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Effect of Bio Fertilization and Phosphate Active Substances of Fennel Seed (*Foeniculum vulgare* Mill)

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***Abstract*:** A factorial experiment was conducted in Al-Watifiyah region of Babylon during 2018 with the aim to observe the effect of bio-fertilizers and phosphate fertilizers on the medicinally effective compounds of fennel seed. The factorial experiment was conducted included the use of four levels of bio-fertilizers, (without addition, mycorrhiza, and *Azotobacter* spp, Mycorrhiza + *Azotobacter* spp). It were symbolized by (A0, A1, A2, A3),The second was the use of four levels of phosphate fertilizer P_2O_5 (0, 40, 60 and 80 kg.ha^{-1}) which were symbolized by (B0,B1,B2,B3). The *Azotobacter* spp was significantly superor with average for limonene ,Anethole ,α-Fenchone as 126.67,293.87 ,77.79 mg.mL^{-1}) respectively, while treatment 60 kg.ha-1 phosphate was significantly superior and gave the highest average for limonene, Anethole, α-Fenchone, ferulic, gallic amounted to 123.98, 275.74, 60.11, 54.66 and 215.65 mg.mL^{-1} respectively, The interaction treatment Azotobacter spp+ phosphate 60 kg.ha-^{1} excelled in most of the studied traits.

***Keywords*:** Fennel, Bio-fertilizer, Phosphate fertilizer

Many medicinal plants can supply inexpensive raw materials used in the manufacture of pharmaceutical compounds to treat many diseases, Fennel IS classified among the medicinal plants that occupied a prominent position in agricultural and industrial production, and therefore it received great care in many producing countries, where it is a natural source of the active substances or so-called secondary metabolites compounds that are involved in the preparation of various medicines. Oils are considered to be one of the most important compounds in the Fennel and are one of the metabolites of secondary metabolism that circulate freely through cell membranes. The carbonyl group is the main component of the oil and is also rich in a group of aldehydes Oils are also one of the most important antioxidants that give the plant great importance and give it high medicinal importance. In addition to being anti-microbial and pathogenic and also has anti-cancer activity (Sharapov et al 2017). Fennel seeds contain varying proportions of carbohydrates, alkaloids, phenols and flavonoids and is also a rich source of dietary fiber and proteins and also contains high levels of important nutrients in addition to containing some antioxidant compounds and microbesand is a food supplement in many plant foods with daily consumption as it is not harmful to health in general (Fennel 2018). The research aims to study the effect of different types of bio fertilizers and phosphate fertilization to increase the production of compounds with a medicinal properties.

MATERIAL AND METHODS

A factorial field experiment was conducted during 2018-2019 in Al-Watifiya region with two factors, the first factor, bio fertilizers at four levels, (without adding, the fungal vaccine *Mycorrhiza*, bacterial vaccine *Azotobacter* spp., *Mycorrhizal* fungal vaccine + *Azotobacter*) and were symbolized by A0, A1, A2, A3. The strength of the fungi was 40 spore.g^{-1} and the vaccine strength for bacteria was 2.8 * 10^{10} per gram. The second factor was the use of four levels of phosphate fertilizer P2O5 (0, 40, 60 and 80 kg.ha^{-1}) and which were symbolized by B0, B1, B2, B3. The crop was sown on 15 October 2018.All the agronomc pratices were followed. The bio-fertilizer was obtained from the Agricultural Research Department of the Ministry of Science and Technology. The seeds were soaked in it for an hour, and the gum Arabic material was applied to fix it on the surface of the seeds. After a month of sowing, the seeds with the bacterial vaccine were reinforced again. The process of thinning was done after the height of the plants was 10 cm. The crop was harvested on 20 May, 2019. The analysis of oil compounds involving limonene Anethole, α-Fenchone, Ferulic, Gallic was done at the end of the growing season from five plants randomly selected in each unit. Soil were anlysed for physical and chemical properties (Table 1).

Extract of volatile oils: High Performance Liquid Chromatography (HPLC) device type (LC-10A shimadzu) was used to estimate the volatile oil of Fennel (*Foeniculum vulgare* Mill) (Badifu 1991). As the device was injected with an oil sample from each of the different treatments, the peaks

of the sample were matched with the peaks of the standard model, and then their quantities were calculated.

Estimating of phenolic and flavonoids: The high-performance chromatography device (HPLC) of Shimadzu company was used. Tests were conducted for the active substances at Ministry of Science and Technology, where the compounds were separated and their quality and quantity were compared to the standard materials on the separation column under the same conditions and according to the concentration separated in the sample (Al-Oubaidi and Al-Khafagi 2018).

Concentration model for sample

$$(ppm) = \frac{\text{Standard model concentration} \times \text{Sample beam space}}{\text{Standard model beam space}} \times \text{Number of times Titration}$$

RESULTS AND DISCUSSION

The *Azotobacter* spp treatment (A2) was significantly excelled gave suprior with highest average of limonene substance (126.67 mg.mL^{-1}) (Table 6) as compared with control (17.71 mg.mL^{-1}). The application of 60 kg.ha^{-1} phosphate (B2) achieved the highest average of 123.98 mg.mL^{-1}, while the lowest average was in control treatment (B0) (62.63 mg.mL^{-1}). The interaction treatment *Azotobacter* spp+ phosphate 60 kg.ha^{-1} (A2B2) achieved the highest average of 179.04 mg.mL^{-1} as compared to the control treatment A0B0 with lowest average of 0.04 mg.mL^{-1}.

The Azotobacter spp treatment A2 significantly superior with highest average of Anethole substance (293.87 mg.mL^{-}

Table 1. Physical and chemical properties of field soils

Traits		Units	Values
pH			7.9
EC		ds.m^{-1}	2.8
Dissolved positive ions	Ca	gm.kg^{-1}	9.3
	Na	gm.kg^{-1}	5.9
	K	gm.kg^{-1}	0.31
Total nitrogen		gm.kg^{-1}	93
Organic matter		gm.kg^{-1}	19.2
soil separates	sand	gm.kg^{-1}	238.7
	Silt	gm.kg^{-1}	362.1
	Clay	gm.kg^{-1}	399.2
Soil Texture			Clay loam
Bulk density		gm.m^{-3}	1.42

Table 2. Chromatographic conditions for the active oils using the HPLC device

Separation column	Type (C-18), its dimensions (50 * 2.0mm.I.D), the syringe size is 3 micromol
Mobile phase	0.1M ammonium acetate Acetonitrile (20:80 V/V)
Flow speed	1.1 ml.min $^{-1}$
Reagent type	Ultraviolet radiation below the wavelength of 285 nanometers

Table 3. Detention time and beam area for oil active compounds

Seq	Subjects	Retention time	Area
1	Limonene	4.72	284132
2	α- Fenchone	6.62	360069
3	Anethole	8.49	312935

Table 4. Chromatographic conditions for active substances using the HPLC

Separation column	Type (C-18), its dimensions (50 * 2.0mm.I.D), the syringe size is 3 micromol
mobile phase	Solvent A 0.1% phosphoric acid Solvent B (6;3;1V/V) Acetonitrile: metand : 0.1% phosphoric acid
Flow speed	1.2 ml.min $^{-1}$
reagent type	Ultraviolet radiation below the wavelength of 280 nanometers

Table 5. Detention time and beam area for active compounds

Seq	Subjects	Retention time	Area
1	Gallic	2.73	126397
2	Caffeic acid	3.56	193575
3	Ferulic acid	5.52	213090
4	Kaempferol	10.38	167916

Table 6. Effect of bio and phosphate fertilizers on limonene (mg mL^{-1}) of fennel seed

Bio fertilizers	Phosphate fertilizers				Average
	B0	B1	B2	B3	
A0	0.04	22.03	32.64	16.12	17.71
A1	90.43	113.19	154.46	99.32	114.35
A2	70.90	136.60	179.04	120.14	126.67
A3	89.13	83.82	129.76	89.02	97.93
Average	62.63	88.91	123.98	81.15	
L.S.D (p=0.05)	A: 5.20	B :5.20	AB: 10.46		

^{1}) (Table 7). compared with control (A0) (45.51 mg.mL^{-1}.0. The application of 60 kg.ha^{-1} phosphate (B2) achieved the highest average of 275.74 mg.mL^{-1}, while the lowest average was in contro(B0) (122.31 mg.mL^{-1}). The interaction treatment *Azotobacter* spp+ phosphate 60 kg.ha^{-1} (A2B2) achieved the highest average for this trait (587.53 mg.mL^{-1}) compared to the control t A0B0 wth lowest average of 41.13 mg.mL^{-1}

The *Azotobacter* spp treatment (A2) was significantly superion with highest average of α-Fenchone (77.79 mg.mL^{-1}) (Table 8) as compared with control (A0) (16.02 mg.mL^{-1}). The application of 60 kg.ha^{-1} phosphate (B2) achieved the highest average of 60.11 mg.mL^{-1}, while the lowest average in control treatment (B0) (30.09 mg.mL^{-1}). The interaction treatment *Azotobacter* spp+ phosphate 60 kg.ha^{-1} (A2B2)achieved the highest average for this trait (110.72 mg.mL^{-}1) as compared to the control treatment A0B0 that achieved the lowest average of 4.98 mg.mL^{-1}.

The mycorrhiza (A1) was significantly r superior with the highest average of ferulic acid (53.22 mg.mL^{-1}) (Table 9). Compared with control (A0) with average of 20.23 mg.mL^{-1}.while treatment 60 kg.ha^{-1} phosphate (B2) achieved the highest average of 54.66 mg.mL^{-1}, while the lowest average was recorded in control treatment (B0) of 24.38 mg.mL^{-1}. The interaction treatment Mycorrhiza + Azotobacter spp + phosphate 80 kg.ha^{-1}(A3B3) achieved the highest average of 64.65 mg.mL^{-1}compared to the control treatment A0B0 with lowest average of 9.15 mg.mL^{-1}.

The mycorrhiza treatment A1 gave significantly highest average ofgphenolic substance (208.48 mg.mL^{-1}) (Table 10) as compared with control (A0) of with average of 104.80 mg.mL^{-1}. The treatment 60 kg.ha^{-1} phosphate (B2) achieved the highest average of 215.65 mg.mL^{-1}, while the lowest average was recorded in control (B0) (119.07 mg.mL^{-1}). The interaction treatment *Azotobacter* spp+ phosphate 60 kg.ha^{-1} (A2B2) recorded the highest average for this trait (244.06 mg.mL$^{-1)}$ as compared to the control A0B0 with lowest average of 44.94 mg.mL^{-1}.

The bio fertilizer positive effect is due to the increase in the absorption efficiency of nutrients `Aand the improvement of the root growth indicators that worked to increase the area of absorption by the root capillaries, which was clearly reflected in the increase of the active compounds. The increase in the proteins and carbohydrates as a result of the of soil microorganisms was reflected in plant tissues, which consequently improved the qualitative level of growth by providing balanced nutrients that improved their growth effect. The phosphorus significantly affected the manufacture and accumulation of proteins and carbohydrates which have a positive effect in increasing secondary metabolism compounds in different plant parts

Table 7. Effect of bio and phosphate fertilizers on Anethole (mg.mL^{-1}) of fennel seed

Bio fertilizers	Phosphate fertilizers				Average
	B0	B1	B2	B3	
A0	41.13	46.46	46.27	48.20	45.51
A1	174.66	198.69	246.54	183.09	200.75
A2	128.88	238.95	587.53	220.13	293.87
A3	144.58	196.91	222.62	158.98	180.77
Average	122.31	170.25	275.74	152.60	
L.S.D	A: 74.431	B : 74.431	AB: 37.216		

Table 8. Effect of bio and phosphate fertilizers on content from α-Fenchone (mg.mL^{-1}) of Fennel seed (Foeniculum vulgare Mill)

Bio fertilizers	Phosphate fertilizers				Average
	B0	B1	B2	B3	
A0	4.98	18.52	26.32	14.26	16.02
A1	51.64	53.19	63.28	55.63	55.94
A2	32.18	92.63	110.72	75.64	77.79
A3	31.56	39.01	40.10	32.35	35.76
Average	30.09	50.84	60.11	44.47	
L.S.D (p=0.05)	A: 4.708	B : 4.708	AB: 9.415		

Table 9. Effect of bio and phosphate fertilizers on ferulic acid content (mg.mL^{-1}) of fennel seed

Bio fertilizers	Phosphate fertilizers				Average
	B0	B1	B2	B3	
A0	9.15	18.99	27.05	25.75	20.23
A1	31.69	47.99	64.87	56.58	50.28
A2	26.97	44.32	62.98	58.24	48.13
A3	29.70	54.76	63.75	64.65	53.22
Average	24.38	41.52	54.66	51.30	
L.S.D (p=0.05)	A: 4.058	B : 4.058	AB: 8.116		

Table 10. Effect of bio and phosphate fertilizers on gallic phenolic substances (mg.mL^{-1}) of Fennel seed (Foeniculum vulgare Mill)

Bio fertilizers	Phosphate fertilizers				Average
	B0	B1	B2	B3	
A0	44.94	98.91	148.97	126.39	104.80
A1	160.12	221.81	237.85	214.13	208.48
A2	132.97	210.69	244.06	203.76	197.87
A3	138.23	217.60	231.70	227.18	203.68
Average	119.07	187.25	215.65	192.86	
L.S.D (p=0.05)	A: 49.617	B : 49.617	AB: 99.234		

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