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STUDYING THE POSSIBILITY OF CULTIVATING *PIMPINELLA ANISUM* L. PLANT UNDER SINAI CONDITIONS BY USING CHEMICAL FERTILIZATION AND MYCORRHIZA

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A field experiment was carried out at El-Maghara Research Station (North Central Sinai), Desert Research Center, during the two successive seasons of 2012/2013 and 2013/2014, to study the effect of biofertilization (mycorrhiza) and chemical fertilization on growth, fruit yield, essential oil and chemical composition of anise (*Pimpinella anisum* L.) plants. This experiment had a split plot design with three replications, the main plots were the mycorrhiza at two levels (non-inoculated and inoculated), and the sub plots were the chemical fertilization (0, 50, 75 and 100% of NPK), to be 100% of chemical fertilization of NPK (200 kg/fed ammonium sulfate + 200 kg/fed calcium super phosphate + 100 kg/fed potassium sulfate, respectively). Obtained data showed that, mycorrhiza application led to a significant increase in plant height, number of umbels/plant, fresh and dry weights/plant, fruit weight/plant, fruit yield/fed, N, P, K and total carbohydrates content. Also, in most cases, using 50 or 75% of the chemical fertilization of NPK achieved an increase in the same characters. The interaction between mycorrhiza and chemical fertilization had a significant effect on all parameters, where the best results of fresh and dry weights/plant, N, K and total carbohydrates content could be achieved with treating the plants with mycorrhiza plus 50% of chemical fertilization of NPK. While, the highest fruit weight/plant, fruit yield/fed and phosphorus content were resulted from inoculated with mycorrhiza plus 75% of chemical fertilization of NPK. Meanwhile, the volatile oil composition, resulted from this treatment, was anethole (74.57%), followed by estragole (7.80%), cis-pseudoisoeugenyl-2-methylbutyrate (7.24%) and longifolene (3.22%).

Keywords: mycorrhiza, chemical fertilization, *Pimpinella anisum* L., anethole, estragole

Anise (*Pimpinella anisum* L.) is a herbaceous annual plant that belongs to Apiaceae family. It is one of the most important medicinal and aromatic plants, native to the Mediterranean region. Anise is primarily grown for its fruits, commercially called seeds. The anise seeds have essential oil as an active substance, while anethole is the most important constituent of anise, which is used in pharmaceutical, food, perfumery and flavoring industries (Ozkan and Chalchat, 2006 and Tuncturk and Yildirim, 2006).

Through this research the anise plant is cultivated for the first time in El-Maghara area, North Central Sinai, Egypt, under sandy soil, using moderately saline irrigation water in order to encourage the cultivation of anise under those conditions and work on the expansion of cultivated areas, in Upper Egypt, especially the governorates of Minya and Assiut, where the cultivation of anise is concentrated. Anise plant is sensitive to salinity, and therefore the purpose of this study was to increase plant tolerance to salinity through inoculation with mycorrhiza, to determine the best level of chemical fertilization with mycorrhiza for production of anise plant under the moderate salinity conditions.

Using biofertilization that contain different microbial strains has led to a decrease in the use of chemical fertilization and has provided high quality products free of harmful agrochemicals for human safety. The beneficial effects, induced by inoculating fungi such as arbuscular mycorrhiza and bacteria, on plant growth are attributed to the improvement of water and nutrient uptake, especially those of limited availability in soil such as nitrogen, phosphorus and micronutrients. Kilian et al. (2005) and Ali et al. (2013) reported that, the effect of inoculation with endomycorrhiza on shoot height, root length, total dry weight of the whole plants, N, P uptake, percentage of infection with mycorrhiza in anise plant were significantly increased. As well as, the interaction between nitrogen fertilization and inoculation with mycorrhiza had significant effect on total dry weight of whole plants and seed yield in the clay soil. Abd El-Mohsen (2008) mentioned that, the maximum benefits of mycorrhiza were obtained when the growing plants were inoculated with VAM mycorrhiza in addition to fertilization with 0.9 g ammonium nitrate (33.5% N)/pot, which represent 112.50 kg/fed, in clay and sandy soils. Seyed and Hossein (2013) reported that, application of mycorrhiza with phosphorus fertilizer, improved yield and other basil plant criteria under water deficit. Therefore, it appears that, the application of mycorrhiza could be promising in the production of basil by the reduction of chemical fertilizer application. It seems using this mycorrhiza in agroecosystems could increase water uptake by its positive effects on root parameters and of course help farmers to save water in arid and semi-arid regions.

MATERIALS AND METHODS

The present study was carried out during the two successive seasons; 2012/2013 and 2013/2014 at El-Maghara Research Station, Desert Research Center, North Sinai Governorate, to investigate the effect of biofertilization (mycorrhiza) and chemical fertilization on vegetative growth, fruit yield, oil production and chemical composition of anise (*Pimpinella anisum*) plant to minimize usage of chemical fertilization.

1. Plant Material and Procedure

Seeds of *Pimpinella anisum* plant were obtained from the Experimental Farm of Faculty of Agriculture, Cairo University. Seeds were sown in the field in 25^{th} and 21^{th} October 2012 and 2013, respectively, at distances of 25 cm between hills (thinned to two plants/hill) and 75 cm between rows. Drip irrigation system was applied in the whole experiment using droppers (4 L/h) every 3 days (for two hours), using a moderate salinity water (2688 ppm).

Soil and water analyses are shown in tables (1 and 2). Soil samples representing the experimental area were taken at 0-30 cm depth, the water analysis was taken from the irrigation water used. Both soil and water samples were analyzed in the Desert Research Center laboratories.

Table (1). Chemical analysis of the soil.

pH	E.C (mmhos /cm)	Soluble cations (mg/L)				Soluble anions (mg/L)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.7	2.80	114.1	36.77	440	12	0	34.07	728.7	340.07

TDS (mg/L)	Total nitrogen (%)	Phosphate (mg/L)
1792	0.42	85.5

Table (2). Water analysis of the irrigation water.

pH	TDS (mg/L)	E.C mmhos/ cm	Soluble cations (mg/L)				Soluble anions (mg/L)			
			Ca^{++}	Mg^{++}	Na	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.5	2688	4.20	188.4	79.79	56	66	0	238.48	923.0	580

2. Fertilization Treatments

An experiment was carried out to investigate the effects of two factors, the first factor was the biofertilization (mycorrhiza), applied either without mycorrhiza (M_0) and with mycorrhiza (M_1). The second factor was the chemical fertilization, which was applied at four levels, the first level was control treatment (without chemical fertilization) (F_0), the second level was at a rate of 50% chemical fertilization of NPK (F_1), the third level at a rate of 75% chemical fertilization of NPK (F_2) and the fourth level at a rate of 100% chemical fertilization of NPK (F_3), to be 100% of chemical fertilization of NPK (200 kg/fed ammonium sulfate + 200 kg/fed calcium super phosphate + 100 kg/fed potassium sulfate, respectively). Both calcium superphosphate and compost were added before the sowing date. Nitrogen as well as potassium fertilizers were added in three equal doses, the first one was applied 45 days after sowing the seeds and the second dose was added after 30 days from the first one, while the third dose was added 30 days after the second one. Organic fertilizer (compost) was added as a basic dose for all experiments at the rate of 10 $m^{3/}$fed, the chemical properties of compost are shown in table (3).

Table (3). The chemical properties of compost.

Humidity	Ash	O.M.	O.C	C/N	N	P	K
26%	9%	65%	36.1%	16:1	2.15%	1.5	1.26
pH	**Trace elements (ppm)**				**Water capacity**	**Na Cl**	
	Fe^{++}	**Mn^{++}**	**Cu^{++}**	**Zn^{++}**			
5.8	1025	115	180	28	250%	1.21%	

The biofertilizer (mycorrhiza) was provided by the unit of Bio-fertilizers, Faculty of Agriculture, Ain Shams University, Egypt. The bio-fertilizer (1 L mixtures of 3 strains of mycorrhizae) was added before sowing the seeds, by adding latex material to seeds, then adding the mycorrhiza. Arbuscular mycorrhizal fungi consisted of a mixture of *Glomus etunicatum*, *Glomus intraradices* and *Glomus monosporum* spores at concentration of 250 spore/ml.

3. Harvesting and Data Recorded

The plants in the two seasons were harvested in 12th April for the first season and 4th April for the second season. Data were recorded for the following parameters: plant height, number of umbels/plant, fresh and dry weights/plant, fruit weight/plant and fruit yield/fed.

4. Statistical Analysis

The layout of the experiment was split plot design with three replications. The main plots were allocated to the biofertilization (mycorrhiza), and the sub plots were specified for the chemical fertilization. Data were subjected to the statistical analysis of variance using Mstat Statistical Software. L.S.D. test at 0.05, that was used to compare the average means of treatments, carried out according to Snedecor and Cochran (1982).

5. Determination of Essential Oil Percentage and GC/MS Analysis of Volatile

The essential oil percentage in anise fruit was determined according to British Pharmacopoeia (1963). The chemical composition of the essential oil was determined using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 mm, 0.251 mm, 0.1 mm film thickness). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system according to Adams (2007).

6. Determination of Nitrogen, Phosphorus, Potassium and Total Carbohydrates Content

Elements contents were determined in the acid digested solution, which was prepared according to Hach et al. (1985). Nitrogen content was determined by modified micro-Kjeldahl method and phosphorus were estimated according to Page et al. (1982). Potassium was estimated using flame photometer method according to Chapman and Pratt (1961). Total carbohydrates percentage was determined according to Chaplin and Kennedy (1994).

RESULTS AND DISCUSSION

1. Vegetative Growth

1.1. Plant height

Data in table (4) showed that, in the first season, inoculation of anise plants with mycorrhiza led to a significant increase in plant height, the highest value was 30.96 cm of inoculated plants. Also, in the second season, an increase in plant height was recorded, but without a significant difference between non-inoculated and inoculated plants, the averages were 40.58 and 41.53 cm, respectively. Regarding the chemical fertilization treatments, in both seasons, using all levels led to a significant increase in plant height compared with the control plants (F_0).

Concerning the interaction between mycorrhiza and chemical fertilization, in the first season, a significant effect on plant height was observed. The tallest plants were 32.40, 32.14 and 31.97 cm in the first season and were obtained by using M_1F_1, M_1F_2 and M_0F_1, respectively. Meanwhile, in the second season, the tallest plants were 44.17, 42.75, 42.00 and 41.63 cm and were obtained by using M_1F_2, M_0F_1, $M_1F_1$and M_1F_3, respectively, without significant differences between them, but the differences between these treatments and the other treatments were significant.

Table (4). Effect of biofertilization (mycorrhiza) and chemical fertilization on plant height of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Plant height (cm)					
	First season (2012/2013)			Second season (2013/2014)		
	M_0	M_1	Mean	M_0	M_1	Mean
F_0	24.26	27.96	26.11	37.33	38.33	37.83
F_1	31.97	32.40	32.19	42.75	42.00	42.38
F_2	29.07	32.14	30.61	41.25	44.17	42.71
F_3	29.84	31.35	30.60	41.00	41.63	41.32
Mean	28.79	30.96		40.58	41.53	
L.S.D at 0.05						
M		2.01			3.25	
F		3.33			1.92	
M × F		4.71			2.72	

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100% of chemical fertilization, respectively.

1.2. Number of umbels/plant

Data represented in table (5) showed that, using inoculation with mycorrhiza led to a significant increase in number of umbels/plant in the first season. The greatest number of umbels was 30.60 umbels/plant, resulted from inoculated plants. Also, in the second season, there was a non-significant increase in the number of umbels/plant. The averages were 35.61 and 37.90 umbels/plant, for non-inoculated and inoculated plants, respectively. Concerning the chemical fertilization treatments, in the first season, the highest number of umbels/plant (31.61umbels/plant) was resulted from F_1, where there was a significant difference between this level and other levels. However, in the second season, the greatest number of umbels/plant (40.75 and 39.71 umbels/plant) was resulted from F_2 and F_3, respectively, without significant difference between them, but the difference between these levels and F_1 and control (F_0) were significant.

Regarding the interaction between mycorrhiza and chemical fertilization, in the first season, the greatest number of umbels/plant (33.40, 33.35, 29.87 and 29.20 umbels/plant) were obtained by using M_1F_2, M_1F_1, M_0F_1 and M_1F_0, respectively, without significant difference between them, but the differences between these treatments and other treatments were significant. Meanwhile, in the second season, the greatest numbers of umbels/plant were 45.50 and 44.75 umbels/plant, and were obtained by using M_1F_2 and M_1F_3, respectively, without significant difference between them, but the differences between these treatments and other treatments were significant. So it can be concluded that, M_1F_2 obtained the greatest number of umbels/plant in both seasons.

Table (5). Effect of biofertilization (mycorrhiza) and chemical fertilization on number of umbels of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Number of umbels/plant					
	First season (2012/2013)			Second season (2013/2014)		
	M_0	M_1	Mean	M_0	M_1	Mean
F_0	18.95	29.20	24.08	34.25	30.00	32.13
F_1	29.87	33.35	31.61	37.50	31.34	34.42
F_2	21.83	33.40	27.62	36.00	45.50	40.75
F_3	20.98	26.43	23.71	34.67	44.75	39.71
Mean	22.91	30.60		35.61	37.90	
L.S.D at 0.05						
M		4.83			7.31	
F		2.98			4.14	
M × F		4.21			5.86	

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100% of chemical fertilization, respectively.

1.3. Fresh and dry weights/plant

Data outlined in tables (6 and 7) showed that, inoculating anise plants with mycorrhiza led to a significant increase in fresh and dry weights/plant compared with non-inoculated plants in both seasons (44.08 and 54.63 g fresh weight /plant and 34.57 and 43.70 g dry weight /plant in the first season, and 49.01 and 52.66 g fresh weight /plant and 32.67 and 40.26 g dry weight /plant in the second season, for non-inoculated and inoculated plants, respectively). Using chemical fertilization, in both seasons the highest values of fresh and dry weights/plant resulted from F_1 with a significant difference between this level and other levels.

The interaction between mycorrhiza and chemical fertilization had a significant effect on fresh and dry weights/plant. The heaviest fresh and dry weights/plant in the first and second seasons resulted from M_1F_1 followed by M_0F_1, with a significant differences between these treatments and other treatments. Whereas the lowest fresh and dry weights/plant were obtained from M_0F_0 (control) in the first and second seasons. So it can be said that, M_1F_1 treatment gave the heaviest fresh and dry weights/plant.

1.4. Fruit weight/plant and fruit yield/fed

The results in tables (8 and 9) clearly indicated that, significant increase in fruit weight/plant and fruit yield/fed resulted from anise plants inoculated with mycorrhiza compared with non-inoculated plants in both seasons (7.86 and 10.25 g/plant and 350.8 and 457.5 kg/fed in the first season, and 7.27 and 9.31 g/plant and 324.3 and 415.4 kg/fed in the second season, for non-inoculated and inoculated plants, respectively). As far as chemical fertilization is concerned, the heaviest fruit weight/plant and fruit yield/fed were obtained with F_1 treatment with a significant difference between this level and other levels in the second season, but, in the first season, there was a non-significant difference between F_1 and F_2.

Table (6). Effect of biofertilization (mycorrhiza) and chemical fertilization on fresh weight (g)/plant of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Fresh weight (g)					
	First season (2012/2013)			Second season (2013/2014)		
	M_0	**M_1**	**Mean**	**M_0**	**M_1**	**Mean**
F_0	35.33	44.33	39.83	39.58	40.42	40.00
F_1	63.50	71.00	67.25	62.25	64.64	63.45
F_2	40.50	58.67	49.59	47.19	53.89	50.54
F_3	37.00	44.50	40.75	47.00	51.67	49.34
Mean	44.08	54.63		49.01	52.66	
L.S.D at 0.05						
M	8.37			2.10		
F	5.72			4.35		
M × F	8.09			6.16		

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100% of chemical fertilization, respectively.

Table (7). Effect of biofertilization (mycorrhiza) and chemical fertilization on dry weight (g)/plant of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Dry weight (g)					
	First season (2012/2013)			Second season (2013/2014)		
	M_0	**M_1**	**Mean**	**M_0**	**M_1**	**Mean**
F_0	25.47	35.47	30.47	28.52	31.67	30.10
F_1	50.80	56.80	53.80	43.16	45.89	44.53
F_2	32.40	46.93	39.67	30.00	43.06	36.53
F_3	29.60	35.60	32.60	29.00	40.42	34.71
Mean	34.57	43.70		32.67	40.26	
L.S.D at 0.05						
M	6.69			4.51		
F	4.57			4.74		
M × F	6.47			6.70		

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100% of chemical fertilization, respectively.

Regarding the interaction between mycorrhiza and chemical fertilization, in the first season, the heaviest fruit weight/plant and fruit yield/fed of 12.93 g/plant and 577.0 kg/fed, respectively, were obtained with M_1F_2 treatment with a significant difference between this treatment and other treatments. In the second season, the heaviest fruit weight/plant and fruit yield/fed (12.39, 12.02 and 10.56 g/plant and 553.0, 536.7 and 471.2 kg/fed, respectively) were resulted from M_1F_2, M_1F_1 and M_0F_1 treatments, respectively, without significant difference between them, but, there were significant differences between these treatments and other treatments, the lowest fruit yield/plant and per fed in both seasons resulted from control treatment.

Table (8). Effect of biofertilization (mycorrhiza) and chemical fertilization on fruit weight/plant of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Fruit weight/plant (g/plant)					
	First season (2012/2013)			**Second season (2013/2014)**		
	M_0	**M_1**	**Mean**	**M_0**	**M_1**	**Mean**
F_0	5.48	7.91	6.70	5.31	6.06	5.69
F_1	10.50	11.49	11.00	10.56	12.02	11.29
F_2	7.99	12.93	10.46	6.69	12.39	9.54
F_3	7.46	8.67	8.07	6.50	6.75	6.63
Mean	7.86	10.25		7.27	9.31	
L.S.D at 0.05						
M	2.01			1.83		
F	0.86			1.36		
M × F	1.21			1.93		

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100 % chemical fertilization, respectively.

Table (9). Effect of biofertilization (mycorrhiza) and chemical fertilization on fruit yield/fed of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Fruit yield/fed (kg/fed)					
	First season (2012/2013)			**Second season (2013/2014)**		
	M_0	**M_1**	**Mean**	**M_0**	**M_1**	**Mean**
F_0	244.8	352.9	298.9	237.2	270.6	253.9
F_1	468.7	512.8	490.8	471.2	536.7	504.0
F_2	356.9	577.0	467.0	298.6	553.0	425.8
F_3	332.9	387.2	360.1	290.2	301.3	295.8
Mean	350.8	457.5		324.3	415.4	
L.S.D at 0.05						
M	90.05			81.77		
F	38.38			61.16		
M × F	54.27			86.49		

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100% chemical fertilization, respectively.

Generally, it can be concluded that, M_1F_2 treatment (inoculating the anise plants with mycorrhiza + 75% of chemical fertilization) recorded the highest fruit weight/plant and fruit yield/fed. So, it is recommended for the highest fruit weight/plant and fruit yield/fed. This increase in all parameters of the vegetative growth in anise plant inoculated with mycorrhiza, may be due to one of the function of the mycorrhiza in the production of materials helpful for plant growth within the rhizosphere area. These materials could be the hormones that mycorrhiza release in the root area and affect its growth and extension positively. Muchovej (2001) noted that, mycorrhiza working to increase production of plant growth hormones; such as cytokinins and gibberellins. Also, the results of the present study could be attributed to the more absorption of nutrients, especially nitrogen, phosphorus and potassium, which reflect more growth activity.

These results agreed with those of many investigators who noticed that, inoculating many plants with mycorrhiza caused increases in growth and fruit yield. In this regard, Abd El-Mohsen (2008) reported that, inoculation with mycorrhiza led to a high significant increase in the seed yield of coriander and anise plants as compared with non-inoculated. Also, the maximum benefits of mycorrhiza were obtained when the growing plants were inoculated with VAM mycorrhiza in addition to fertilization with 112.5 kg/fed ammonium nitrate. Aliabadi et al. (2008) reported that, the highest water use efficiency was achieved under the application of mycorrhiza accompanied by 70 kg/ha phosphorus. Behzad and Hossein (2010) mentioned that, mycorrhiza and phosphorus application had a significant effect on seed yield of coriander plant. Ali et al. (2013) reported that, the effect of inoculation with the used endomycorrhiza on shoot height, root length, total dry weight of the whole plants was significantly increased in anise plants. Masoumi et al. (2015) showed that mycorrhiza inoculation with both *Glomus mosseae* and *Glomus interaradices* strains improved plant growth of anise in salinity conditions. Salehi et al. (2015) indicated that, inoculated cumin plants with the mycorrhiza species produced heavier and greater grain than non-inoculated plants. Also**,** chemical fertilization caused increases in growth and fruit yield**.** Yassen et al. (2010) on anise plant, deduced that, application of nitrogen fertilizer was effective in increasing all tested growth parameters compared with unfertilized treatment. Khalid (2013) reported that, the most effective dose of nitrogen was 200 kg/ha of ammonium sulfate, resulting in a positive increase in vegetative growth characters for anise plant.

2. Volatile Oil

2.1. Oil percentage

Data presented in table (10) showed that, in both seasons, there was a non-significant difference between non-inoculated and inoculated plants with mycorrhiza concerning oil percentage. These results disagreed with some investigators; such as Masoumi et al. (2015) who showed that, mycorrhiza inoculation with both *Glomus mosseae* and *Glomus interaradices* strains improved essential oil of anise plant in salinity conditions.

Regarding chemical fertilization, in both seasons, it was clear that, the highest oil percentage (1.65 and 2.09% in the first and second seasons, respectively) were obtained from plants received F_2 (75% of chemical fertilization of NPK). There were significant differences between this level and other levels. These results are in agreement with those obtained by Yassen et al. (2010) on anise plant that showed that application of nitrogen fertilizer was effective in increasing oil percentage compared with unfertilized treatment. Khalid (2013) reported that, the most effective dose of nitrogen was 200 kg/ha of ammonium sulfate, resulting in a positive increase in the content of essential oil of anise plant.

Table (10). Effect of biofertilization (mycorrhiza) and chemical fertilization on oil percentage of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments	Volatile oil percentage (%)					
	First season (2012/2013)			Second season (2013/2014)		
	M_0	**M_1**	**Mean**	**M_0**	**M_1**	**Mean**
F_0	1.00	1.05	1.03	1.00	1.50	1.25
F_1	1.60	1.20	1.40	2.37	1.50	1.94
F_2	2.00	1.30	1.65	2.50	1.67	2.09
F_3	1.10	1.70	1.40	1.27	2.37	1.82
Mean	1.43	1.31		1.79	1.76	
L.S.D at 0.05						
M	0.36			0.30		
F	0.15			0.24		
M × F	0.22			0.35		

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100% of chemical fertilization, respectively.

The interaction between mycorrhiza and chemical fertilization, indicated that, in both seasons, the highest oil percentage (2.00 and 2.50% in the first and second seasons, respectively) were recorded from the plants treated with M_0F_2 (75% of the chemical fertilization of NPK only without inoculated anise plants with mycorrhiza). So this treatment is recommended for the highest volatile oil percentages.

2.2. Oil yield/fed

From the data shown in table (11) it may be remarked that, anise plants inculcated with mycorrhiza showed a non-significant increase in the oil yield/fed in both seasons. Concerning the chemical fertilization, in both seasons, the highest oil yield/fed resulted from F_1 and F_2, without significant differences between them, but there were a significant difference between these levels and the highest level of chemical fertilization (F_3) or control treatment (F_0).

Table (11). Effect of biofertilization (mycorrhiza) and chemical fertilization on oil yield/fed of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

	Oil yield/fed (L/fed)					
	First season (2012/2013)			Second season (2013/2014)		
Treatments	**M_0**	**M_1**	**Mean**	**M_0**	**M_1**	**Mean**
F_0	2.45	3.74	3.10	2.37	4.06	3.22
F_1	7.48	6.14	6.81	11.08	8.05	9.57
F_2	7.14	7.51	7.33	7.46	9.22	8.34
F_3	3.67	6.58	5.13	3.67	7.13	5.40
Mean	5.19	5.99		6.15	7.12	
L.S.D at 0.05						
M		1.50			1.46	
F		0.77			1.28	
M × F		1.09			1.81	

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100 % chemical fertilization, respectively.

Regarding the interaction between mycorrhiza and chemical fertilization, in the first season, the highest oil yields/fed; 7.51, 7.48, 7.14 and 6.58 L/fed were resulted from M_1F_2, M_0F_1, M_0F_2 and M_1F_3, respectively, without significant differences between them. While, there were a significant difference between these treatments and the other treatments. In the second season, the highest oil yield/fed; 11.08 L/fed was resulted from M_0F_1, with a significant difference between this treatment and other treatments.

In conclusion, treating anise plants with 50% of chemical fertilization only without inoculation with mycorrhiza (M_0F_1) gave the highest oil yield/fed. So, this treatment is recommended for the highest oil yield/fed.

3. Chemical Composition

3.1. Nitrogen, phosphorus, potassium and total carbohydrates content

Data in table (12) indicated that, the highest nitrogen, potassium and total carbohydrates content resulted from M_1F_1 (treating the anise plants with mycorrhiza plus 50% of chemical fertilization), and were reflected on vegetative growth; especially fresh and dry weights/plant.

Also, it is noticed that, phosphorus content was increased when treating anise plants with M_1F_2 (inoculation with mycorrhiza plus 75% of chemical fertilization), which was reflected on inflorescences process and increase in the number of umbels/plant with this treatment, which led to the highest fruit weight/plant and fruit yield/fed. It may explain the role of phosphorus in inflorescences process.

Table (12). Effect of biofertilization (mycorrhiza) and chemical fertilization on N, P, K and total carbohydrates content of anise (*Pimpinella anisum* L.) plants, during 2012/2013 and 2013/2014 seasons.

Treatments		N %		P %		K %		Total carbohydrates	
		1st season	2nd season	1st season	2nd season	1st season	2nd Season	1st season	2nd season
M_0	F_0	1.37	1.03	0.37	0.25	1.35	1.78	12.32	13.13
M_0	F_1	1.37	1.37	0.42	0.29	1.81	1.81	13.94	13.85
M_0	F_2	2.06	2.06	0.40	0.25	1.63	1.56	19.14	19.80
M_0	F_3	1.72	1.37	0.32	0.25	1.46	1.51	20.16	20.70
M_1	F_0	1.72	1.54	0.47	0.50	1.56	1.60	19.95	20.70
M_1	F_1	3.46	3.37	0.49	0.51	1.85	1.92	23.42	22.23
M_1	F_2	2.37	2.06	0.53	0.54	1.81	1.81	22.23	21.80
M_1	F_3	1.03	1.62	0.31	0.27	1.71	1.67	19.38	21.12

M_0 and M_1 = without and with mycorrhiza F_0, F_1, F_2 and F_3 = at 0, 50, 75 and 100 % chemical fertilization, respectively.

Generally, treating the anise plants with mycorrhiza and adding 50 or 75% of chemical fertilization (M_1F_1 and M_1F_2) achieved the highest benefit from inoculated anise plants with mycorrhiza, which proved the importance of mycorrhiza in increasing the content of nutrients in anise plant and their reflection on the vegetative growth and fruit yield.

These results are in line with those reported by Aliabadi et al. (2008), who showed that, water use efficiency was increased under application of mycorrhiza that can increase absorption of phosphorus and water under drought conditions. Hossein et al. (2008) stated that, AM is able to enhance the growth of coriander under water stress through enhancing P uptake. Abd El-Mohsen (2008) proved that inoculation of coriander and anise plants with mycorrhiza plus fertilization with 112.5 kg/fed ammonium nitrate had positive effects on percentage of N and P in the plants, and percentage of N, P and protein in seeds. Behzad and Hossein (2010) mentioned that, mycorrhiza and phosphorus application had significant effects on P content of coriander plant. Ali et al. (2013) on anise plant, found that, the interaction effect between inoculation with mycorrhiza fungi and nitrogen fertilization was significant with N and P uptake.

Generally, it was noticed that, the contents of nitrogen, phosphorus, potassium and total carbohydrates were raised with the inoculation of anise plants with mycorrhiza, especially with three levels of chemical fertilization

(0, 50 and 75%) compared with non-inoculated plants. Also, there was a decrease in the content of these characters with the highest level of chemical fertilization (100%). This is may be due to the decrease in efficiency or count of mycorrhizal fungi with high levels of chemical fertilization, which was reported by some researches; Ali et al. (2013) on anise plant, reported that, the mineral fertilization decreased the percentage of infection with mycorrhiza at the rate of 75 kg/fed of ammonium nitrate.

3.2. Essential oil constituents

Four volatile oil samples were selected for the discretion of the components, that are representing the highest volatile oil percentages resulted from M_0F_2 treatment and the control treatment (M_0F_0). Also, samples of the highest oil yield/fed resulted from M_0F_1 treatment and the highest fruit yield/fed resulted from M_1F_2 treatment were selected. The samples of the essential oil during the second season were subjected to GC-MS analysis. The main compounds are shown in table (13). It can be shown that 29 compounds were identified. The main compounds of essential oil were anethole, estragole, cis-pseudoisoeugenyl-2-methylbutyrate, p-cymene, longifolene and spathulenol, which represent around 94.82 - 96.27% from anise oil.

It can be seen from M_0F_2 treatment, that the major component was anethole (68.29%), followed by estragole (11.58%), cis-pseudoisoeugenyl-2-methylbutyrate (9.18%), longifolene (3.81%), spathulenol (1.72%) and p-cymene (1.12%). Also, as for the control plants (M_0F_0) the major component was anethole (77.27%), followed by estragole (12.95%), cis-pseudoisoeugenyl-2-methylbutyrate (3.78%) and longifolene (2.07%). But, when using 50% of chemical fertilization only without mycorrhiza, the major component was anethole (65.78%), followed by p-cymene (11.30%), estragole (9.29%), cis-pseudoisoeugenyl-2-methylbutyrate (5.47%) and longifolene (2.70%). Meanwhile, concerning the anise plants inoculated with mycorrhiza + 75% of chemical fertilization, the main component was anethole (74.57%), followed by estragole (7.80%), cis-pseudoisoeugenyl-2-methylbutyrate (7.24%) and longifolene (3.22%).

It was noticed that, the highest value of anethole content (77.27%) was achieved by control plants (M_0F_0). There were a decrease in anethole content with using the chemical fertilization only without mycorrhiza (65.78 and 68.29% resulted from M_0F_1 and M_0F_2 treatments, respectively). But when using mycorrhiza + 75% of chemical fertilization, it led to raising the anethole content (74.57%) compared with the treatment without mycorrhiza + 75% of chemical fertilization, which gave 68.29%.

Also, it is noted that, the highest content of estragole (12.95%) was resulted from control plants, while the lowest content (7.80%) was recorded by using mycorrhiza + 75% of chemical fertilization, which led to an increase in the quality of the volatile oil resulted from this treatment.

Table (13). Chemical composition of the essential oil of four treatments using GC-MS.

No.	Compound name	M_0F_0	M_0F_1	M_0F_2	M_1F_2
1	α- Pinene	-	0.28	-	-
2	p-Cymene	-	11.30	1.12	0.30
3	Camphor	-	-	-	0.36
4	γ-Terpinene	-	1.04	-	-
5	Linalool	-	0.39	-	-
6	1,8-Cineole	-	-	0.49	0.26
7	Geyrene	0.19	-	-	-
8	Estragole	12.95	9.29	11.58	7.80
9	Anethole	77.27	65.78	68.29	74.57
10	1-(2-Hydroxyphenyl)1butanone	-	-	0.26	-
11	α –Elemene	0.12	-	-	-
12	Longifolene	2.07	2.70	3.81	3.22
13	(trans) Isoeugenol Methyl Ether	-	0.22	0.34	0.45
14	Methyl eugenol	0.13	-	-	-
15	Zingiberene	0.14	0.28	0.42	0.45
16	α -Himachalene	0.22	0.34	0.72	0.13
17	α -Bisabolene	0.18	0.28	0.40	0.34
18	Isolongifolene,4,5dehydro	0.64	0.62	0.59	1.62
19	Isolongifolene,4,5,9,10dehydro	0.18	0.12	-	-
20	8-methoxy-1-acetonaphthone	-	-	0.17	-
21	8-Isopropenyl1,3,3,7tetramethylbicyclo[5.1.0]oct5en2-one	0.13	-	-	
22	Isospathulenol	0.19	0.20	-	-
23	Longifolenaldehyde	-	0.16	-	0.25
24	α -Himachalen oxide	0.17	-	0.23	0.23
25	(-) Spathulenol	1.12	1.12	1.72	1.87
26	Himachalol	0.15	0.18	-	-
26	Aromadendrene oxide(2)	0.09	-	-	0.13
27	α -Bisabolol	0.11	0.11	0.18	0.31
28	3-Methoxy-5-hydroxy-2-oxophthalide	0.09	0.12	0.19	0.20
29	cis-pseudoisoeugenyl-2-methylbutyrate	3.78	5.47	9.18	7.24
	Total	**99.92**	**100.00**	**99.69**	**99.73**

CONCLUSION

Although anise is sensitive to salinity, as observed clearly in the results of control plants in this experiment, the production of anise succeeded under moderate salinity of irrigation water (2688 ppm) when using mycorrhiza plus chemical fertilization. To obtain the best results from oil yield/fed of anise plants in sandy soil, it is recommended**,** using 50% of chemical fertilization only without using mycorrhiza, but the disadvantage of this treatment, was ascertained by a low content of anethole (68.29%). Meanwhile, to obtain the highest fruit weight/plant, fruit yield/fed and high oil yield/fed, anise plants should be treated with mycorrhiza plus 75% of chemical fertilization, however, the volatile oil resulted from this treatment was characterized by its lowest estragole content (7.80%) and high content of anethole (74.57%) compared with other treatments.

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دراسة إمكانية زراعة نبات الينسون تحت ظروف سيناء بإستخدام التسميد الكيميائي والميكروهيزا

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في تجربة حقلية تمت بمحطة بحوث المغارة بشمال سيناء التابعة لمركز بحوث الصحراء خلال موسمين متتالبين ٢٠١٢ /٢٠١٣ و٢٠١٣ /٢٠١٤. أجريت دراسة لتأثير التسميد الحيوى (الميكروهيزا) والتسميد الكيماوي على نمو ومحصول الثمار والزيت الطيار والتركيب الكيميائي لنبات الينسون. كان تصميم التجربة عاملية منشقة مرة واحدة مع ثلاث مكررات بحيث تكون الميكروهيزا في القطع الرئيسية بمستويين (معاملة وبدون معاملة) والتسميد الكيماوي في القطع المنشقة بأربع مستويات (بدون ، ٥٠، ٧٥ و١٠٠٪ من التسميد الكيماوي من نتروجين، فوسفور، بوتاسيوم) بحيث تكون ١٠٠٪ من التسميد الكيماوي عبارة عن (٢٠٠ كجم/فدان سلفات نشادر +٢٠٠ كجم/فدان سوبر فوسفات الكالسيوم +١٠٠ كجم/فدان سلفات بوتاسيوم). أظهرت النتائج أن المعاملة بالميكروهيزا أدت إلى زيادة معنوية في إرتفاع النبات، عدد النورات/نبات، الوزن الطازج والجاف/نبات، وزن الثمار/نبات ومحصول الثمار/فدان، المحتوى من النتروجين والفوسفور والبوتاسيوم والكربوهيدرات الكلية. أيضًا في غالبية الأحيان فإن إضافة الأسمدة الكيميائية بمعدل ٥٠ أو ٧٥٪ من التسميد الكيماوي حققت زيادة في نفس الصفات. كان للتداخل بين الميكروهيزا والتسميد الكيماوي تأثير معنوي على كل الصفات، وأفضل النتائج من الوزن الطازج والجاف/نبات، المحتوى من النتروجين والبوتاسيوم والكربوهيدرات الكلية والتي تحققت من معاملة النباتات بالميكروهيزا +٥٠٪ من التسميد الكيماوي. في حين أن أعلى محصول ثمار للنبات والفدان وأعلى محتوى من الفوسفور تحقق من معاملة نبات الينسون بالميكروهيزا + ٧٥٪ من التسميد الكيماوي، وكان الزيت الطيار الناتج من هذه المعاملة يحتوى على الأنيثول (٧٤,٥٧٪) تلاه أستراجول (٧,٨٠٪)، سيس-بسيدوايزوجينول-٢-ميثيل بيوتريت (٧,٢٤٪) ولونجيفولين (٣,٢٢٪).