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EFFECT OF BIOFERTILIZATION AND ROCK PHOSPHATE, ON GROWTH AND OIL PRODUCTION OF *Trachyspermum ammi* (L.) SPRAGUE (AJOWAN) PLANT UNDER SINAI CONDITIONS

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ABSTRACT

The current study was conducted during two seasons of 2012/2013 and 2013/2014 at El-Maghara Research Station (North Sinai), Desert Research Center to study the effect of two different biofertilizer mixtures of [*Azotobacter chroococcum* + *Bacillus megatherium* var. *phosphaticum* (B_1)] and [*Azospirillum brasilense* + *Bacillus megatherium* var. *phosphaticum* (B_2)] three application methods of yeast [foliar spray (Y_1), soil drench (Y_2) or a soil drench + foliar spray (Y_3)] and two levels of rock phosphate [200 and 300 kg/fed. (R_1) and (R_2), respectively], on growth and oil production of Ajowan (*Trachyspermum ammi* (L.) Sprague) plant, in a split split plots design. The main plots were the methods of adding yeast, the sub plots were the levels of rock phosphate, and the sub sub plots were the mixtures of bio-fertilizers. The results showed that, adding the yeast as a soil drench significantly increased the plant height, number of umbels/plant and volatile oil percentages. But, adding yeast as a soil drench + foliar spray gave the best results of fresh and dry weights/plant, fruit weight/plant and fruit yield/fed. The second level of rock phosphate (300 kg/fed.) caused an increase in plant height and volatile oil percentages. While, the first level (200 kg/fed.) achieved an increase in the number of umbels/plant, fresh and dry weights/plant, fruit weight/plant, fruit yield/fed. and oil yield/fed. As for the biofertilizers, using *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2) led to the best results of plant height, number of umbels/plant, fruit weight/plant and fruit yield/fed. Meanwhile, *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) increased the fresh and dry weights/plant, volatile oil percentages and oil yield/fed. The highest volatile oil percentage resulted from treating the plants with $Y_2R_1B_1$, the main component of the volatile oil resulted from this treatment was P-cymene (45.83%), followed by γ-terpinene (30.19%), thymol (18.96%) and α-pinene (3.14%). While, the highest fruit yield/fed. resulted from $Y_3R_1B_2$ (using yeast as a soil drench and foliar spray treatments plus 200 kg rock phosphate plus mixture of *A. brasilense* + *B. megatherium*).

Key words: *Biofertilizer, rock phosphate, yeast, Trachyspermum ammi, Ajowan, thymol, volatile oil.*

1- INTRODUCTION

Ajowan plant (*Trachyspermum ammi* (L.) Sprague) is an important seed spice, belongs to family Apiaceae. Seeds are 2-3 mm, long, greyish brown in colour. Its characteristic odor and taste due to the presence of an essential oil (2-4%). Ajowan oil is a principal source of thymol, (Rathore *et a*l., 2014). Ajowan is used as germicide, antiseptic, cough syrups, throat lozenges, asthma, in digestion and gas relief, ulcers, carminative, laxative and in the treatment of abdominal tumors, enlargement of spleen, piles, vomiting, abdominal pains, anti-platelet activity, antioxidant activity, antiviral activity, insecticidal activity and anti-tussive activity, (Rashmi *et al*. 2011).

Applying biofertilizers led to a decrease in the use of chemical fertilizers and provided high quality products free of harmful agrochemicals for human safety, (Mahfouz and Sharaf El-din 2007). Nitrogen fixing bacteria, such as; *A. chroococcum* and *A. lipoferum*, were found to have not only the ability to fix nitrogen, but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients and photosynthesis, (Mahfouz and Sharaf El-din, 2007) and (El Ghadban *et al.*, 2006). And increase the supply or availability of primary nutrients to the host plant, (Wu *et al.*, 2005). For the synthesis of antibiotics, enzymes and fungicidal compounds, (Ahmad *et al.*, 2006). phosphorus is an essential constituent of nucleic acids and stimulates root growth as well as increase nodule activity in plants. Nitrogen fixers and phosphate solubilizers contribute through biological fixation of nitrogen, solubilization of fixed nutrients and enhanced uptake of plant nutrients (Gupta *et al.,* 2015).

Therefore, the present study was conducted to evaluate the effect of bio-fertilizers (nitrogen fixers, phosphate solubilizer and yeast) and rock phosphate on growth, yield, essential oil and chemical composition of Ajowan plants under Sinai conditions. Also, to study the antimicrobial activity of Ajowan essential oil against some pathogenic bacteria.

2. MATERIALS AND METHODS

This investigation was carried out during two successive seasons; 2012/2013 and 2013/2014 at El-Maghara Research Station, Desert Research Center, North Sinai Governorate, Egypt, to investigate the effect of application method of yeast, two levels of rock phosphate and two mixtures of bio-fertilizers on growth, yield of fruits and chemical composition of Ajowan (*Trachyspermum ammi* (L.) Sprague) plant.

2.1. Plant material and procedure

Seeds of Ajowan plant were obtained from the Experimental Farm of the Faculty of Agriculture, Cairo University, Seeds were sown in the field on the 25th and the 21st of October 2012 and 2013, respectively, at distances of 35 cm between hills (thinned to one plant /hill) and 70 cm between rows. Drip irrigation system was adopted in the experiment using droppers (4 l/h) every 3 days (for two hours), using moderate saline water (2688 ppm). Compost was added as basic dose at the rate of 15 m^3/fed., (Table A).

Table (A): The chemical properties of compost used

Weight/m^3	Humidity%	Ash %	O.M. %	O.C %	N %	P %	K %	pH	C/N
639 kg	25	74.03	25.97	15.06	1.38	0.49	0.59	5.91	16:1

2.2. Fertilization treatments

An experiment was carried out to test the effects of three factors, the first was the application methods of the yeast (5 g/l) as a foliar spray (Y_1), soil drench (Y_2) or as a soil drench + foliar spray (Y_3). Adding yeast at three times, the first was applied when the plant height was approximately 15 cm and the second was added after 30 days from the first one, while the third was added 30 days after the second one. The second factor was the rock phosphate (22 % P_2O_5), which was applied at two levels, (200 kg/fed. (R_1) and 300 kg/fed. (R_2)). Both rock phosphate and organic fertilizer were added before sowing. The third factor was the biofertilization, using two mixtures (1- *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and 2- mixture of *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). The bio-fertilizers were added in two times, the first was applied on sowing seeds and the second application was added after 60 days from the first one. Fresh liquid cultures of 48 hrs pure local strains of both *A. chroococcum*, *A. brasilense* and *B. megatherium* var. *phosphaticum* isolated from rhizosphere of soil of El- Maghara district, purified and identified according to Bergey's Manual (1994) were used as biofertilizers in the form of mixed inoculations at the rate of ~10^8 c.f.u./ml for each strain.

2.3. Harvested and data recoded

The plants in the two seasons were harvested at 24th May for the first season and 28th May for the second. 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., calculated at 16000 plant densities/fed.

2.4. Statistical analysis

The layout of the experiment was split split plots design with three replications, the main plots were designated as the application methods of yeast, the sub plots were the levels of rock phosphate and the sub sub plots were the mixtures of bio-fertilizers. The data obtained were subjected to the statistical analysis of variance using Mstate Statistical Software. L. S. D. test at 0.05 was used to compare the means of treatments, according to Snedecor and Cochran (1982).

2.5. Chemical analysis

2.5.1.Determination of essential oil percentage and GC/Mass analysis of volatile oil

The essential oil percentage in ajowan fruits was determined according to the British

Pharmacopoeia (1963). The chemical compositions of the essential oil were determined using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). The quantification of all the identified components was determined 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).

2.5.2 Determination of nitrogen, phosphorus, potassium and total carbohydrates

Element contents were determined in the acid digested solution, which was prepared according to Hach *et al.* (1985). Nitrogen content was determined by the modified micro-Kjeldahl method, potassium was estimated using flame photometer method and phosphorus content was estimated according to Page *et al.* (1982). Total carbohydrate percentages in fruits were determined according to Chaplin and Kennedy (1994).

2.6. Soil and water analysis

Soil analyses are shown in Table (B). Soil samples representing the experimental area were taken at 0-30 cm depth. Water analysis is shown in Table (C) taken from the irrigation water used from El-Maghara Station.

2.7. Microbial determinations

Soil samples of Ajowan (*Trachyspermum ammi*) plant rhizosphere were collected at the end of the first and the second seasons and analyzed for total microbial counts according to (Nautiyal *et al.*,2000). For counting and growing phosphate dissolving bacteria using Pikovskaya's agar medium (PVK) (Goenadi *et al.,* 2000). For counting and growing azotobacters, modified Ashby's media (Hill, 2000). For counting and growing *Azospirillum* on Doberiners media, (Dobereiner, 1997).

2.8. Antimicrobial activity

The antimicrobial activity of Ajowan (*Trachyspermum ammi*) essential oil was detected against some pathogenic microorganisms namely, *Staphyllococcus aureus*, *Candida albicans*, *Salmonella typhi*, *Escherichia coli*, *Rhizoctonia solani* and *Fusarium solani.* These microorganisms were provided by Soil Microbiology Unit, Desert Research Center, Cairo, Egypt. The antimicrobial activity was determined by agar diffusion technique using filter paper discs according to the method of Irob *et al*., (1996).

3- RESULTS AND DISCUSSION

3.1. Vegetative growth

3.1.1. Plant height

Data in Table (1) showed that, in both seasons, applying yeast as a drench (Y_2) to the ajowan plants led to the highest significant increase in plant height, the tallest plants were 71.42 and 66.14 cm in the first and the second seasons, respectively. Concerning the rock phosphate treatments, in both seasons, the tallest plants were detected from (R_2). This increase was significant in the first season. Regarding the bio-fertilizers, the tallest plants resulted from (B_2), but without a significant difference between the two mixtures of bio-fertilizers in both seasons.

Table (B): 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^{--}
7.70	2.80	114.10	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 (C): Water analysis of the irrigation water

pH	E.C mmhos/cm	Soluble cations mg/l				Soluble anions mg/l			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.5	4.20	188.40	79.79	560	66	0	238.48	923.02	580
(TDS), mg/l		TOC, %		Nitrate, mg/l		Phosphate, µg/l			
2688		Nil		Nil		Nil			

Table (1): Effect of yeast, rock phosphate and biofertilization on plant height and number of umbels of Ajowan (*Trachyspermum ammi*) plants, during 2012 and 2013 seasons.

Treatments		**Plant height (cm)**						**Number of Umbels**					
		First season			**Second season**			**First season**			**Second season**		
		B_1	**B_2**	**Mean**	**B_1**	**B_2**	**Mean**	**B_1**	**B_2**	**Mean**	**B_1**	**B_2**	**Mean**
Y_1	**R_1**	54.69	51.68	**53.19**	61.22	63.22	**62.22**	53.50	75.15	**64.33**	55.00	70.33	**62.67**
	R_2	55.84	51.04	**53.44**	63.78	58.25	**61.02**	66.60	67.90	**67.25**	68.78	76.44	**72.61**
	Mean	**55.27**	**51.36**		**62.50**	**60.74**		**60.05**	**71.53**		**61.89**	**73.39**	
Y_2	**R_1**	68.72	68.96	**68.84**	63.44	63.67	**63.56**	79.60	81.40	**80.50**	78.44	82.33	**80.39**
	R_2	68.72	79.28	**74.00**	68.27	69.20	**68.74**	61.00	71.90	**66.45**	64.78	64.34	**64.56**
	Mean	**68.72**	**74.12**		**65.86**	**66.44**		**70.30**	**76.65**		**71.61**	**73.34**	
Y_3	**R_1**	52.32	55.04	**53.68**	63.89	65.44	**64.67**	55.80	79.07	**67.44**	59.56	77.66	**68.61**
	R_2	50.80	65.36	**58.08**	60.34	64.00	**62.17**	77.40	66.30	**71.85**	74.78	73.00	**73.89**
	Mean	**51.56**	**60.20**		**62.12**	**64.72**		**66.60**	**72.69**		**67.17**	**75.33**	
Mean	**R_1**	58.58			62.85	64.11		62.97	78.54		64.33	76.78	
Mean	**R_2**	58.45			64.13	63.82		68.33	68.70		69.44	71.26	
L.S.D. at 0.05													
Y× R		4.54			7.52			2.70			9.41		
Y× B		6.58			4.42			5.11			5.43		
R× B		5.37			3.61			4.17			4.44		
Y× R × B		9.31			6.26			7.22			7.69		
		Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
Mean of Y		53.31	71.42	55.88	61.62	66.14	63.42	65.79	73.47	69.64	67.64	72.47	71.25
Mean of R		58.57	61.84		63.48	63.97		70.75	68.52		70.55	70.35	
Mean of B		58.51	61.89		63.49	63.96		65.65	73.62		66.89	74.02	
L.S.D. at 0.05													
Y		3.73			1.43			5.73			9.08		
R		2.62			4.34			1.56			5.43		
B		3.80			2.55			2.95			3.14		

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

Interactions between yeast, the rock phosphate and the bio-fertilizers, showed that, the tallest plants (79.28 and 69.20 cm in the first and the second seasons, respectively) resulted from$Y_2R_2B_2$ treatment with a significant difference between this treatment and other treatments.

3.1.2. Number of umbels/plant

Data in Table (1) showed that, adding yeast to the ajowan plants in drenching form (Y_2) caused a significant increase in the number of umbels/plant in the first season. The same trend was observed in the second one, but, without a significant difference among the three treatments applied. Regarding the rock phosphate, the highest number of umbels/plant was obtained from the first level (R_1) in both seasons, but, there was a significant difference in the first season only.

Concerning the bio-fertilizers, adding (B_2) led to a significant increase in the number of umbels/plant in both seasons.

Concerning the interactions among the treatment of yeast, the rock phosphate and the bio-fertilizers, in both seasons, the highest numbers of umbels/plant were obtained from the treatments $Y_2R_1B_2$, $Y_3R_1B_2$ and $Y_3R_2B_1$, without significant differences among them.

3.1.3. Fresh and dry weighs/plant:

The results recorded on fresh and dry weights/plant (Table 2) indicated that, applying yeast Y_3 (drench and foliar spray) or Y_2 (drench) to the plants led to significant increases in fresh and dry weights/plant in both seasons, compared with Y_1 (foliar spray). Adding 200 kg/fed. rock phosphate (R_1) to the plants significantly increased the fresh and dry weights/plant in both seasons. Also, applying *A. chroococcum* + *B. megatherium* (B_1) caused a significant increase in fresh and dry weights/plant.

The interactions among yeast, rock phosphate and bio-fertilizers, revealed that, treating ajowan plants with $Y_2R_1B_1$ (drench yeast + 200 kg rock phosphate + *A. chroococcum* + *B. megatherium*) gave the heaviest fresh and dry weights/plant in both seasons.

3.1.4. Fruit weight/plant and fruit yield/fed.

Data in Table (3) clearly indicated that, treating ajowan plants with Y_3 (drench and foliar spray treatment) led to a significant increase in fruit weight/plant (27.00 and 21.36 g/plant in the first and the second seasons, respectively) and fruit yield/fed. (432.0 and 341.8 kg/fed. in the first and the second seasons, respectively) compared with drench or foliar spray only. Using the low level of rock phosphate (R_1) caused a significant increase in fruit weight/plant (23.24 and 20.24 g/plant) and fruit yield/fed. (371.8 and 323.8 kg/fed.) in the first and second seasons, respectively). Meanwhile, applying *A. brasilense* + *B. megatherium* (B_2) led to a non-significant increment in fruit weight/plant and fruit yield/fed.

Interactions had a significant effect on fruit weight/plant and fruit yield/fed. In both seasons, the heaviest fruit weight/plant (31.56 and 26.40 g/plant in the first and second seasons, respectively) and fruit yield/fed. (504.9 and 422.4 kg/fed. in the first and second seasons, respectively) were formed on the plants received $Y_3R_1B_2$ (i.e.: drench soil and foliar spray + 200 kg rock phosphate + *A. brasilense* + *B. megatherium*). So, we recommend this treatment for the highest fruit weight/plant and fruit yield/fed.

3.2. Volatile Oil

3.2.1. Oil percentage

From the data shown in Table (4) it may be noticed that, adding the yeast by drench treatment (Y_2) led to a significant increment in volatile oil percentages in both seasons (2.87 and 2.68 %, in the first and second seasons, respectively). Also, the high level of rock phosphate [(R_2) 300 kg/fed.] significantly increased the volatile oil percentages (2.65 and 2.57 %, in the first and the second seasons, respectively). Meanwhile, using mixtures of bio-fertilizers (*A. chroococcum* + *B. megatherium*) gave higher oil percentage (2.62 and 2.51%, in the first and the second seasons, respectively) compared with the mixtures of (*A. brasilense* + *B. megatherium*), but, this increase was significant in the first season, only.

Concerning the interactions, there was a significant effect on oil percentage, in the first season, as the highest volatile oil percentages were resulted from $Y_2R_1B_1$, $Y_2R_2B_1$ and $Y_2R_2B_2$, giving 3.07, 2.98 and 2.90%, without significant difference among them. Meanwhile, in the second season, treating the plants with $Y_2R_1B_1$, $Y_1R_2B_1$ and $Y_3R_2B_2$ led to the highest volatile oil percentages (2.88, 2.70 and 2.70 %), without significant difference among them. The lowest volatile oil percentages (1.85 and 2.02 %, in the first and the second seasons, respectively) resulted from $Y_1R_1B_2$ treatment in both seasons.

In conclusion, the treatment of $Y_2R_1B_1$ (*i,e;* adding yeast by drench treatment + 200 kg/fed. rock phosphate + using mixtures of bio-fertilizers (*A. chroococcum* + *B. megatherium*)

Table (2): Effect of yeast, rock phosphate and biofertilization on fresh and dry weights/plant of Ajowan (*Trachyspermum ammi*) plants, during 2012 and 2013 seasons.

Treatments		Fresh weight/plant (g)						Dry weight/plant (g)					
		First season			Second season			First season			Second season		
		B_1	B_2	Mean	B_1	B_2	Mean	B_1	B_2	Mean	B_1	B_2	Mean
Y_1	R_1	161.3	150.7	**156.0**	195.0	183.3	**189.2**	70.83	64.17	**67.50**	74.17	70.83	**72.50**
	R_2	154.7	145.3	**150.0**	195.0	160.0	**177.5**	74.17	60.83	**67.50**	58.75	55.83	**57.29**
	Mean	**158.0**	**148.0**		**195.0**	**171.7**		**72.50**	**62.50**		**66.46**	**63.33**	
Y_2	R_1	215.0	141.7	**178.3**	273.3	178.3	**225.8**	104.4	67.78	**86.11**	83.33	76.67	**80.00**
	R_2	187.5	135.0	**161.3**	156.7	148.3	**152.5**	78.89	70.00	**74.44**	62.50	60.00	**61.25**
	Mean	**201.3**	**138.3**		**215.0**	**163.3**		**91.67**	**68.89**		**72.92**	**68.33**	
Y_3	R_1	200.0	173.3	**186.7**	218.3	205.0	**211.7**	97.78	75.55	**86.67**	79.17	76.67	**77.92**
	R_2	175.0	165.0	**170.0**	205.0	196.7	**200.9**	86.11	67.78	**76.94**	81.67	77.50	**79.58**
	Mean	**187.5**	**169.2**		**211.7**	**200.9**		**91.94**	**71.67**		**80.42**	**77.08**	
Mean	R_1	192.1	155.2		228.9			91.02	69.17		78.89	74.72	
Mean	R_2	172.4	148.4		185.6			79.72	66.20		67.64	64.44	
L.S.D. at 0.05													
Y× R		16.35			16.94			10.01			8.98		
Y× B		12.28			23.28			12.17			6.97		
R× B		10.03			19.00			9.93			5.60		
Y× R × B		17.37			32.92			17.22			9.86		
		Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
Mean of Y		153.0	169.8	178.3	183.3	189.2	206.3	67.50	80.28	81.81	64.90	70.63	78.75
Mean of R		173.7	160.4		208.9	176.9		80.09	72.96		76.81	66.04	
Mean of B		182.3	151.8		207.2	178.6		85.37	67.69		73.26	69.58	
L.S.D. at 0.05													
Y		12.55			21.80			12.86			9.69		
R		9.44			9.77			5.77			5.18		
B		7.09			13.44			7.02			4.02		

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

Table (3): Effect of yeast, rock phosphate and biofertilization on fruit weight/plant and fruit yield/feddan of Ajowan (*Trachyspermum ammi*) plants, during 2012 and 2013 seasons.

Treatments		Fruit weight/plant (g)						Fruit yield/feddan (kg)					
		First season			Second season			First season			Second season		
		B_1	B_2	Mean	B_1	B_2	Mean	B_1	B_2	Mean	B_1	B_2	Mean
Y_1	R_1	16.78	23.56	**20.17**	15.78	20.80	**18.29**	268.4	376.9	**322.7**	252.5	332.8	**292.7**
	R_2	22.00	17.11	**19.56**	17.38	16.57	**16.98**	352.0	273.8	**312.9**	278.1	265.1	**271.6**
	Mean	**19.39**	**20.34**		**16.58**	**18.69**		**310.2**	**325.4**		**265.3**	**299.0**	
Y_2	R_1	24.00	20.70	**22.35**	22.06	19.32	**20.69**	384.0	331.1	**357.6**	352.9	309.2	**331.0**
	R_2	17.72	17.11	**17.42**	16.62	15.56	**16.09**	283.6	273.8	**278.7**	266.0	248.9	**257.5**
	Mean	**20.86**	**18.91**		**19.34**	**17.44**		**333.8**	**302.5**		**309.5**	**279.1**	
Y_3	R_1	22.83	31.56	**27.20**	17.06	26.40	**21.73**	365.4	504.9	**435.2**	273.1	422.4	**347.8**
	R_2	28.56	25.06	**26.81**	24.34	17.63	**20.99**	456.9	400.9	**428.9**	389.5	282.1	**335.8**
	Mean	**25.70**	**28.31**		**20.70**	**22.02**		**411.2**	**452.9**		**331.3**	**352.3**	
Mean	R_1	21.20	25.27		18.30	22.17		339.3	404.3		292.8	354.8	
Mean	R_2	22.76	19.76		19.45	16.59		364.1	316.1		311.2	265.4	
L.S.D. at 0.05													
Y× R		2.87			1.78			45.98			28.55		
Y× B		2.74			1.35			43.88			21.70		
R× B		2.23			1.10			35.83			17.72		
Y× R × B		3.87			1.92			62.05			30.70		
		Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
Mean of Y		19.86	19.88	27.00	17.63	18.39	21.36	317.8	318.1	432.0	282.1	294.2	341.8
Mean of R		23.24	21.26		20.24	18.02		371.8	340.1		323.8	288.3	
Mean of B		21.98	22.51		18.88	19.38		351.7	360.2		302.0	310.1	
L.S.D. at 0.05													
Y		0.73			2.14			11.82			34.30		
R		1.66			1.03			26.55			16.49		
B		1.58			0.78			25.33			12.53		

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

gave the highest volatile oil percentages in both seasons. So, we recommend this treatment for the highest volatile oil percentages.

3.2.2 Oil yield/fed.

Data in Table (4) showed that, using drench + foliar spray treatments of yeast (Y_3) led to a significant increase in oil yield/fed. in both seasons, but, in the first season, there was non-significant difference between Y_3 and Y_2. However, adding 200 kg rock phosphate (R_1) caused a significant increase in oil yield/fed., but this increase was significant in the second season only.

Interactions had a significant effect in oil yield/fed., in first season, the highest significant increase oil yield/fed. resulted from the treatments $Y_3R_1B_2$, $Y_2R_1B_1$and $Y_3R_2B_1$ (12.11, 11.80 and 11.30 L/fed, respectively), without significant differences among them. Also, the same trend was obtained in the second season, as the highest oil yield/fed resulted from $Y_2R_1B_1$, $Y_3R_1B_2$ and $Y_3R_2B_1$ (10.16, 9.92 and 8.99 l/fed, respectively), without significant differences among them.

Therefore treatments $Y_3R_1B_2$, $Y_2R_1B_1$ and $Y_3R_2B_1$ (both drench and foliar spray treatments plus 200 kg rock phosphate plus using a mixture of bio-fertilizers (*A. brasilense* + *B. megatherium*), or adding yeast by drench treatment plus 200 kg/fed rock phosphate plus using mixture of bio-fertilizers (*A. chroococcum* + *B. megatherium*) or using both drench and foliar spray treatments plus 300 kg rock phosphate plus using mixture of bio-fertilizers (*A. chroococcum* + *B. megatherium*) gave the highest oil yield/fed in both seasons. So we recommend them for the highest oil yield/fed.

Generally, the previous results can be summarized as follows: the foliar spray and the soil drench treatments of the yeast together gave the highest fresh and dry weights/plant, fruit weight and fruit yield/fed., while the soil drench treatment of the yeast only led to the highest plant height, the number of umbels/plant and volatile oil percentage.

Regarding the rock phosphate, it was found that, the high level (300 kg/fed.) caused a significant increase in volatile oil percentages. While, the first level 200 kg/fed. caused a significant increase in fresh and dry weights/plant, fruit weight/plant and fruit yield/fed..

Using the mixture of bio-fertilizers (*A. chroococcum* + *B. megatherium*) significantly increased the fresh and dry weights/plant and volatile oil percentage. Whereas, adding (*A. brasilense*+ *B. megatherium*) had no-significant effect on plant height, fruit weight/plant and fruit yield/fed. and a significant increase in the number of umbels/plant.

Generally, concerning the interactions between yeast and rock phosphate, the tallest plants were obtained by using Y_2R_2, also using Y_2R_1 caused a significant increase in the number of umbels/plant. Meanwhile, the highest values of fresh and dry weights/plant resulted from using Y_3R_1 and Y_2R_1. Using Y_3R_1 and Y_3R_2 recorded the heaviest fruit weight/plant and fruit yield/fed.

Meanwhile, the interactions between yeast and bio-fertilizers, showed that, using Y_2B_2 and Y_2B_1 led to a significant increase in plant height and the number of umbels/plant. However, using Y_2B_1 led to the heaviest fresh weights/plant and the highest volatile oil percentage as well as the highest dry weights/plant resulted from Y_3B_1. Also, significant increases in fruit weight/plant and fruit yield/fed. were obtained with using Y_3B_2.

The interactions between rock phosphate and bio-fertilizers, using R_1B_2 caused a significant increment in the number of umbels/plant, fruit weight/plant and fruit yield/fed. Concerning the fresh and dry weighs/plant, the highest values resulted from R_1B_1.

The previous results for vegetative growth, fruit yield, oil percentage and oil yield illustrated the importance of yeast. Also, the treatments of adding yeast were effective in many characters, which may be due to the size of the plant benefiting from yeast depending on the method of addition of yeast. It is clear that, yeast has a great role in the revitalization of the plant growth. It is a natural biofertilizer, it causes various promoting effects on the plants and it is a natural source of cytokinins, which stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and vitamin B, (Ezz El-Din and Hendawy, 2010).

Also, the biofertilizers representative in *A. chroococcum, A. brasilense* and *B. megatherium* var. *phosphaticum* all share in promoting vegetative growth and fruit yield, which may be due to *Azotobacter* can promote plant growth, including phytohormone production, phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients and photosynthesis, also, N_2 fixation and stimulation of nutrient uptake, (El Ghadban *et al*., 2006;

Table (4): Effect of yeast, rock phosphate and biofertilization on oil percentage and oil yield/feddan of Ajowan (*Trachyspermum ammi*) plants, during 2012 and 2013 seasons.

Treatments		Oil percentage						Oil yield/feddan (L)					
		First season			Second season			First season			Second season		
		B_1	B_2	Mean	B_1	B_2	Mean	B_1	B_2	Mean	B_1	B_2	Mean
Y_1	R_1	2.40	1.85	**2.13**	2.20	2.02	**2.11**	6.44	6.97	**6.70**	5.56	6.71	**6.13**
	R_2	2.55	2.43	**2.49**	2.70	2.52	**2.61**	9.00	6.64	**7.82**	7.51	6.67	**7.09**
	Mean	**2.47**	**2.14**		**2.45**	**2.27**		**7.72**	**6.81**		**6.53**	**6.69**	
Y_2	R_1	3.07	2.52	**2.79**	2.88	2.64	**2.76**	11.80	8.38	**10.10**	10.16	8.16	**9.16**
	R_2	2.98	2.90	**2.94**	2.67	2.54	**2.60**	8.48	7.96	**8.22**	7.08	6.31	**6.69**
	Mean	**3.03**	**2.71**		**2.77**	**2.59**		**10.14**	**8.17**		**8.62**	**7.23**	
Y_3	R_1	2.27	2.37	**2.32**	2.32	2.35	**2.33**	8.28	12.11	**10.19**	6.33	9.92	**8.12**
	R_2	2.47	2.55	**2.51**	2.30	2.70	**2.50**	11.30	10.23	**10.76**	8.99	7.62	**8.31**
	Mean	**2.37**	**2.46**		**2.31**	**2.53**		**9.79**	**11.17**		**7.66**	**8.77**	
Mean	R_1	2.58	2.25		2.47	2.34		8.84	9.16		7.35	8.26	
Mean	R_2	2.67	2.63		2.56	2.59		9.60	8.28		7.86	6.87	
L.S.D. at 0.05													
Y× R		0.14			0.21			1.10			0.73		
Y× B		0.22			0.22			1.79			1.01		
R× B		0.18			0.18			1.46			0.82		
Y× R × B		0.31			0.31			2.54			1.43		
		Level 1	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**	**Level 1**	**Level 2**	**Level 3**
Mean of Y		2.31	2.87	2.41	2.36	2.68	2.42	7.26	9.16	10.48	6.61	7.93	8.22
Mean of R		2.41	2.65		2.40	2.57		8.99	8.94		7.81	7.36	
Mean of B		2.62	2.44		2.51	2.46		9.22	8.72		7.60	7.57	
L.S.D. at 0.05													
Y		0.08			0.07			0.38			0.90		
R		0.08			0.12			0.63			0.42		
B		0.12			0.12			1.03			0.58		

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

Mahfouz and Sharaf El-din 2007 and El-Shazly, 2010). Nitrogen fixers and phosphate solubilizers contribute through biological fixation of nitrogen, solubilization of fixed nutrients and enhanced uptake of plant nutrients, (Gupta *et al*., 2015).

The present results are in agreement with those reported by Selim *et al.* (2013) on fennel plant, Ramadan and Ragab (2015) on caraway and El-Naggar *et al.* (2015) on basil, showed that, yeast application significantly increased vegetative growth characters, oil production and chemical compositions.

Darzi (2012) on coriander mentioned that, nitrogen fixing bacteria (*Azotobacter*, A*zospirillum* alone and together) showed significant effects on umbel number/ plant and seed yield. The maximum seed yield was obtained with *Azospirillum* inoculation. Darzi *et al.* (2012) on anise, demonstrated that, phosphate solubilizing bacterium showed significant effects on umbel number/plant and seed yield. Ghilavizadeh *et al.* (2013) found that, the highest seed yield, essential oil content and essential oil yield of ajowan plant were obtained by using a mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum*. Habibi and Talaei (2014) on ajowan, showed that, the greatest plant highest, seed yield and essential oil yield were obtained by a treatment of biological phosphate (*Pseudomonas putida*) + chemical phosphorus (50 kg./ha P_2O_5).

3.3. Nitrogen, phosphorus, potassium and total carbohydrates content

Data in Table (5) clearly indicated that, concerning treating ajowan plants with yeast as a foliar spray treatment (Y_1), the highest nitrogen content in fruits was obtained with the first level of rock phosphate (R_1) and *A. brasilense* + *B. megatherium* (B_2), but, when using the second level of rock phosphate (R_2), the highest nitrogen content was obtained with *A. chroococcum* + *B. megatherium* (B_1). Meanwhile, when using yeast as a soil drench treatment (Y_2) or a soil drench + foliar spray (Y_3), the highest nitrogen content was obtained with *A. brasilense* + *B. megatherium* (B_2) regardless the level of rock phosphate.

Concerning phosphorus content, when using yeast as a foliar spray treatment (Y_1) or a soil drench treatment (Y_2) with treating the plants by *A. chroococcum* + *B. megatherium* (B_1) led to the highest content of phosphate regardless the level of rock phosphate. But, using the yeast as a soil drench + foliar spray (Y_3) with *A. brasilense* + *B. megatherium* (B_2) led to the highest content of phosphorus regardless the level of rock phosphate.

As far as the potassium content, the highest content was always with treating plants by *A. brasilense* + *B. megatherium* (B_2) compared with *A. chroococcum* + *B. megatherium* (B_1), also, with 300 kg/fed. rock phosphate (R_2) compared with 200 kg/fed. rock phosphate (R_1).

Regarding the total carbohydrates content, the lowest content obtained with using yeast as a soil drench treatment (Y_2), but using foliar spray treatment (Y_1) or a soil drench + foliar spray (Y_3) led to the highest content of total carbohydrates, meaning that, the foliar spray method is associated with increasing the total carbohydrates content. In both seasons, the greatest total carbohydrates percentages (39.70 and 39.03 % in the first and second seasons, respectively) resulted from the treatment $Y_1R_1B_2$. It can be seen that, this treatment gave in both seasons less the volatile oil percentage, this may be due to the accumulation of carbohydrates and its non-entry into the metabolic processes which ends the composition of the volatile oil.

Generally, the previous results can be summarized as follows the highest values of total carbohydrates content resulted from using, the soil drench of yeast treatment plus the rock phosphate at the rate 300 kg/fed., or adding yeast by foliar spray treatment or using foliar spray plus drench soil treatments with rock phosphate at 200 kg/fed.

From the above results, it is clear that, yeast has a major role in increasing the absorption of nutrients, thus increasing its content in plants. This was supported by Ramadan and Ragab (2015) they revealed that, active dry yeast application resulted in increasing chemical constituents (N, P, K, Ca, Mg, Fe, Zn and Mn % in shoot and root), total carbohydrates in caraway plant. Also, bio-fertilizers lead to increased absorption of nutrients through nitrogen fixation and phosphate solubilization which was confirmed by Wu *et al*. (2005), (El Ghadban *et al*. 2006), Mahfouz and Sharaf El-din, (2007) and Gupta e*t al*. (2015).

Table (5): Effect of yeast, rock phosphate and bio-fertilizer on N, P, K and total carbohydrates content of Ajowan (*Trachyspermum ammi*) plants, during 2012 and 2013 seasons.

Treatments			N %		P %		K %		Total carbohydrates %	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Y_1	R_1	B_1	1.03	0.69	0.46	0.51	0.75	0.72	34.90	34.03
Y_1	R_1	B_2	1.37	1.03	0.45	0.46	0.78	0.75	39.70	39.03
Y_1	R_2	B_1	2.74	2.40	0.41	0.57	0.75	0.71	32.73	32.85
Y_1	R_2	B_2	0.86	1.37	0.37	0.56	0.85	0.78	36.40	33.63
Y_2	R_1	B_1	1.03	1.37	0.45	0.46	0.67	0.72	19.35	21.83
Y_2	R_1	B_2	1.54	3.09	0.39	0.39	0.75	0.79	17.08	16.18
Y_2	R_2	B_1	1.03	1.37	0.45	0.45	0.73	0.75	22.43	21.75
Y_2	R_2	B_2	1.37	3.09	0.43	0.42	0.75	0.82	21.09	20.80
Y_3	R_1	B_1	1.03	1.37	0.41	0.49	0.60	0.64	30.45	31.03
Y_3	R_1	B_2	1.51	1.72	0.43	0.52	0.75	0.75	35.73	29.03
Y_3	R_2	B_1	1.72	2.06	0.45	0.56	0.85	0.81	30.10	29.60
Y_3	R_2	B_2	1.72	2.23	0.48	0.57	0.89	0.82	30.53	29.70

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

3.4. Analysis of Ajowan volatile oil components by GC-MS

Data represented in Table (6) showed the results obtained by using Gas chromatography/mass spectrometry (GC-MS) analysis for four treatments. Selected four volatile oil samples to discretion of the components, representing ($Y_2R_1B_1$) treatment, which caused the highest volatile oil percentages, and ($Y_1R_1B_2$) treatment, which obtained the least volatile oil percentages. Also showing the highest volatile oil percentages resulted from three methods of application of the yeast, foliar spray method ($Y_1R_2B_1$), a soil drench method ($Y_2R_1B_1$) and a soil drench + foliar spray methods ($Y_3R_2B_2$). The samples of the essential oil during the second season were subjected to (GC-MS) analysis.

Thirty compounds were identified, the main compounds of essential oil were P-cymene, γ-terpinene, thymol and α-pinene, which represent from 97.38 - 98.12 % of Ajowan oil.

It can be seen from ($Y_1R_1B_2$), that the major component was γ-terpinene (51.66 %), followed by P-cymene (23.75 %), thymol (18.03 %) and α-pinene (3.94 %). Treated ajowan plants by ($Y_1R_2B_1$), the major component was P-cymene (46.35 %), followed by γ-terpinene (25.44 %), thymol (23.66%) and α-pinene (2.03%). Also, when using ($Y_2R_1B_1$) treatment, the major component was P-cymene (45.83%), followed by γ-terpinene (30.19%), thymol (18.96%) and α-pinene (3.14%). Meanwhile, treated ajowan plants by ($Y_3R_2B_2$), the major component was γ-terpinene (39.97 %), followed by thymol (30.43%), P-cymene (23.35 %) and α-pinene (3.67 %). It should be noticed that, this treatment recorded a high content of phosphorus and potassium in fruits.

Comparing the four samples, it was clear that, always with the bio-fertilizer B_1 (*A. chroococcum* + *B. megatherium*), the main component is P-cymene (45.83 - 46.35 %) compared with B_2 (*A. brasilense* + *B. megatherium*) which gave (23.35 - 23.75 %).

Also, with the high level of rock phosohate R_2 (300 kg/fed.) it was noticed that, the high content obtained from thymol (23.66 – 30.43 %) compared with R_1 (200 kg/fed.) which gave (18.03 - 18.96 %).

From the above mentioned results, it is clear that, fertilization by rock phosphate has a powerful effect on volatile oil components of the oxygenated compounds and this was confirmed by Hendawy *et al.* (2010) on thyme they found that, the highest value of oxygenated compounds was obtained from plants received rock phosphate at 150 kg/fed. compared with the control treatment.

Table (6): Chemical composition of the essential oils of four treatments using GC-MS

No.	Compound name	$Y_1R_1B_2$	$Y_1R_2B_1$	$Y_2R_1B_1$	$Y_3R_2B_2$
1	α -Thujene	-	0.14	0.18	0.65
2	l-Phellandrene	0.50	-	-	-
3	α -pinene	**3.94**	**2.03**	**3.14**	**3.67**
4	α -Myrcene	-	0.77	0.46	0.75
5	P-Cymene	**23.75**	**46.35**	**45.83**	**23.35**
6	4-Terpinenyl acetate	-	-	-	0.12
7	γ -Terpinene	**51.66**	**25.44**	**30.19**	**39.97**
8	Cis Sabinene hydrate	0.11	0.04	0.04	0.05
9	α -terpinolene	-	-	0.13	-
10	Cyclohexene,1-methyl-4-(1-methylethylidene)	-	0.09	-	-
11	p-Menth-2-en-1-ol, trans-	-	0.06	0.29	0.08
12	trans-p-Mentha-2,8-dienol	-	0.23	-	-
13	trans-2-Caren-4-ol	0.25	-	-	0.23
14	α -Terpineol	-	0.11	0.08	0.10
15	Thymol	**18.03**	**23.66**	**18.96**	**30.43**
16	Bicyclo[3.1.0]hexane-6-methanol,2-hydroxy-1,4,4-trimethyl-	-	0.08	-	-
17	Piperitenone	-	-	0.04	-
18	Verbenone	0.09	-	-	-
19	Muurola-4 (14),5-diene, cis-	-	-	0.05	-
20	2-Tridecanone	0.21	0.23	-	0.07
21	Caryophyllene oxide	0.08	0.07	-	-
22	3-Methoxy-6-oxaestra-1,3,5(10),8(9),14(15)-pentaen-17-one	-	0.06	-	-
23	4-Fluorobiphenyl-d(5)	-	-	-	0.13
24	Silane, trimethylphenyl-(CAS)	0.17	-	-	-
25	Homotrypticene	0.08	0.06	-	-
26	α -Isomethyl ionone	-	0.07	-	-
27	Coraxeniolide-B	0.16	0.13	0.16	0.10
28	4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	0.09	-	-	-
29	1,8-Dimethoxy-3-methylanthracene-9,10-dione	0.21	-	-	-
30	1-Methoxy-2-mesitylacenapthylene	0.10	-	-	-
	Total	99.52	99.62	99.55	99.70
	Unknown	0.48	0.38	0.45	0.30

3.5. Microbial determinations in Ajowan plants rhizosphere soil.

Total microbial counts: Data presented in Table (7) showed the changes in microbial counts in rhizosphere of Ajowan. The initial total microbial counts in the soil before cultivation were 93×10^5 cfu/g dry soil. Counts tended to increase with different treatments. The highest mean counts were associated with $Y_3R_2B_1$ being 188 $\times10^5$ cfu. /g dry soil. However, slight differences in the total microbial counts were abserved with different treatments. The enhancement in microbial activity is good parameters for soil improvement indices. Plant growth promoting rhizobacteria (PGPR) like *Azotobacter* and *Azospirillum* produces growth promoting substance which enhances plant growth, lateral roots and root hairs which increase nutrient absorbing surface, (Metin *et al.*, 2014).

Azotobacter densities: Inoculation with heavy suspension of *Azotobacter* led to a rather pronounced increase in densities recorded at the 1^{st} and the 2^{nd} seasons. The effect diminished with the prolongation of the plant growth period. The lowest densities of *Azotobacter* (Table 7) were recorded with $Y_1R_1B_2$ and a slight difference in *Azotobacter* densities was recorded with the different treatments. The promoting effect due to the application of *A. chroococcum* was not only due to nitrogen fixation, but also to the production of plant growth promoting

Table (7): Effect of yeast, rock phosphate and biofertilization on microbial determinations of Ajowan (*Trachyspermum ammi*) plants rhizosphere during 2012 and 2013 seasons.

Treatments			Total microbial counts $\times10^5$cfu/gdry soil		Azotobacter densities($\times10^3$cells/g dry soil		Azospirillum counts $\times10^3$cells/g dry soil		PDB counts ($\times10^2$cfu/gdry soil)	
			1^{st} season	2^{nd} season	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season	1^{st} season	2^{nd} season
Y_1	R_1	B_1	155	184	84	90	108	113	67	71
Y_1	R_1	B_2	142	165	61	65	91	97	63	68
Y_1	R_2	B_1	168	193	95	102	116	122	74	80
Y_1	R_2	B_2	137	150	84	86	104	116	72	81
Mean			150.5	173	85.5	89.5	104.8	112	69	75
Y_2	R_1	B_1	142	180	65	69	98	102	43	48
Y_2	R_1	B_2	128	153	79	80	79	83	39	41
Y_2	R_2	B_1	129	168	72	75	90	103	55	62
Y_2	R_2	B_2	122	151	66	73	81	94	46	55
Mean			130.3	163	66	71.3	87	96.5	45.8	51.5
Y_3	R_1	B_1	175	219	98	107	137	159	84	87
Y_3	R_1	B_2	178	194	91	103	119	140	82	86
Y_3	R_2	B_1	188	225	102	119	185	191	86	94
Y_3	R_2	B_2	174	185	97	116	157	162	79	82
Mean			178.8	205.8	97	111.3	149.5	163	82.8	87.3
L.S.D. at 0.05			7.4		4.3		6.5		1.4	

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

substances, production of amino acids, organic acids, vitamins and antimicrobial substances which increase soil fertility, microbial community and plant growth, Singh *et. al.,* 2013.

Azospirillum densities: data in Table (7) showed the estimation of *Azospirillum* densities in rhizosphere area of Ajowan plants, *Azospirillum* densities tended to increase at the 1^{st} and 2^{nd} seasons. The highest counts were recorded with $Y_3R_2B_1$ compared with the other treatments. Biofertilization with *Azospirillum* increased its densities in mixtures treatment compared with the control treatment. These results agreed with there obtained by Abd El Gawad (2008).

Phosphate Dissolving Bacteria counts (PDB): Concerning phosphate dissolving bacterial counts, inoculation treatments with *A. chrococcum* and *A. brasilense* gave the highest mean value for counting at the 1^{st} and the 2^{nd} seasons for different treatments These results are in agreement with those obtained by Ashrafuzzaman *et al*., 2009) who reported that, inoculation with the plant growth promoting rhizobacteria (*Azotobacter, Azospirillum, Bacillus megaterium* and *Rhizobium*) had a stimulation effect on the population of rhizosphere microorganisms by increasing their numbers by more than 50% from the intial.

3.6. Antimicrobial activity of Ajowan essential oil against some common pathogenic microbes

Ajowan, one of the aromatic seed spices, is generally used for medicinal purposes. There are near 30 compounds in Ajowan, but Thymol, the most important and main phenolic compound in Ajowan, has been reported to be a germicide (anti-bacterial and anti-fungal) and an antispasmodic agent. Also, ethanol and acetone extracts were found to be effective against many bacteria, including *Pseudomonas species*, *E. coli*, *Bacillus subtilis* and *S. aureus*, (Usha *et al*., 2012). In previous studies the antibacterial activity was assessed, using agar well diffusion method and MIC.

The current results revealed that, the bacterial growth, including *Escherichia coli*, *S. typhi*, *Candida albicans* and *S. aureus* were inhibited by Ajowan oils which had antimicrobial activity on four different bacteria. Antimicrobial activity of Ajowan essential oil (extracted from 2^{nd} season) against some human and plant pathogenic microbes was detected and represented in (Table 8). Application of biofertilizers increase the antagonistic activity of Ajowan essential oil against some pathogenic microbes. This result is compatible with the finding of (Mood *et al*., 2014).

Table (8): Antimicrobial activity of Ajowan (*Trachyspermum ammi*) essential oil against some pathogenic bacteria.

Treatments			*E. coli*	*S. typhi*	*S. aureus*	*C. albicans*
			Inhibition zone diameter cm			
Y_1	R_1	B_1	4.8	3.4	5.8	5.2
Y_1	R_1	B_2	2.6	3.1	3.4	5
Y_1	R_2	B_1	3.7	2.9	5.1	4.9
Y_1	R_2	B_2	3.1	2.4	3.5	4.8
Y_2	R_1	B_1	3.2	2.2	5.3	4.9
Y_2	R_1	B_2	1.8	1.6	3.9	4.1
Y_2	R_2	B_1	3.5	2.5	4.5	4.5
Y_2	R_2	B_2	1.7	2	3.8	2.7
Y_3	R_1	B_1	**5.2**	**3.7**	**6.3**	**6.1**
Y_3	R_1	B_2	4.7	3.3	4.8	4.7
Y_3	R_2	B_1	3.4	3	6.2	5.3
Y_3	R_2	B_2	3.1	3	4.2	4.6

Y_1, Y_2 and Y_3 = yeast as a foliar spray (Y_1), soil drench (Y_2) and as a soil drench + foliar spray (Y_3). B_1 and B_2 = *A. chroococcum* + *B. megatherium* var. *phosphaticum* (B_1) and *A. brasilense* + *B. megatherium* var. *phosphaticum* (B_2). R_1 and R_2 = rock phosphate (200 and 300 kg/fed., respectively)

Recommendations

To obtain the best results from fruit, weight/plant, fruit yield/fed. and oil yield/fed. of ajowan plants, it is recommended that, treating ajowan plants with $Y_3R_1B_2$ [both soil drench and foliar spray treatments plus 200 kg/fed. rock phosphate plus using mixtures of bio-fertilizers *A. brasilense* + *B. megatherium*]. To obtain the highest volatile oil percentages, are treated the plants with $Y_2R_1B_1$ [adding yeast by drench treatment (Y_2) plus 200 kg/fed. rock phosphate plus using mixtures of bio-fertilizers (*A. chroococcum* + *B. megatherium*)]. Also, it is clear that, the highest thymol content (30.43%) in volatile oil resulted from $Y_3R_2B_2$ treatment.

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تأثير التسميد الحيوى وصخر الفوسفات على نمو وإنتاج الزيت لنبات النخوة الهندى تحت ظروف سيناء

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ملخص

تمت هذه الدراسة خلال موسمين متتالبين 2013/2012 ، 2014/2013 بمحطة بحوث المغارة بشمال سيناء التابعة لمركز بحوث الصحراء لدراسة تأثير التسميد الحيوى [خليط من الازوتوباكتر والباسيلس (B_1) ، وخليط من الازوسبيرليم والباسيلس (B_2)] والخميرة [اضافه بالرش الورقي (Y_1)، اضافه ارضيه (Y_2)، اضافه ارضيه + رش ورقي (Y_3)] والتسميد بصخر الفوسفات [بمستويين 200، 300كجم/فدان (R_1) و (R_2) على الترتيب] على نمو وإنتاجية الزيت لنبات النخوة الهندى. كان تصميم التجربة قطع منشقة مرتين بحيث تكون الخميرة في القطع الرئيسية وصخر الفوسفات في القطع المنشقة والتسميد الحيوى في القطع تحت المنشقة. اظهرت النتائج ان الأضافة الأرضية للخميره أدت الى زيادة معنويه في ارتفاع النبات، عدد النوارات/نبات، نسبة الزيت الطيار. أدت الأضافه الأرضيه والرش الورقى معاً الى زيادة معنوية في الوزن الطازج والجاف/نبات، وزن الثمار/نبات، محصول الثمار/فدان. بالنسبه لصخر الفوسفات فان المستوى الثانى (300كجم/فدان) أدى الى زيادة في ارتفاع النبات ونسبة الزيت الطيار، بينما أدى المستوى الاول (200كجم/فدان) الى زيادة في عدد النورات/نبات، الوزن الطازج والجاف/نبات، وزن الثمار/نبات، محصول الثمار/فدان، محصول الزيت/فدان. بالنسبه للتسميد الحيوى فأن استخدام خليط الازوسبيرليم والباسيلس (B_2) أدى الى الحصول على افضل النتائج من ارتفاع النبات، عدد النورات/نبات، وزن الثمار/نبات، محصول الثمار/فدان. بينما خليط الازوتوباكتر والباسيلس (B_1) احدث زياده معنويه فى الوزن الطازج والجاف/نبات، نسبة الزيت الطيار، محصول الزيت/فدان. تم الحصول على اعلى نسبة زيت طيار من المعاملة $Y_2R_1B_1$ (الأضافة الأرضية للخميرة+ 200كجم/فدان صخر فوسفات+ التسميد الحيوى بالازوتوباكتر والباسيلس)، وكانت مكونات الزيت الطيار الناتج منها البارا-سيمين 45.83%، يليها جاما-تربينين 30.19%، ثيمول 18.96%، الفا-بينين 3.94%. بينما نتج أعلى محصول ثمار/فدان من معاملة النباتات بـ $Y_3R_1B_2$ (اضافة ارضية+ رش ورقى للخميره معاً +200كجم/فدان صخر فوسفات+ التسميد الحيوى بالازوسبيرليم والباسيلس).

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