the maximum values of oleic acid (15.86%) followed by the combined treatment AMF inoculum and phosphate rock (13.11%). Additionally, the highest values of palmitic acid by the combined treatment AMF inoculum and phosphoric acid and the combined treatment AMF inoculum with MAP as it (8.15 and 7.27%), respectively.

The majors compounds of chia oil was stated by various studies like **Ayerza and Coates (2004)** found that the majors compound were α-linolenic (63.2%), linoleic (18%), oleic (3.4%), palmitic (7.25%) and stearic (3.4%); **Segura-Campos *et al.*, (2014)** stetaed that chia oil contains α-linolenic (68.52%), linoleic (20.40%), oleic (2.43%), palmitic (7.74%) and stearic (0.29%) and **Silva *et al.*, (2016)** stadied quantification of fatty acids in the chia seed oils obtained with different solvents and stated that α-linolenic aranged (61.48 - 62.92%), linoleic (18.10 - 19.76%), oleic (6.9 - 6.87%), palmitic (9.13 - 9.95%) and stearic (2.92 - 2.99%)

IV. Discussion:

Mycorrhizal symbioses are essential for the sustainable management of agricultural ecosystems (**Barrios 2007 and Smith and Read 2008**). Arbuscular mycorrhizal fungi play an important role in plant growth, health and productivity. AMF fungi help plants to absorb nutrients, especially the less available mineral nutrients such as copper, molybdenum, phosphorus and zinc (**Chanda *et al.*, 2014**)

AMF fungi have been shown to have benefits to host plants by increasing herbivore tolerance, pollination, soil stability, and heavy metal tolerance (**Hart and Trevors 2005**). Arbuscular mycorrhizal fungi help plant species to uptake water and nutrients and make physiological changes to increase growth and productivity of host plants, AMF fungi play a key role in soil fertility and plant nutrition, enhancing the uptake and translocation of mineral nutrients (P, N, S, K, Ca, Fe, Cu and Zn) from soil to host plants, by means of an extensive below ground hyphal network, which spreads from colonized roots into the soil environment **(Smith and Read 2008 and Raei and Weisany 2013)**

Infection of arbuscular mycorrhizal fungi causes specific physiological changes in host cells. The amount of mitochondria increase threefold and migrate toward the arbuscule, the nucleus increases in size, and nuclear chromatin decondenses, increased numbers of mitochondria and plastids lead to increased energy production (**French 2017**). Plastids also increase in number and stromules become more abundant; they can move toward arbuscules, forming a net-like structure over the fungus (**Buee *et al.*, 2000 and Lohse *et al.*, 2005**).

Table 7. Effect of arbuscular mycorrhizal fungi, phosphorus sources and their interaction treatments on oil percentage (%), oil yield plant^{-1} (g) and oil yield feddan^{-1} (kg) of chia (*Salvia hispanica* L.) plants during 2016/2017 and 2017/2018 seasons

	Oil percentage (%)						Oil yield plant $^{-1}$ (g)						Oil yield feddan $^{-1}$ (kg)					
	First Season			Second season			First season			Second season			First season			Second season		
AMF / P. Sources	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean	Uninoculum	Inoculum	Mean
Without phosphorus	21.55	25.35	23.45	24.05	24.90	24.48	0.53	0.94	0.75	0.76	0.90	0.83	22.16	37.62	28.89	30.44	35.92	33.18
Phosphate rock	26.30	29.15	27.73	24.02	28.20	26.20	0.80	1.16	0.98	0.97	1.18	1.08	32.20	46.36	39.28	38.81	47.24	43.02
Super phosphate	27.25	29.25	28.25	24.60	28.35	26.47	1.02	1.79	1.41	1.25	1.59	1.42	40.91	71.62	56.26	50.17	63.62	56.89
MAP	28.10	30.55	29.33	25.65	30.05	27.85	1.80	2.71	2.25	1.74	2.51	2.12	71.92	108.4	90.15	69.52	100.33	84.93
Phosphoric acid	27.80	30.25	29.02	24.75	28.25	26.50	1.65	2.40	2.02	1.56	2.12	1.84	65.91	95.90	80.90	62.25	84.91	73.58
Mean	26.20	28.91		24.65	27.95		1.17	1.80		1.26	1.66		46.62	71.98		50.24	66.41	
L.S.D at 0.05 %																		
AMF		1.77			1.79			0.38			0.26			15.35			10.45	
P. Sources		2.79			2.83			0.60			0.41			23.90			16.52	
AMF * P. Sources		3.95			4.00			0.84			0.58			33.80			23.37	

AMF = arbuscular mycorrhizal fungi and P. Sources = phosphorous sources

Table 8. Effect of AMF, phosphorus sources and their interaction treatments on fixed oil constituents of chia (*Salvia hispanica* L.) during 2017/2018 season

No	R.T	C atom	Fatty acids	Control	Phosphate rock	Super phosph-ate	MAP	Phosphoric acid	AMF + no phosphorus	AMF + Phosphate rock	AMF + Super phosphate	AMF + MAP	AMF + Phosphoric acid
1	5.7	**C4**	**Butaric**	--	0.14	0.06	--	--	--	0.03	--	--	--
2	7.26	**C6**	**Caporic**	0.15	0.07	0.10	--	--	--	0.27	--	0.05	--
3	9	**C8**	**Caprilic**	0.16	0.15	0.02	--	0.08	--	0.17	--	0.06	--
4	9.9	**C10**	**Capric**	0.23	0.12	0.06	--	0.10	0.17	0.12	0.02	0.13	--
5	10.5	**C 11**	**Undecylic acid**	--	--	--	--	--	3.84	--	--	--	0.56
6	14.01	**C12.0**	**Lauric acid**	--	0.06	0.09	--	--	3.63	0.12	0.03	0.19	3.15
7	17.7	**C14.0**	**Myristic acid**	--	0.06	0.03	--	--	0.68	0.06	0.05	0.06	0.18
8	19.27	**C 15:0**	**Pentadecanoic**	--	--	0.03	--	--	0.30	--	0.03	0.10	--
9	21.6	**C16:0**	**Palmitic acid**	7.23	7.26	6.79	6.30	6.58	7.17	6.89	6.50	7.27	8.15
10	22.96	**C16:1**	**Palmitoleic**	--	0.17	0.17	--	--	--	0.19	--	0.12	--
11	23.17	**C 17:0**	**Margaric acid**	--	0.07	--	--	--	--	--	--	--	0.25
12	23.85	**C 17:1**	**Heptadecaenoic**	--	--	--	--	--	--	--	--	--	0.15
13	24.86	**C18:0**	**Stearic acid**	--	--	--	--	--	--	--	0.26	--	--
14	26.92	**C18:1**	**Oleic acid**	8.56	7.17	6.19	7.77	10.20	6.62	13.11	7.02	15.86	7.92
15	27.79	**C18:2**	**Linoleic acid**	19.85	20.57	21.36	19.62	16.50	18.04	15.82	19.36	16.81	19.54
16	28.61	**C18:3**	**α-linolenic acid**	60.35	62.39	63.23	59.89	63.70	58.15	61.81	59.24	58.46	54.96
17	28.77	**C18:3**	**γ-Linolenic acid**	0.62	1.07	1.27	--	1.43	--	1.02	4.40	0.65	0.50
18	29.58	**C20:0**	**Arachidic acid**	1.65	0.32	0.47	6.41	0.58	--	0.26	0.90	0.11	3.4
19	30.38	**C 20:1**	**Gadoleic**	--	0.15	--	--	0.46	--	0.05	0.87	--	0.58
20	31.34	**C20:4w6**	**Arachidonic**	--	--	--	--	--	--	--	--	--	0.32
21	31.73	**C20:3w9**	**Mead acid**	--	--	--	--	--	--	--	--	--	0.32
22	33.33	**C 22:0**	**Behenic**	0.74	0.14	0.05	--	0.34	--	--	1.00	--	--
23	35.33	**C 24:0**	**Lignoceric**	0.44	--	--	--	--	--	--	--	--	--
Total		**99.98**	**99.91**	**99.92**	**99.99**	**99.98**		**98.6**	**99.92**	**99.68**	**99.87**		**99.98**

AMF = arbuscular mycorrhizal fungi

Fig. 1: G.L.C. of chia fixed oil constituents from control treatment:

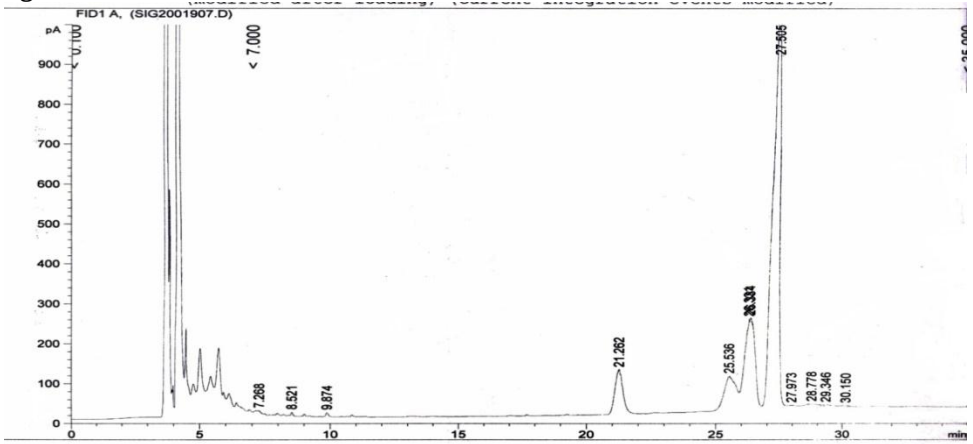


Fig. 2: G.L.C. of chia fixed oil constituents from Phosphate rock treatment:

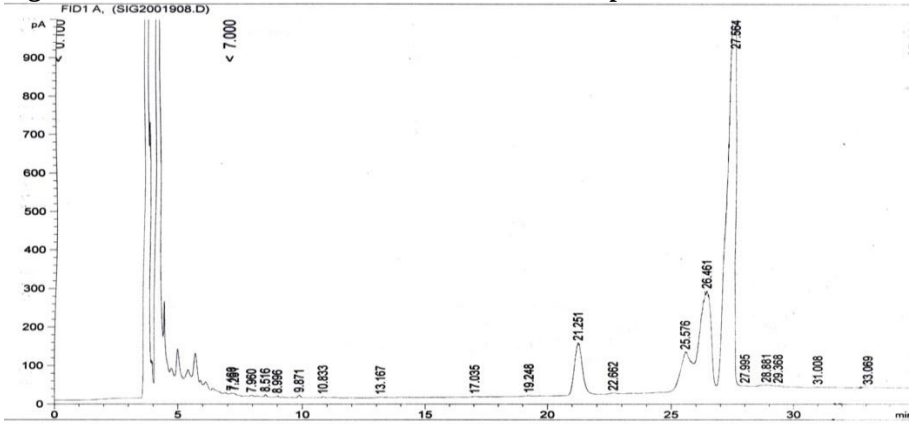


Fig. 3: G.L.C. of chia fixed oil constituents from Super phosphate treatment:

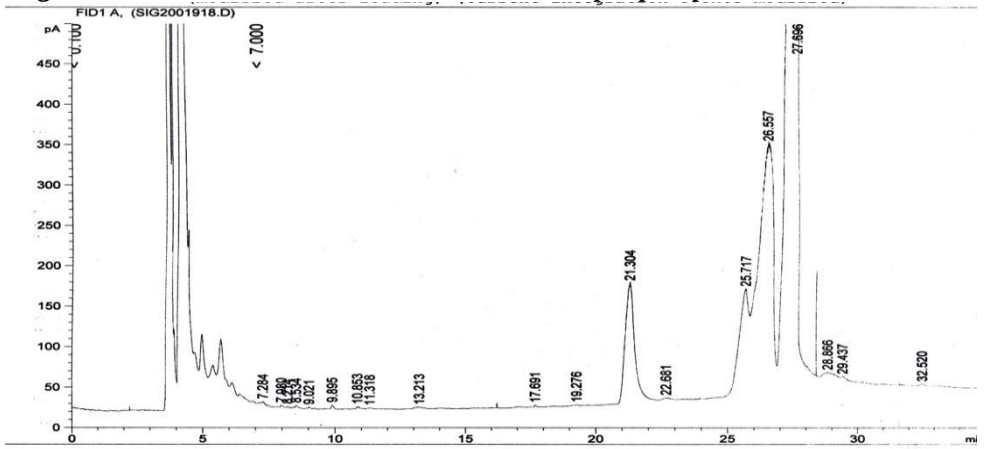


Fig. 4: G.L.C of chia fixed oil constituents from MAP treatment:

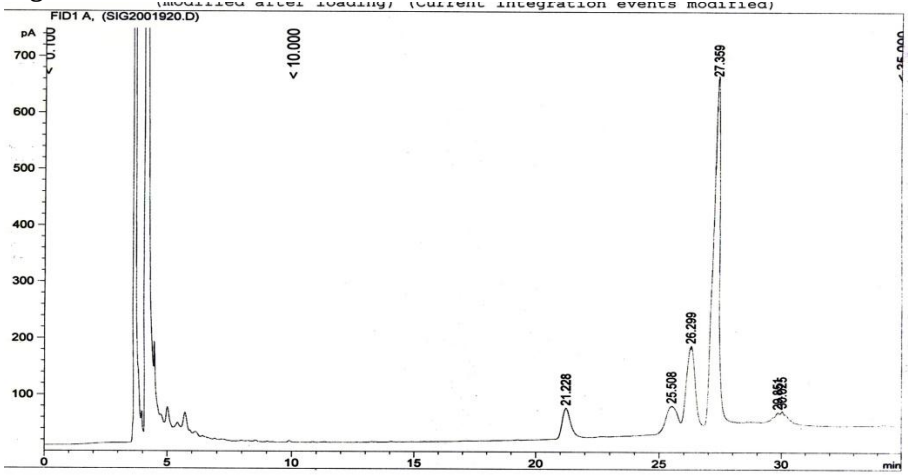


Fig. 5: G.L.C. of chia fixed oil constituents phosphoric acid treatment:

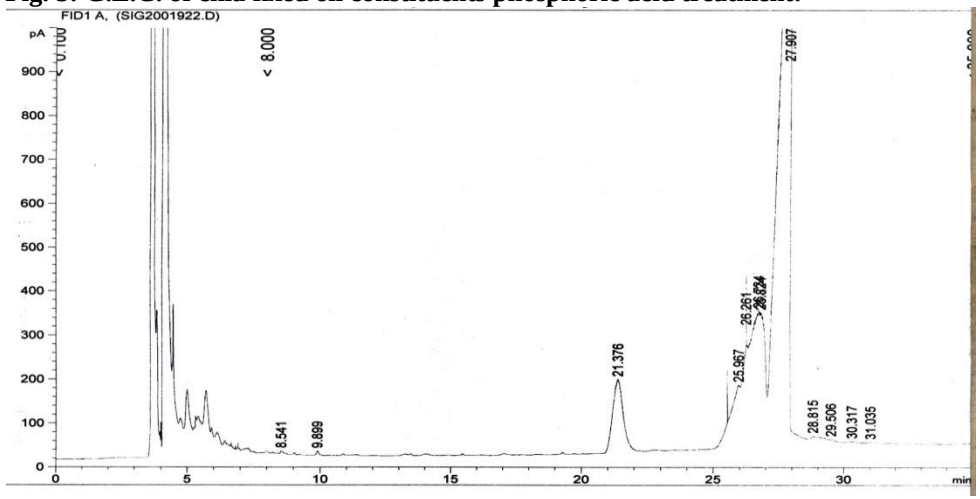


Fig. 6: G.L.C. of chia fixed oil constituents from AMF + Without phosphorus treatment:

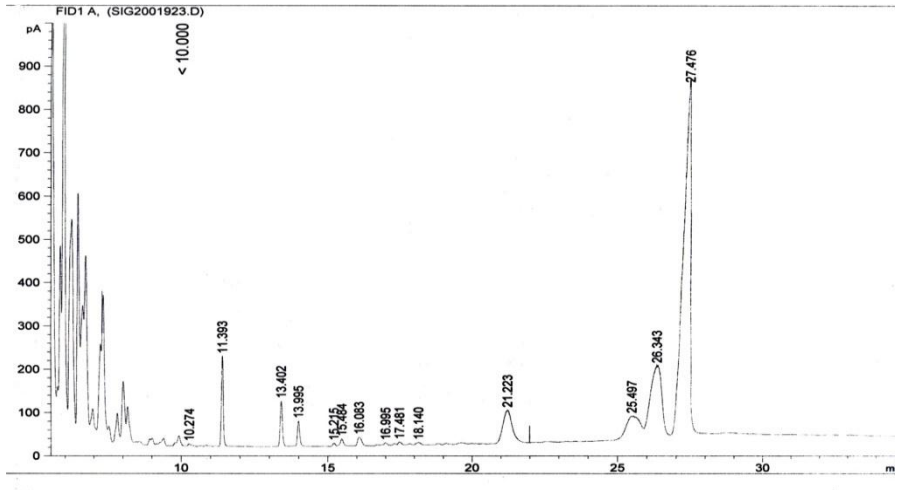


Fig. 7: G.L.C. of chia fixed oil constituents from AMF + Phosphate rock treatment:

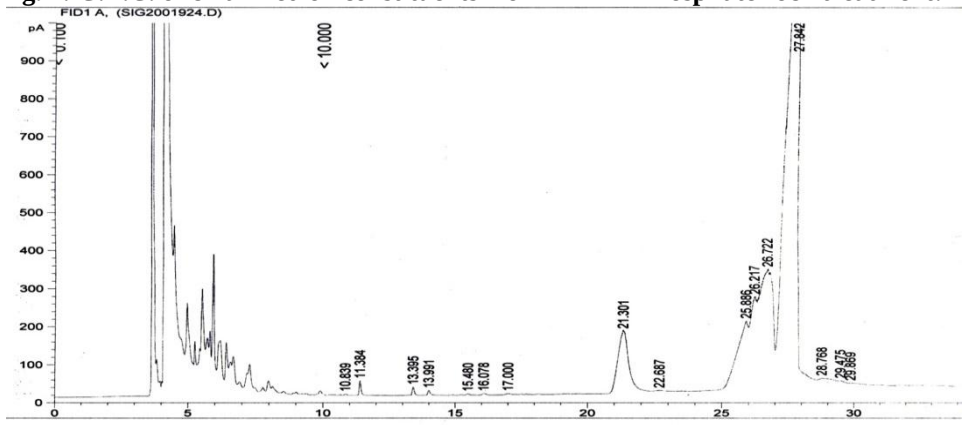


Fig. 8: G.L.C of chia fixed oil constituents from AMF + Super phosphate treatment:

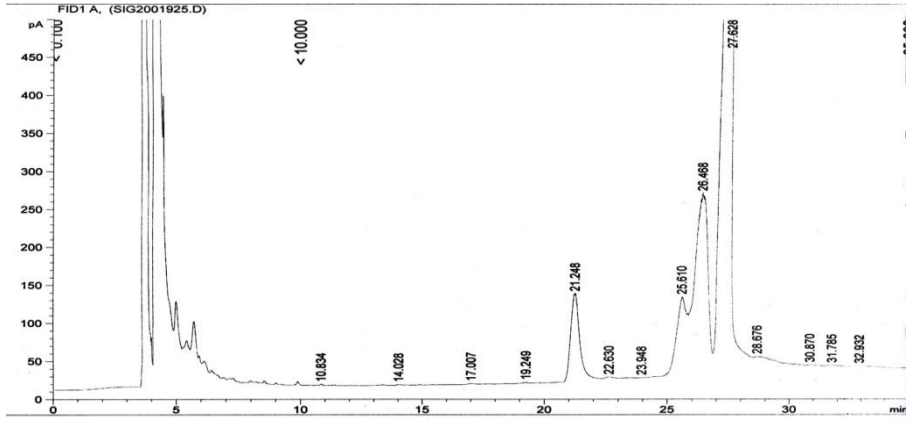


Fig. 9: G.L.C.of chia fixed oil constituents from AMF + MAP treatment:

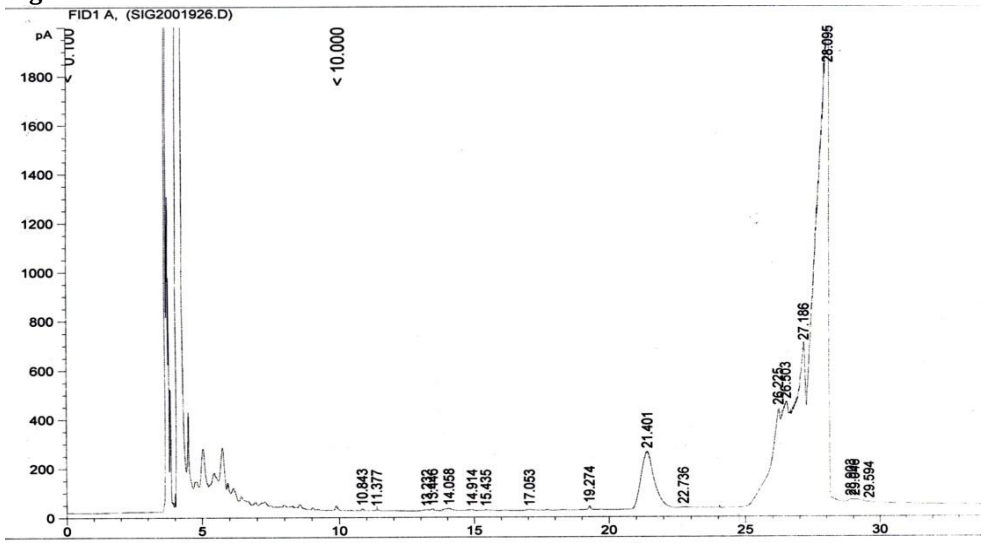
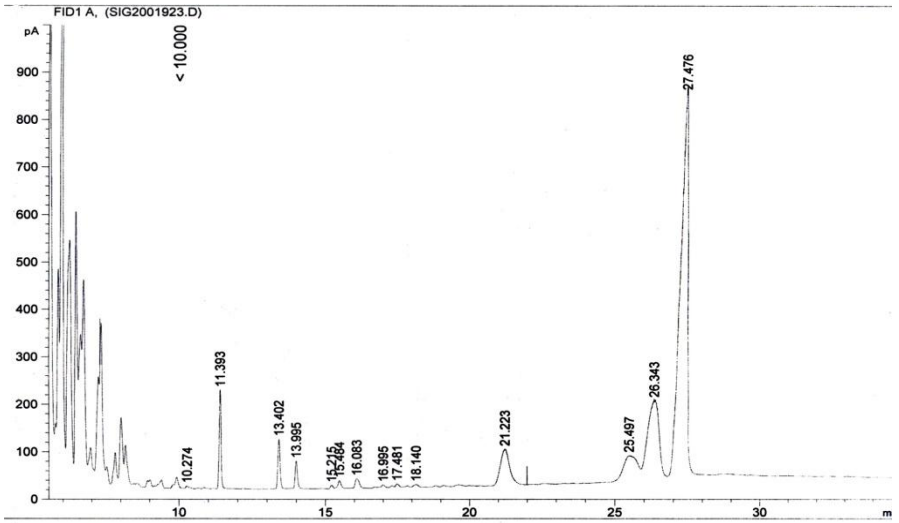


Fig. 10: G.L.C. of chia fixed oil constituents from AMF + Phosphoric acid treatment:



Similar results of mycorrhizal fungi were obtained by **Sajjadnia *et al.,*** (**2013**) showed that, essential oil percentage of fennel seeds only affected by mycorrhizal inoculation treatment, **Khalil and El-Noemani** (**2015**) stated that the inoculation oregano (*Origanum vulgare* L.) plants with mycorrhizal fungi (AMF) showed positive effects on plant height, number of branches, stem diameter, fresh weight, dry weight, oil percentage, chlorophyll a, chlorophyll b, chlorophyll a + b and carotenoids. **Pedone-Bonfim *et al.,* (2018)** reported that compared to non-mycorrhizal plants, mycorrhized *M. Tenuiflora* seedlings showed the greater photosynthetic performance and content of soluble carbohydrates and secondary metabolites. **Pankaj *et al.,* (2018)** studied was framed to assess the essential oil yield and quality of four varieties of palmarosa under the salt affected soil with the intervention of arbuscular mycorrhizal fungi (AMF). The essential oil yield (7.04-12.70 g kg^{-1} fresh biomass) and geraniol yield (5.71-10.56 g kg^{-1} fresh biomass) were significantly affected by AMF inoculation. Significantly higher essential oil and geraniol yields were observed in var. Tripta due to AMF intervention (*Funneliformis mosseae* and *Glomus aggregatum*).

In the earlier studies in the literature, phosphorus (P) is required in relatively large amounts for the biosynthesis of primary and secondary metabolites **(Marschner 2002)**, Seeds have the highest concentration of P in a mature plant, and P is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid **(Khalid and Shedeed 2015)**. Phosphorus fertilization significantly induced a higher chlorophyll production of *Salvia chamelaeagnea* **(Lefever *et al.,* 2017). Pedone-Bonfim *et al.,* (2018)** reported that the plant growth is severely restricted at low P levels, but the addition of AMF appears to remove this limiting factor. Although *M. tenuiflora* responds to levels of phosphate fertilization, it responds well to mycorrhizal inoculation, which promotes benefits for secondary metabolite content in this plant.

In this respect, **(Grant *et al.,* 2005)** Plants require adequate P from the very early stages of growth for optimum crop production. Phosphorus supply to the crop is affected by soil P, P fertilizer management and by soil and environmental conditions influencing P phytoavailability and root growth. Phosphorus uptake in many crops is improved by associations with arbuscular mycorrhizal fungi. Cropping system and long-term input of P through fertilizers and manures can influence the amount and phytoavailability of P in the system and the development of mycorrhizal fungi associations. Optimum yield potential requires an adequate P supply to the crop from the soil or from P additions. Where early-season P supply is low, P fertilization may improve P nutrition and crop yield potential. Alternately, under low-P conditions, encouragement of arbuscular mycorrhizal associations may enhance P uptake by crops early in the growing season, improving crop yield potential and replacing starter fertilizer P applications. Soil P supply that exceeds P requirements of the crop may preclude mycorrhizal fungi development. To encourage arbuscular mycorrhizal fungi association, threshold levels of soil solution P that restrict mycorrhizal development must not be exceeded. Sustainable P management practices must be applied both in conventional and in alternative biologically based agricultural systems.

These results of phosphorus sources are in close agreement with those reported **Dadkhah (2012)** showed that inoculation AMF of bio-fertilizers applied with 50% recommended dosage of NP (super phosphate), increased vegetative growth (plant height, number of umbel per plant, plant dry weight) of fennel plants compared to chemical fertilizer treatments only, **Kilic *et al.,* (2012)** on *Thymus vulgaris* L. mentioned that using phosphoric acid in as a source of phosphor significantly increased green herb yield (kg ha^{-1}) and k content in the second season.

The other traits were recorded have significant increasing in both season like N %, P %, oil %, thymol, paracymen and carvacrol in both seasons, **Awad Alla *et al.,* (2013)** showed that coriander plants were significantly responded to Egyptian phosphate rock increased the vegetative growth expressed as plant height, number of branches, fresh and dry weights of aerial parts of the plant. Also, it significantly increased fruit yield. This increase was parallel to the gradual increase in the rate of Egyptian phosphate rock from 0 to 150 kg/fed., **Rahimi *et al.,* (2013**) on two basil variety, **Ackerman *et al.,* (2013**) on canola, stated that differences in P uptake among P sources were detected at the highest P rate where P uptake was significantly greater for MAP and PCMAP, biomass yields of canola were similar for all P sources (mean 6.9 g pot^{-1}) when applied at the low rate of 9.5 mg P pot^{-1}, they were 28% lower for MDS and PS compared with MAP and PCMAP at 47.5 mg P pot^{-1}, **Ahmed *et al.,* (2014)** on damsisa found that calcium superphosphate at rate 100 kg/fed. increased plant height (cm), number of branches, herb fresh per plant, dry weights per plant, herb fresh/feddan, dry weights per feddan and plot compared to control an rate at 200 kg/feddan, **Soliman *et al.,* (2016)** on baobab (*Adansonia digitata* L.) used different sources of phosphorous [monoammonium phosphate (MAP), diammonium phosphate (DAP) or hydroxyapatite nanoparticles (nHA)] and unfertilized seedlings (control). The results indicate that MAP inceased significantly no. of leaves, leaf area (cm^2), root length (cm), total fresh weight (g $plant^{-1}$), total dry weight (g $plant^{-1}$), N (%), P (%), K (%), crude protein (%) and ascorbic acid (mg g^{-1} fresh weight) compared to control **Said-Al Ahl and Hussien (2016)** on *Satureja montana* L. 'carvacrol' chemotype found that in the two seasons,

herb dry weight/plant, essential oil % and oil yield in two cuts were significantly increased with the rise in nitrogen and/or phosphorus fertilizers (calcium super phosphate), **Azman *et al.*, (2018)** on *Centella asiatica* stated that, MAP monoammonium phosphate inhanced N, P and K content, no. of leaves, no. of branches no. of flowers, total fresh biomass and total dry biomass compared with untreated plants (control), **Hassan *et al.*, (2018)** on caraway (*Carum carvi*, L.) plants showed that, using different sources of rock phosphate at all treatments led to a significant increase in plant height compared with control in both seasons total herb dry weight, fruit yield/plant**,** fruit yield/feddan, essential oil yield/plant, essential oil/feddan and phosphorus percentage and **Mary *et al.*, (2018)** on chia (*Salvia hispanica* L.), indicate that an increase in the yield level at a spacing of S3: 60 x 45cm (597.59 kg ha^{-1}) and a fertilizer level of F3: 90: 60: 75 kg NPK ha^{-1} (623.60 kg ha^{-1}). Significantly higher seed yield (676.58 kg ha^{-1}) was obtained in the treatment combination S3F3- 60 x 45 cm with 90: 60: 75 kg NPK ha^{-1}. Total dry matter (168.35 g $plant^{-1}$) was increased at a spacing of 60 x 45 cm and fertilizer level of 90: 60: 75 kg NPK ha^{-1} compared to other treatments.

Conclusively, from the aforementioned results, it could be recommended that the combinations of arbuscular mycorrhizal fungi (AMF) with MAP could be used to improve on growth, seeds yield, chemical compositions, fixed oil productivity and fixed oil constituents of chia (*Salvia hispanica* L.) plant.

References

Ackerman, J. N.; Zvomuya, F.; Cicek, N. and D. Flaten (2013). Evaluation of manure-derived struvite as a phosphorus source for canola. Canadian Journal of Plant Science, 93: 419-424.

Ahmed, Sh. K. ; Shalaby I. A. and R. K. Mortada (2014). Effect of calcium superphosphate and sulphur fertilizer on the growth, herb yield and active ingredient of *Ambrosia maritime*, L. Middle East Journal of Applied Sciences, 6 (2): 308-314.

Ali, N. M.; Yeap, S. K.; Ho, W. Y.; Beh, B. K.; Tan, S. W. and S. G. Tan (2012). The Promising Future of Chia, *Salvia hispanica* L., Journal of Biomedicine and Biotechnology, 2012: p1-9.

A. O. A. C. (1970). Official Methods of Analyses of Association of Official Agriculture Chemists. Washington, D. C., 10th ed.

Awad Alla, S. S.; Ali, H. M. H. and M. M. Abou-Zeid (2013). Effect of Egyptian rock phosphate and phosphate dissolving bacteria on coriander plant growth and yield**.** Egyptian Journal of Horticulture, 40 (1): 63-79.

Ayerza, R. (2013). Seed composition of two chia (*Salvia hispanica* L.) genotypes which differ in seed color. Emirates Journal of Food and Agriculture, 25: 495-500.

Ayerza, R. and W. Coates (2000). Dietary levels of chia influence on yolk cholesterol, lipid content and fatty acid composition, for two strains of hens. Poultry Science, 78: 724-739.

Ayerza, R. and W. Coates (2004). Composition of chia (*Salvia hispanica*) grownin six tropical and subtropical ecosystems of South America. Tropical Science, 44: 131-135.

Ayerza, R. and W. Coates (2005). Effect of ground chia seed and chia oil on plasma total cholesterol, LDL, HDL, triglyceride content and fatty acid composition when fed to rats. Nutrition Research, 11: 995-1003.

Azman, N. A. N.; Malek, N. A. N. N.; Noor, N. S. M. and M. A. Javed (2018). Improving the growth of *Centella asiatica* using surfactant modified natural zeolite loaded with NPK nutrients. Asian Journal of Agriculture and Biology, 6 (1): 55-65.

Badr El-Din S. M. S; Attia, M. and S. A. Abo-Sedera (1999). Evaluation of several substrates for mass multiplication of arbuscular mycorrhizal (AM) fungi grown on onion. Egyptian Journal of Microbiology, 34: 57-61.

Barker, S. J. and D. Tagu (2000). The roles of auxins and cytokinins in mycorrhizal symbioses. Journal of Plant Growth Regulation, 19 (2): 144-154.

Barrios, E. (2007). Soil biota, ecosystem services and land productivity. Ecological Economics, 64: 269-285.

Bedini, S.; Pellegrino, E.; Avio, L.; Pellegrino, S.; Bazzoffic, P.; Arges, E. and M. Giovannetti (2009). Changes of soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. Soil Biology and Biochemistry, 41 (7): 1491-1496.

Black, C. A.; Evans, D. O.; Ensminger, L. E.; White, J. L.; Clark, F. E. and R. C. Dinauer (1982). Methods of Soil Analysis. part 2. Chemical and microbiological properties. 2nd Ed. Soil Sci., Soc. of Am. Inc. Publ., Madison, Wisconsin, U. S.A.

Bresson, J. L.; Flynn, A. and Heinonen, M.; Hulshof, K.; Korhonen, H.; Lagiou, P.; Løvik, M.; Marchelli, R.; Martin, A.; Moseley, B.; Przyrembel, H.; Salminen, S.; Strain, J. J.; Strobel, S.; Tetens, I.; Berg, H.; Loveren, H. and H. Verhagen (2009). Opinion on the safety of chia seeds (*Salvia hispanica* L.) and ground whole chia seeds as a food ingredient. The European Food Safety Authority Journal, 996: 1-26.

Buee, M., Rossignol, M., Jauneau, A., Ranjeva, R., and G. Bécard (2000). The presymbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root

exudates. Molecular Plant-Microbe Interactions, 13 (6): 693-698.

Cahill, J. P. and M. C. Provance (2002). Genetics of qualitative traits in domesticated chia (*Salvia hispanica* L.). Journal of Heredity, 93 (1): 52-55.

Chanda, D.; Sharma, G. D. and D. K. Jha (2014). The potential use of arbuscular mycorrhiza in the cultivation of medicinal plants in Barak Valley, Assam: A Review. Current World Environment, 9 (2): 544-551.

Chaphlin, M. F. and J. F. Kennedy (1994). Carbohydrate Analysis, 2nd ed. Oxford University Press, New York.

Conde, L. D.; Chen, Z.; Chen, H. and H. Liao (2014). Effects of phosphorus availability on plant growth and soil nutrient status in the rice/soybean rotation system on newly cultivated acidic soils. American Journal of Agriculture and Forestry, 2 (6): 309-316.

Coorey, R.; Grant, A. and V. Jayasena (2012). Effect of chia flour incorporation on the nutritive quality and consumer acceptance of chips. Journal of Food Research, 1 (4): 85-95.

Cottenie, A.; Verloo, M.; Velghe, M. and R. Camerlynck (1982). Chemical Analysis of Plant and Soil. Laboratory of Analytical and Agrochemistry. State Univ. Ghent, Belgium.

Dadkhah, A. (2012). Effect of chemicals and bio-fertilizers on yield, growth parameters and essential oil contents of funnel *(Foeniculum vulgare* Miller.). Journal of Medicinal Plants and by Products, 1 (2): 101-105.

French, K. E. (2017). Engineering mycorrhizal symbioses to alter plant metabolism and improve crop health. Frontiers in Microbiology, 8: 1403.

Grant, C.; Bittman, S.; Montreal, M.; Plenchette, C. and C. Morel (2005). Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. Canadian journal of plant science, 85: 3-14.

Hach, C. C.; Bowden, B. K.; Kopelove, A. B. and S. T. Brayton (1987). More powerful peroxide Kjeldahl digestion method. Association of Official Analytical Chemists, 70: 783-787.

Hart, M. M. and J. T. Trevors (2005). Microbe management: application of mycorrhyzal fungi in sustainable agriculture. Frontiers in Ecology and the Environment, (3): 533-539.

Hassan, E. A.; Hamad, E. H. A.; Ibrahim, A. E. and R. A. A. Khalil (2018). Impact of rock phosphate sources and their solubility by some microorganisms on *Carum carvi* plants productivity. Middle East Journal of Applied Sciences, 8 (3): 787-797.

Ixtaina, V. Y.; Nolasco, S. M. and M. C. Tomas (2008). Physical properties of chia (*Salvia hispanica* L.) seeds. Industrial Crops and Products, 28 (3): 286-293.

Jackson, M. L. (1973). Soil Chemical Analysis. Prentice-Hall of Indian Private, New Delhi, 1-485.

Ju, X. T.; Kou, C. L.; Christie, P.; Dou, Z. X. and F. S. Zhang (2007). Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. Environmental Pollution, 145: 497-506.

Khalid, A. Kh. and M. R. Shedeed (2015). Effect of NPK and foliar nutrition on growth, yield and chemical constituents in *Nigella sativa* L. Journal of Materials and Environmental Science, 6 (6): 1709-1714.

Khalil, S. E. and A. A. El-Noemani (2015). Effect of bio-fertilizers on growth, yield, water relations, photosynthetic pigments and carbohydrates contents of *Origanumnn vulgare* L. plants grown under water stress conditions. American-Eurasian Journal of Sustainable Agriculture, 9 (4): 60-73.

Kilic, C. C.; Anaç, D.; Eryüce, N. and Ö. G. Kilic (2012). Effect of potassium and phosphorus fertilization on green herb yield and some quality traits of *Thymus vulgaris* L. African Journal of Agricultural Research, 7 (48): 6427-6431.

Lefever, K.; Laubscher, C. P.; Ndakidemi, P. A. and F. Nchu (2017). Effects of pH and phosphorus concentrations on the chlorophyll responses of *Salvia chamelaeagnea* (Lamiaceae) grown in hydroponics. InTech, Chlorophyll, pp. 79-92.

Lohse, S.; Schliemann, W.; Ammer, C.; Kopka, J.; Strack, D. and T. Fester (2005). Organization and metabolism of plastids and mitochondria in arbuscular mycorrhizal roots of *Medicago truncatula*. Plant Physiology, 139: 329-340.

Malhotra, H.; Vandana; Sharma, S. and R. Pandey (2018). Plant Nutrients and Abiotic Stress Tolerance (eds.), chapter: Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess, 171-190 pp.

Marschner, H. (2002): Mineral Nutrition of Higher Plants. 2nd edition, Academic press, Amsterdam, Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo.

Mary, J.; Veeranna, H. K.; Girijesh, G. K.; Dhananjaya, B. C. and S. Gangaprasad (2018). Effect of different spacings and fertilizer levels on growth parameters and yield of chia (*Salvia hispanica* L.). International Journal of Pure & Applied Bioscience, 6 (2): 259-263.

Murphy, J. and J. H. Riley (1962). A modified single solution for the determination of phosphate in natural waters. Analytica Chimica Acta, 27: 31-36.

Pankaj, U.; Verma, R. S.; Yadav, A. and R. K. Verma (2018). Effect of arbuscular mycorrhizae species on essential oil yield and chemical

composition of palmarosa (*Cymbopogon martinii*) varieties grown under salinity stress soil. Journal of Essential Oil Research, 31 (2): 145-153.

Pedone-Bonfim, M. V. L.; Alves da Silva D. K.; Silva-Batista, A. R.; Oliveira, A; Almeida, J. R. G.; Yano-Melo, A. M. and L. Costa-Maia (2018). Mycorrhizal inoculation as an alternative for the sustainable production of *Mimosa tenuiflora* seedlings with improved growth and secondary compounds content. Fungal Biology, 122 (9): 918-927.

Peiretti, P. G. and F. Gai (2009). Fatty acid and nutritive quality of chia (*Salvia hispanica* L.) seeds and plant during growth. Animal Feed Science and Technology, 148 (2-4): 267-275.

Peiretti, P. G. and G. Meineri (2008). Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. Meat Science, 80 (4): 1116-1121.

Raei, Y. and W. Weisany (2013). Arbuscular mycorrhizal fungi associated with some aromatic and medicinal plants. Bulletin of Environment, Pharmacology and Life Sciences, 2 (11): 129-138.

Raghothama, K. G. and A. S. Karthikeyan (2005). Phosphate acquisition. Plant Soil, 274: 37-49.

Rahimi, M.; Poor, M. R. A.; Ramroudi, M. and M. A. Rasoolizadeh (2013). Effects of potassium and phosphorus fertilizers on arsenic accumulation and plant growth of two basil cultivars. Bulletin of Environment, Pharmacology and Life Sciences, 2 (5): 79-85.

Rainwater, F. H. and L. L. Thatcher (1960). Methods for collection and analysis of water samples, U.S. Geol. Surv. Water Supply Papers, 1454.

Reyes-Caudillo, E.; Tecante, A. and M. A. Valdivia-Lopez (2008). Dietary fiber content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. Food Chemistry, 107 (2): 656-663.

Said-Al Ahl, H. A. H. and M. S. Hussien (2016). Effect of nitrogen and phosphorus application on herb and essential oil composition of *Satureja montana* L. 'carvacrol' chemotype. Journal of Chemical and pharmaceutical Research, 8 (6): 119-128.

Sajjadnia, N.; Mirshekari, B. and R. Amirni (2013). Sustainable production of fennel (*Foeniculum vulgare* Mill.) by seed inoculation with mycorrhizae strains under drought stress conditions. International Journal of Biosciences, 3 (12): 169-174.

Santos, R.; Girardi, C. G.; Pescador, R. and S. L. Stürmer (2010). Effects of arbuscular mycorrhizal fungi and phosphorus fertilization on post vitro growth of micropropagated *Zingiber officinale* Roscoe. Revista Brasileira de Ciencia do Solo, 34: 765-771.

Saric, M.; Kastrori, R.; Curic, R.; Cupina, T. and I. Gric (1967). Chlorophyll determination. University of "Noveon Sadu Praktikum iz Fiziologize Biljaka", Belgrade, Serbia.

Segura-Campos, M. R.; Ciau-Solís, N.; Rosado-Rubio, G.; Chel-Guerrero, L. and D. Betancur-Ancona (2014). Physicochemical characterization of chia (*Salvia hispanica*) seed oil from Yucatán, México Agricultural Sciences, 5: 220-226.

Silva, C.; Garcia, V. A. S. and C. M. Zanette (2016). Chia (*Salvia hispanica* L.) oil extraction using different organic solvents: oil yield, fatty acids profile and technological analysis of defatted meal. International Food Research Journal, 23 (3): 998-1004.

Smith, S. E. and D. Read (2008). Mycorrhizal symbiosis. Academic Press, London, UK.

Smith, S. E.; Jakobsen, I.; Gronlund, M. and F. A. Smith (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant physiology, 156 (3): 1050-1057.

Snedecor, G. W. and W. G. Cochran (1989). Statistical methods. 7th Ed. Iowa State Univ. Press. Ames Iowa, USA.

Soliman, A. Sh.; Hassan, M.; Abou-Elella, F.; A. H. Ahmed, H. and S. A. El-Feky (2016). Effect of nano and molecular phosphorus fertilizers on growth and chemical composition of baobab (*Adansonia digitata* L.). Journal of Plant Sciences, 11 (4-5): 52-60.

Stahl, E. E. (1967). Thin Layer Chromatography. A Laboratory Hand Book. Published by Springer Verlag, New York. 14-37.

Stewart, W. M.; Hammond, L. L. and S. J. V. Kauwenbergh (2005). Phosphorus as a Natural Resource. Phosphorus: Agriculture and the Environment, Agronomy Monograph No. 46; American Society of Agronomy, Crop Science Society of America, Soil Science Society of America: Madison, WI, USA, P 3-22.

Van der Heijden, M. G. A; Rinaudo, V.; Verbruggen, E.; Scherrer, C.; Bàrberi, P., and M. Giovannetti (2008). The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. 16th IFOAM Organic World Congress, Modena, Italy, June 16-20.

Veresoglou, S. D.; Shaw, L. J. and R. Sen (2011). *Glomus intraradices* and *Gigaspora margarita* arbuscular mycorrhizal associations differentially affect nitrogen and potassium nutrition of *Plantago lanceolata* in a low fertility dune soil. Plant Soil, 340 (1-2): 481-490.

Wang, X. R.; Shen, J. B. and H. Liao (2010). Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? Plant Science, 179: 302-306.

Zanqui, A. B.; Morais, D. R.; Silva, C.M.; Santos, J. M., Chiavelli, L. U. R.; Bittencourt, P. R. S; Eberlin, M. N.; Visentainer, J. V.; Cardozo-Filho, L. and M. Matsushita (2015). Subcritical extraction of *Salvia hisp*anica L. oil with n-propane: composition, purity and oxidation stability as compared to the oils obtained by conventional solvent extraction methods. Journal of the Brazilian Chemical Society, 26: 282-289.

تأثير الميكروريزا وبعض صور الفسفور على نمو، محصول البذور، التركيب الكيماوى وإنتاجية الزيت الثابت ومكوناته لنبات الشيا (*Salvia hispanica* L.)

إيمان مختار أبو الغيط[1]، أحمد سعيد محمد يوسف[1]، محمد أحمد عبدالوهاب[2]، يسرى فهمى يوسف محمد[1]، وليد محمد عبدالعليم مغيث[2]

(1)قسم البساتين – كلية الزراعة بمشتهر – جامعة بنها

(2)قسم النباتات الطبية والعطرية– مركز بحوث الصحراء

أجريت هذه الدراسة فى المزرعة البحثية لجامعة هليوبوليس (محافظة الشرقية، مصر) خلال موسمين متتابعين 2017/2016 و 2018/2017. وهدفت الدرسة إلى التحقق من إمكانية تحسين النمو، محصول البذرة وإنتاجية الزيت الثابت ومكوناته لنبات الشيا النامي تحت ظروف التربة الرملية بإستخدام الميكروريزا وبعض صور الفسفور مثل صخر الفوسفات، السوبر فوسفات، المونوأمونيوم فوسفات (ماب) و حمض الفسفوريك (30 وحدة من خامس أكسيد الفسفور للفدان). بذور الشيا لقحت بفطر الميكروريزا *Glomus, mosseae* and *fasciculatum* قبل الزراعة بمعدل 20 جم /5 جرام بذرة وبعد 10 أيام من الزراعة حقنت فى التربة بمعدل 3.5 جم للجورة.

أظهرت النتائج أن نباتات الشيا الملقحة بالميكروريزا أعطت أعلى زيادة معنوية فى معظم الصفات محل الدراسة (إرتفاع النبات، عدد الافرع للنبات، الوزن الطازج والجاف للنبات، محصول البذرة للنبات والفدان، وزن ال 1000 بذرة، كلوروفيل "أ ، ب"، والكاروتينات الكلية، النتروجين، الفسفور، البوتاسيوم، والكربوهيدرات الكلية، نسبة الزيت الثابت، محصول الزيت الثابت للنبات والفدان ومحتوى الزيت الثابت من الأحماض الدهنية، بينما النباتات الغير ملقحة (كنترول) أعطت أقل القيم من هذه الصفات. أثرت صور الفسفور معنوياً على الصفات السابقة ذكرها خاصة المونوأمونيوم فوسفات (ماب) فى كلا الموسمين. أشارت النتائج أن معاملة الجمع بين التلقيح بالميكروريزا والماب أعطت أعلى النتائج فى معظم الصفات المدروسة مثل كلوروفيل "أ ، ب"، والكاروتينات الكلية، النتروجين، الفسفور، البوتاسيوم، والكربوهيدرات الكلية، نسبة الزيت الثابت، ومحصول الزيت الثابت للنبات والفدان مقارنة بباقى المعاملات فى كلا الموسمين. أدى التحليل الكروموتوجرافى للزيت الثابت إلى التعرف على 23 مركب، كان المركب الأساسى هو الحمض الدهنى ألفا لينولينك (54.96 الى 63.23 %). كانت المركبات الرئيسية للزيت من الأحماض الدهنى هى ألفا لينولينك، اللينوليك، الأوليك و البالميتك. ويمكن أن نخلص إلى أن التلقيح بالميكروريزا مع التسميد بالمونو أمونيوم فوسفات (ماب) كان الأفضل لتحسين النمو، التركيب الكيماوى، محصول البذور وإنتاجية الزيت الثابت ومكوناته لنبات الشيا (*Salvia hispanica* L.).