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The Effect of Organic and Bio-Fertilization on some Physical and Chemical Properties of *Calendula officinalis* L. Plant

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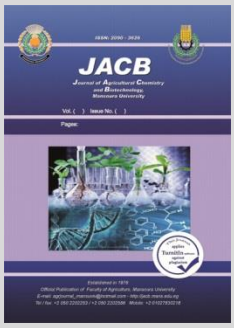

ABSTRACT

The effect of organic fertilization (using three rates of poultry manure) and biological fertilization (using a nitrogen-fixing bacterial strain *Azospirillum brasilense*, mycorrhizal (VAM) fungi and another bacterial strain dissolving phosphate(*Bacillus megatherium*) on *Calendula officinalis* L. plant during two consecutive seasons were studied. The results showed that all organic and biological fertilization treatments improved different vegetative growth characteristics and enhanced flowering factors. The various chemical components (Chlorophyll A&B) and carotenoids, as well as an increase in carbohydrates, minerals percentage and oleanolic acid. Hence, organic and biological fertilizers can be used as an alternative to chemical fertilization, which reduces production costs and pollution the agricultural environment and leads to improvement of the natural and chemical properties.

***Keywords*:** *Calendula officinalis* L., organic manure, *Azospirillum brasilense*, *Bacillus megatherium*, chlorophyll A&B, carotenoids and oleanolic acid.

INTRODUCTION

Calendula officinalis L., plant is an important ornamental plant belonging to family *Asteraceae*. It is cultivated for outdoors winter annual landscaping as a source of color in the gardens and cut flowers. Moreover, it is considered to be one of the valuable medicinal plants, which contains a lot of compounds such as oleanolic acid, phytosterols, glycosides carotenes (tetraterpenes), salicylic acid, phenolic acid, sugars, flavonoids, bitter principies, saponins, chlorogeneic acid, trace of pyrogallol tannins, fatty acids in the seeds and essential oil of antibiotic activity (Vidal-Ollivier *et al*., 1989). Compounds similar to pyrthins and series of triterpeniods glycosides in the roots (Rizk and Al-Nowaihi, 1989)

Calendula officinalis L., plant is becoming very important cultivated medicinal plant for the local use and export. Chemical nutrients especially phosphorus, nitrogen and potassium are very important for plants, because nitrogen, phosphor partake in structure of several components of the whole plants (protein, hormones, amino acids, enzymes, nucleic acids, fats and regulation of water conditions). However, using intense chemical fertilization cause serious problems on human health by pollution of the whole environmental conditions (soil, air and drainage water) (Sharaf EL-Din *et al*.,2019).The organic fertilizers are utilized for the change of soil texture, supplying nutrients and they are considered save for human health. Organic matter improves the aeration and drainage of compacted soils, the water holding capacity and increases the soil exchange capacity i.e. its ability to absorb nutrients(Bryan and Lance, 1991).The significant effect of bio-fertilizers may be due to the effect of different strain groups and nutrients mobilizing microorganisms which help in availability of metals and their forms in the composted material and increased levels of extractable minerals(EL-Kramany*et al*., 2000).

The objective of this study is to investigate the effect of organic manure and some bio-fertilizers treatments (*Azosperillium*, *Bacillus megatherium* and Mycorrhiza) on vegetative growth, flowering and chemicals composition of *Calendula officinalis* L., plant.

MATERIALS AND METHODS

This investigation was carried out in a private farm in El- Santa,El-Gharbia Governorate, Egypt, during the two successive seasons 2019/2020 and 2020/2021.

The soil used:

Experimental

The soil used was sandy soil. Its chemical and physical characteristics are presented in Table (1).

Table 1. Physical and chemical properties of the experimental soil.

A-Physical properties:-

Soil Mixture	Clay%	Silt%	Fine sand%	coarse sand %
Sand	11.00	6.00	10.10	73.30

B-Chemical properties:-

Chemical analysis	K	Na	Mg	Ca	SO_4	Cl	HCO_3	SP	pH	E.c
Sand	0.40	4.20	2.20	5.10	7.20	3.5	3.00	25.00	7.55	1.25

Plant Material:

Calendula officinalis L., seeds were obtained from Horticulture Research Institute of Medicinal and Aromatic Plants Section, Giza, Egypt, and then sown in the nursery for beds on 5th September for both seasons. Seedlings were transplanted after 45 days at 20 October to the field. The experiment was carried in pots 35cm. Each treatment contained there replicates and each replicate consisted of ten pots.

Bio-Fertilizers:

Cultures of the microorganisms, nitrogen fixer bacteria (*Azospirillum brasilense*) and phosphate solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum*) were isolated and purified also characteristics separately in Microbiol lab. of Botany Dept. Fac. of Agric., Al-Azhar Univ., according to(Abd El-Malek and Ishac, 1968; Dobreiner *et al.*, 1976). But potassium and phosphor solubilizing bacteria (*Bacillus megaterium* var.

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phosphaticum) and Vesicular arbscular mycorrhizal (VAM) fungi were obtained from cultural collection of Agric. Microbiol. Dept. National Research Centre, Egypt. The bio-fertilizers were applied after grown according to mentioned previously authors, each plant was inoculated with 3 ml of bacterial suspension from the three bacteria strains *Azospirillum brasilense* and *Bacillus megaterum* after transplanting. Vesicular arbscular mycorrhizal (VAM) fungi, which contained three effective strains representing [*Glomus etunicatum*, *Glomus intraradices* and *Glomus fasciculatum*] VAM fungi were used for soil inoculation after transplanting containing about 200 VAM spores/plant.

Organic Fertilizer used:

Organic fertilization as poultry manure was provided from a private farm for the production of broiler poultry in El-Santa,El-Gharbia Governorate, Egypt. It was applied at three levels 20, 40 and 60 m³/fed. The chemical analysis of poultry manure is presented in Table (2).

Table 2. Chemical analysis of poultry manure.

Chemical Analysis	pH	E.C. mM	N %	P %	K %	C/N ratio	Fe ppm	Mn ppm	Cu ppm	Zn Ppm
Poultry manure	6.69	7.09	4.1	1.78	1.11	9.3:1	149	179	92	111

The experimental design:

The layout of the experiment was a complete randomized blocks design during the two seasons in three replicates for each treatment with sterile soil.

Treatments:

The fertilization treatments were used as follows:

Control untreated, Poultry manure at (20,40 and 60 m³ / fed.), Azospirillum + (*Bacillus megatheriu)* . (Azos+ Bacillu)), Azospirillum + VAM. (Azos+VAM) and Azospirillum + Bacillus)+ VAM.(Azos+ Bacillus)+VAM).

These treatments were carried out in the two successive seasons. The compost was applied at the level of 7.5 ton / fed. as a carrier to bio fertilizers.

Plant data recorded:

The following data were recorded for the two seasons:

Plant height (cm), as the main plant stem, Number of branches/plant, Dry weight (g/plant),Flowering date, considered at the first opened flowering head, Number of flowering heads/plant, diameter flowering heads (cm.) and flowering period, recorded from the first opened flowering head until the end of the flowering.

Chemical data recorded in plant

a) Chlorophyll :

Both chlorophylls (A and B) and total carotenoids were determined spectrophotometrically according to the methods described by Şükran *et al.*, (1998).

b) Carbohydrates :

Total carbohydrates (%) was determined by using phenol-sulphuric acid method according to (Dubois *et al*., 1956).

c) Nitrogen:

Total nitrogen (%) was determined according to the method described by (A.O.A.C, 1995).

d) Potassium and phosphorus:

Potassium and phosphorus (%)were determined according to (Cottenie *et al*., 1982).

Oleanolic acid content was estimated in the dry flowering heads according to (El-Gaingihi*et al*., 1982)

Statistical analysis:

The statistical analysis was performed according to (Snedecor and Cochran, 1980) using M-state program version 4. analysis of variance (ANOVA) with Duncan's Multiple Range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effect of organic and bio-fertilizers on some vegetative characters.

The data in Table (3) revealed that, in most cases, the studied vegetative growth characters, plant height (cm.), branches number/ plant and herb dry weight (g) /plant of *Calendula officinalis* L. plants were significantly increased in both seasons due to the use of poultry manure and bio-fertilization in comparison to the untreated plants. Corresponding data showed an increase in plant height due to poultry manure treatments in comparison to bio-fertilizers treatments and to the control especially, when poultry manure was used at the rate of 40 and 60 m³/fed., which produced 87.47 and 90.73 cm. in the 1st season, 86.50 and 88.43 cm. in the 2nd season, respectively. However, application of poultry manure at 40 m³/fed. gave the highest values for number of branches (86.73) in the 1st season, while application of poultry manure 60 m³/fed. gave the highest values for number of branches (93.00) in the 2nd season. Data illustrated showed that, the maximum value of herb dry weight (249.36 g/plant in the 1st season) was obtained as a result of poultry manure 60 m³/fed. Moreover, treatments of poultry manure at 20, 40 and 60 m³/fed. gave the highest values of herb dry weight in the 2nd season recording 254.48, 257.35 and 276.94 g/plant, respectively.

Table 3. Effect of poultry manure, *Azospirillum*, *Bacillus* andVAM on vegetative growth characteristics of *Calendula officinalis* L., plants during two seasons.

Treatments	Plant height(cm.)		Branches number/plant		Herb dry weight (g/plant)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	37.33^{d}	38.93^{d}	29.30^{e}	28.67^{f}	84.94^{c}	94.41^{d}
Poultry 20 m³/fed.	72.50^{b}	76.10^{b}	68.37^{bc}	77.67^{c}	210.47^{ab}	254.48^{a}
Poultry 40 m³/fed.	87.47^{a}	86.50^{a}	86.73^{a}	85.33^{b}	234.84^{ab}	257.35^{a}
Poultry 60 m³/fed.	90.73^{a}	88.43^{a}	80.40^{ab}	93.00^{a}	249.36^{a}	276.94^{a}
Azos+B 3 ml each.	67.63^{b}	64.30^{c}	54.12^{cd}	55.67^{d}	201.09^{ab}	154.62^{c}
Azos+VAM 3 ml each.	56.57^{c}	56.60^{c}	45.97^{de}	46.67^{e}	210.36^{ab}	178.71^{bc}
Azos+ B +VAM 3ml each.	66.30^{b}	63.10^{c}	59.03^{cd}	60.33^{d}	186.51^{b}	197.81^{b}

a to f Means having different letters exponents in column are significantly different ($P \leq 0.05$)

The enhancing effects of organic manures on plant height, number of branches and dry weight of herb may be attributed to the role of organic manure in holding moisture and maintaining sufficient pore spaces to permit good air circulation and drainage of the excessive water produced, and composts from residues of plants and animals dropping, is one type of humus, which contributes to the soil fertility (Schachtschable, 1979). Organic fertilizer contains a little or no soluble salt and can be used in high levels without causing root damage, that may occur if the organic fertilizer is utilized to apply corresponding quantities for plant nutrient (Cooke, 1972).

The results take the same line with Hammam (1996) on *Pimpinell aanisum*, El-Sayed *et al.* (2002) on *Origanumm ajoranum*, Helmy and Zarad (2003) on *Borago officinalis*, Atta-Alla*et al.* (2005) on *Capsicum annuum*.

1. Effect of organic and bio-fertilizers on some flowering aspects.

Concerning the effect of organic and bio-fertilizers on flowering characters, it could be noticed that the studied

flowering growth characters; number of flowering heads/plant, flowering head diameter/plant (cm.) (Table (4)), flowering start (days) and flowering period (days) (Table (4) were significantly increased due to the use of organic and bio-fertilization in both seasons in comparison to the unfertilized plants. Data presented in Table (4) showed that, the highest number of flowering heads (36.33) and diameter of flowering head (6.30 cm.) in the 1st season, when poultry manure at 60 m³/fed. was applied. The same treatment also resulted in the highest values number of flowering heads (36.33) and diameter of flowering head (6.63 cm.) in the 2nd season.

Table 4. Effect of poultry manure, *Azospirillum*, *Bacillus* andVAM on number of flowering heads and flower diameter (cm.) of *Calendulaofficinalis*, L. plants during two seasons.

Treatments	Number flowering heads / plant		Diameter flowering heads (cm.)	
	1st season	2nd season	1st season	2nd season
Control	5.00^{c}	5.67^{c}	3.50^{c}	3.87^{c}
Poultry 20 m³/fed.	23.33^{ab}	24.00^{ab}	5.73^{ab}	6.03^{ab}
Poultry 40 m³/fed.	25.00^{ab}	26.00^{ab}	5.23^{b}	5.63^{ab}
Poultry 60 m³/fed.	36.33^{a}	36.33^{a}	6.30^{a}	6.63^{a}
Azos+B 3 ml each.	13.67^{bc}	12.33^{bc}	5.53^{ab}	5.27^{b}
Azos+VAM 3 ml each.	13.33^{bc}	12.00^{bc}	5.60^{ab}	5.43^{b}
Azos+B+VAM 3ml each.	17.33^{bc}	15.67^{bc}	5.30^{ab}	5.17^{b}

a to c Means having different letters exponents in column are significantly different (P ≤ 0.05)

Data in Table (5) reveal that, all applications (poultry manure; 20, 40 and 60 m³ /fed. and bio-fertilizers; Azos+B, Azos+VAM and Azos+B+VAM) started to flowering earlier (43, 43, 41, 43, 43, and 43 days) in the 1st season, respectively, versus poultry manure 30 m³/fed. and Azos+B+VAM which recorded 38 days for both in the 2nd season, decreasing the start of flowering with 7-11 days as compared with the untreated plants. The flowering period extended longer time in all treatments (poultry manure; 20, 40 and 60 m³ /fed. and bio-fertilizers; Azos+B, Azos+VAM and Azos+B+VAM) that, recorded 124,124,126, 124,124 and 124 days during the 1st season, respectively, versus poultry manure 30 m³/fed, AzosBc+VAM which recorded 128 days for both treatments during the 2nd season as compared with untreated plants (117 days).

Increasing the vegetative growth of plants are reflected in increasing flowers yield and improving quality and nutritive value. The enhancement of number of flowers/ plant and flower diameter may be due to beneficial effect of organic manure bacteria and mycorrhiza on the soil properties, in addition to the role of increasing nitrogen in the initiation of new cells. The beneficial effect of N_2-fixers and P- dissolving bacteria on the plant's development can be attributed not only to the N_2-fixation and dissolving phosphate process, but also to the production of growth promoting substances. Several soil microorganisms possess the capability to synthesize gibberellins (Rademacher, 1994).

Our results were in harmony with those found by El-Maadawy (2007) on *Tageteserecta*, Vieira *et al*. (1999) on *Calendula officinalis*.

Table 5. Effect of poultry manure, *Azospirillum*, *Bacillus* and VAM on flowering start (days) and flowering period (days) of *Calendulaofficinalis* L. plants during two seasons.

Treatments	Flowering start (days)		Flowering period (days)	
	1st season	2nd season	1st season	2nd season
Control	50^{a}	49^{a}	117^{a}	117^{c}
Poultry 20 m³/fed.	43^{b}	47^{ab}	124^{b}	119^{bc}
Poultry 40 m³/fed.	43^{b}	38^{c}	124^{b}	128^{a}
Poultry 60 m³/fed.	41^{b}	40^{bc}	126^{b}	126^{ab}
Azos+B3 ml each.	43^{b}	40^{bc}	124^{b}	126^{ab}
Azos+VAM3 ml each.	43^{b}	44^{abc}	124^{b}	122^{abc}
Azos+B+VAM3 ml each.	43^{b}	38^{c}	124^{b}	128^{a}

a to c Means having different letters exponents in column are significantly different (P ≤ 0.05)

3. Effect of organic and bio-fertilizers on chemical composition.

Effect of organic and bio-fertilizers on chemical compositionin leaves and flowers

Carotenoids and Chlorophyll:

The effect of organic and bio-fertilizers treatments on photosynthetic pigments, carotenoids in the flowers and Chlorophyll A&B in the leaves of marigold plants are displayed in Table (6). The results showed that, photosynthetic pigments (mg/g fresh weight)were significantly increased due to the application of the organic and bio-fertilization in both seasons, in comparison with the unfertilized plants (control). The highest value of carotenoids in the flowers was 0.98 mg/g fresh weight in the 1st season when poultry manure was applied at 60 m³/fed., while the highest values of carotenoids in the flowering heads were 0.99 and 0.99 mg/g fresh weight in the 2nd season when poultry manure was 40 and 60 m³/fed. Moreover, the application of Azos+VAM and Azos+B+VAM gave the highest values of Chlorophyll A which recorded 3.21and 3.16 mg/g fresh weight in the 1st season, respectively, while, application of Azos+B, Azos+VAM and Azos+B+VAM resulted in the highest values of Chlorophyll A which recorded 3.09, 3.18 and 3.13 mg/g fresh weight in the 2nd season, respectively. Application of poultry manure at 60 m³/fed. and Azos+B+VAM resulted in the highest content of Chlorophyll B (1.20 and 1.13 mg/g fresh weight) in the 1st season, while it was1.16 and 1.10 mg/g fresh weight, respectively.

Table 6. Effect of poultry manure, *Azospirillum*, *Bacillus* and VAM on carotenoids content inthe flowering head and Chlorophll A, B (mg/g) of *Calendula officinalis* L. plants during two seasons.

Treatments	Carotenoid content (mg/g fresh weight)		CholorophyllA (mg/g fresh weight)		CholorophyllB (mg/g fresh weight)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	0.87^{c}	0.85^{c}	2.35^{d}	2.29^{c}	0.93^{c}	0.96^{d}
Poultry 20 m³/fed.	0.90^{bc}	0.91^{bc}	2.78^{c}	2.81^{b}	0.99^{bc}	0.97^{cd}
poultry 40 m³/fed.	0.96^{ab}	0.99^{a}	2.97^{b}	3.00^{ab}	1.10^{ab}	1.07^{abc}
poultry 60 m³/fed.	0.98^{a}	0.99^{a}	3.05^{ab}	3.08^{ab}	1.20^{a}	1.16^{a}
Azos+B 3ml each.	0.87^{c}	0.89^{bc}	3.05^{ab}	3.09^{a}	0.91^{c}	0.93^{d}
Azos+VAM 3ml each.	0.93^{abc}	0.92^{b}	3.21^{a}	3.18^{a}	0.97^{c}	0.99^{bcd}
Azos+ B+VAM3ml each.	0.92^{abc}	0.90^{bc}	3.16^{a}	3.13^{a}	1.13^{a}	1.10^{ab}

a to d Means having different letters exponents in column are significantly different (P ≤ 0.05).

From the physiological view, the obtained results could be attributed to the role of the organic fertilizers as a constituent of pyridines, which are in turn constituents of chlorophyll and cytocromes (Jooet *al.,* 1999; Mostafa, 2002).These results were in agreement with those obtained by Hammam (1996) on *Pimpinella anisum*,Jacoup (1999) on *Thymus vulgaris*, El-Sherbenyet *al.* (2005) on *Sideritismontana*,Swaefyet *al.* (2007) on pepper mint and Matter (2009) on *Hibiscus subdariffa* plant.

Effect of organic and bio-fertilizers on carbohydrates percentage (%) and oleanolic acid in the flowering heads (mg/g dry weight):

Carbohydrates percentage and oleanolic acid content in the flowering heads of marigold (Table 7) were greatly and significantly increased in response to organic and bio-fertilizers in comparison to the unfertilized plants in the two seasons. The highest values of carbohydrates percentage were 62.40 and 64.21% in both seasons, respectively; when poultry manure was applied at 60 m³/fed., which gave the highest values of oleanolic acid in flower (0.43 mg/g dry weight) in the 1st season. Moreover, applications of poultry manure at 40 and 60 m³/fed. and bio-fertilization with Azos+VAM and Azos+B+VAM resulted in the highest values of oleanolic acid in the flowering heads in the 2nd season, which recorded 0.43, 0.44, 0.44 and 0.43 mg/g dry weight, respectively. This may be explained that, organic fertilizers are reknowed for their ability to chelate soil nutrients, improve nutrient uptake, especially phosphorous, sulfur and nitrogen, reduce the need for nitrogen fertilization, remove toxins from soil, stimulate soil biological activity, solubilize minerals, improve soil structure, act as a storehouse of N, P, S and Zn (Frank and Roeth, 1996). Organic manure contains microorganisms for example, *Azotobacter* and *Azosprillum*, which plays an important role to fix nitrogen and release phytohormones such as, IAA, GA and Cytokinins; which promote the growth, content of dry matter and nutrients absorption (Reynders and Volassak, 1982).

Effect of organic and bio-fertilizers on minerals percentage:

Relevant data in Table (8) showed the percentage of nitrogen, phosphorus and potassium in dry herb of *Calendula officinalis* L. plants as affected by organic and bio-fertilizers. The three elements were significantly increased due to the use of all organic and bio-fertilizers treatments in the two seasons in comparison with control plants. Corresponding data showed an increase in nitrogen percentage due to poultry manures treatments in comparison to bio-fertilizers treatments and to the control. When poultry manure was used at the rates of 20, 40 and 60 m³/fed. the values of nitrogen percentages were 3.60, 3.75 and 3.79 % in the 1st season, and 3.72, 3.80 and 3.92 % in the 2nd season, respectively. However, application of poultry manure at 60 m³/fed. gave the highest value of phosphorus (0.35 %) in the 1st season, while applications of poultry manure 20, 40 and 60 m³/fed. gave the highest values of phosphorus contents (0.32, 0.34 and 0.37 % in the 2nd season, respectively). In case of potassium the maximum values were obtained as a result of poultry manure 40 and 60 m³/fed. (2.27 and 2.29 % for 1st season) and (2.30 and 2.33 % for 2nd season, respectively).

The previously obtained results were in harmony with those reported by,Sakr (2001) on *Menta piperita*, Abd El-Latif (2002) on *Carumcarvi*, Haroun and Hussein (2003) on *Lupinustermis*,Zaied*et al.* (2003), on wheat, Atta-Alla*et al.* (2005) on *Capsicum annuum*,Abdelaziz *et al.* (2007) on *Rosmarinus officinalis*,Azzaz and Hassan (2008) on fennel.

Table 7. Effect of poultry manure, *Azospirillum*, *Bacillus* andVAM on carbohydrate percentage and oleanolic acid of *Calendulaofficinalis* L. plants during two seasons.

Treatments	Carbohydrate percentage (%)		Oleanolic acid in flowers (mg/g dry weight)	
	1st season	2nd season	1st season	2nd season
Control.	50.23^{d}	52.10^{e}	0.33^{b}	0.34^{b}
Poultry 20 m³/fed.	58.01^{b}	58.84^{c}	0.39^{ab}	0.39^{ab}
Poultry 40 m³/fed.	60.08^{ab}	61.36^{b}	0.41^{ab}	0.43^{a}
Poultry 60 m³/fed.	62.40^{a}	64.21^{a}	0.43^{a}	0.44^{a}
Azos+B 3ml each.	55.11^{c}	56.71^{d}	0.37^{ab}	0.39^{ab}
Azos+VAM 3ml each.	58.91^{b}	60.80^{bc}	0.39^{ab}	0.44^{a}
Azos+B+VAM 3ml each.	59.18^{b}	60.00^{bc}	0.41^{ab}	0.43^{a}

$^{a\ to\ e}$**Means having different letters exponents in column are significantly different (P ≤ 0.05).**

Table 8. Effect of poultry manure, Azospirillum, Bacillus andVAM on nitrogen, phosphorus and potassium percentagein dry herb of *Calendulaofficinalis* L. plants during two seasons.

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.88^{c}	1.94^{d}	0.19^{c}	0.21^{c}	1.13^{d}	1.16^{d}
Poultry 20 m³/fed.	3.60^{a}	3.72^{a}	0.30^{ab}	0.32^{a}	1.94^{b}	1.90^{b}
Poultry 40 m³/fed.	3.75^{a}	3.80^{a}	0.33^{ab}	0.34^{a}	2.27^{a}	2.30^{a}
Poultry 60 m³/fed.	3.79^{a}	3.92^{a}	0.35^{a}	0.37^{a}	2.29^{a}	2.33^{a}
Azos+B 3ml each.	2.48^{b}	2.54^{c}	0.31^{ab}	0.30^{ab}	1.50^{c}	1.44^{c}
Azos+VAM 3ml each.	2.59^{b}	2.73^{bc}	0.25^{bc}	0.24^{bc}	1.23^{cd}	1.35^{c}
Azos+ B+VAM3ml each.	2.66^{b}	2.92^{b}	0.29^{ab}	0.29^{ab}	1.36^{cd}	1.34^{c}

$^{a\ to\ d}$**Means having different letters exponents in column are significantly different (P ≤ 0.05)**

REFERENCES

A.O.A.C. (1995). Agricultural Chemicals, Contaminants, Drugs. 16th ed. Association of Official Analytical Chemists, Washington, D.C., USA. Methods of Analysis. Vol. (1).

Abd El-Latif, T.A. (2002). Effect of organic manure and biofertilizer on caraway plants (*Carumcarvi, L.*). J. Agric. Sci. Mansoura Univ., 27(5): 3459-3468.

Abd El-Malek, Y. and Y.Z. Ishac (1968). Evaluation of methods used in counting azotobacters. J. Appl. Bact., 31(4): 267-275.

Abdelaziz , M. E.; Hanafy, A. H. Ahmed, M.; Shaaban, M . and Pokluda, R. (2007). Fresh weight and yield of lettuce as affected by organic manure and bio-fertilizers. Conference of Organic Farming, CzechUniv.Agric., Czech Republic, 212-214.

Atta-Alla, H.; Waly, A.K.; Khaleil, A.M.and El-Zony, E. (2005). Effect of some organic manures on the vegetative growth, fruits and chemical composition of *Capsicum annum*, L. VAR. Santoka. proc. 6th Arabian Conf.Hort., Ismailia , Egypt.

Azzaz, N. A. E. and Hassan, E. A. (2008). The chemical constituents, and vegetative and yielding characteristics of fennel plants treated with organic and bio-fertilizer AS A. substitute of mineral fertilizer. J. BioLChem.Environ. Sci., March 2008, Vol 3(1): 411-454.

Bryan, H.H. and Lance, C.J. (1991).Compost trials on vegetables and tropical crops.Biocycle, 27: 36-37

Cooke, G.W. (1972). Fertilizing for maximum yield. Richard caly (The Chauer Press) LTD. Bungary, Ssuffok, Great Britain, PP.457.

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

Dobereiner, J.; Marrieland, E. I. and Nery, M. (1976). Ecological distribution of Spirillum lipoferumBeijerinck. Can. J. Microbial., 22(10):1464-1473.

Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebors, P.A. and Smith, F. (1956). Colourimetric method for determination of sugars and related substance. Analytical Chem.28 (3): 350-356.

El-Gaingihi, S.E.; Abdalla, N.M. and Sidrak, I. (1982). The effect of fertilization levels on flowering, oleanolic acid and phytosterol content of Calendula officinalis, L. Pharmazie 37(7): 511-514.

EL-Kramany, M.F.; Ahmed, M.K.; Bahr, A.A. and Kabesh, M.O. (2000).Utilization of biofertilizers in field crop production. Egypt J. Appl. Sci., 15(11): 137

El-Maadawy, E.I.(2007) Response of summer annual flowering plants to chemical, organic and bio-fertilization treatments. II- African marigold (*Tageteserecta*) plants. J. product. and Dev. 12 (1): 173- 199.

El-Sayed, A.A.; Mansour, H.A. and El-Maadawy, E.I. (2002). Effect of organic and inorganicfertilization on herb and oil productivity of spearmint and majoram. Zagazig J. Agric. Res. 29 (6): 1859-1888.

El-Sherbeny, S.E.; Khalil, M.Y. and Naguib, N.Y. (2005). Influence of compost levels and suitable spacings on the productivity of *Sideritismontana L*. plants recently cultivated under Egyptian conditions. Bulletin of Faculty of Agriculture,-Cairo-University. 56 (2): 373-392.

Frank, K.D. and Roeth, F.W.(1996). Using soil organic matter to help make fertilizer and pesticide recommendation. In: Soil organic matter: Analysis and interpretation. Soil Science society of American special publication No. 46, P.33.

Hammam, K.H.A. (1996). Effect of nitrogenous fertilization and irrigation on growth, yield and active constituents of anise plants (*Pimpinella anisum*) M.Sc. Thesis, Fac. of Agric., Cairo. Univ., Egypt.

Haroun, S.A. and Hussein, M.H. (2003). The promotive effect of algae biofertilizers on growth, protein pattern and some metabolic activities of *Lupinustermis* plants grown in siliceous soil, Asian J. Plant Sci., 2, 944-951.

Helmy, L.M. and Zarad, S.S. (2003) Effect of different rates of some organic manures on the productivity of borage plant in sandy soil. J. Agric. Sci., Mansoura Univ. 28(5): 3911-3926.

Jacoub, W.R. (1999). Effect of some organic and non-organic fertilizers on growth, oil yield and chemical composition of (*Ocimumbasilicum, L.* and *Thymus vulgaris*, P.) plants. ph.D. Thesis, Fac. of Agric. Cairo Univ., Egypt.

Joo, Y. H.; Senanayake, Y. D.A. and Sangakkara, U.R. (1999). Effect of EM on the production of crops and waste treatment in Korea. Fifth International Conference on KyuseiNaturre.

Magda, H.Mostafa (2002). Studies on feitilization of washington navel orange trees. Ph.D. Thesis. Fac. Of Agric., Moshtohor, Zagazig Univ., Egypt. Pp. 61-68.

Matter, F. M. A. (2009). Response of roselle plants (*Hibisus sabdariffa, L.*) to chemical and Bio – fertilizers. J. Agric. Sci Mahsouraunir, 34(2): 1129-1139.

Rademacher, W. (1994). Gibberelln formation in microorganisms. Plant Growth Regulation .15(3): 303-314.

Reynders, L. and Volassak, K. (1982). Use of *Azospirillumbrasilense* as bio-fertilizer in intensive wheat cropping, plant and Soil, 217- 223. Roberfroid M. "Introducing inulin-type fructans". Br J Nutr 93 Suppl 1: S13–25.

Rizk, A.M. and Al Nowaihi, A.S. (1989). The phytochemistry of the Horticultural plants of Quarter. The sci. and applied Researches Center Univ., Quarter (1989) page 47-51.

Sakr, W. R. A. (2001). Effect of some organic and inorganic fertilizers on *Mentha piperita*. M. Sc. Thesis, Fac. of Agric., Cairo Univ., Egypt.

Schachtschable, S. (1979). Lchrobuch der B.O. deskund, Printed in Germany (F.R.G) Fedinaudenke, Stuttgart.

Sharaf EL-Din, M. N.: Ahmed, H. A.; Shalan, M. M. and. Hussien, H. M.(2019). The effect of Organic and Bio-Fertilization on Vegetative Growth and Yield of Thyme *(Thymus vulgaris*, L.)" J. of Plant Production, Mansoura Univ., Vol 10 (12):1175 -1185, 2019.

Snedecor, W. C. and Cochran, W. G.(1980). Statistical Methods. 7 th ed., 2 nd printing. The Iowa State Univ. Press, Ames, Iowa, U.S.A.

SwaefyHend, M.F.; Weaam R.A.; Sakr; Sabh, A.Z. and Ragab, A.A. (2007). Effect of some chemical andbio-fertilizers on peppermint plants grown in sandy soil. , Annals. , Agric. Sci. Ain Shams Univ. Cairo. 52(2): 451-463.

Vidal-Ollivier, E.; Balansard, G.; Faure, R. and Babadjamian, A. (1989). Revised structures of triterpenoid saponins from the flowers of calendula officinalis. Journal products (LLOYDIA) 52(5):1156-1159.Biological Abstracts Vol., 89, ISS. 3 ref. 29476.

Vieira, M.C.; Heredia, N.A.S. and Ramos, M.B.M. (1999). Marigold (*Calendula officinalis*, L.) growth and flower head yield as function of semide compost poultry house litter and phosphorus fertilization. RevistaBrasilieira de plants medicines, 1(2): 45-51. (Hort. Abst., 70(4): 3545).

Zaied, K. A.; Abdelhady, A. H.; Aida, H. A. and Nassef, M. A. (2003). Yield and nitrogen assimilation of winter wheat inoculated with new recombinant inoculants of rhizobacteria, Pakistan J. Biological Sci., 6, 344-358.

تاثير التسميد العضوي والحيوي علي بعض الخواص الطبيعيه والكيميائيه لنبات الأقحوان

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تم دراسة تأثير التسميد العضوي(بإستخدام ثلاث معدلات من سبلة الدواجن) والتسميد الحيوي(بإستخدام سلالة بكتيرية مثبتة للنيتروجين وسلالة بكتيرية أخري مذيبة للفوسفات والميكروهيزا)علي نبات الأقحوان خلال موسمين متتاليين .أظهرت النتائج أن جميع معاملات التسميد العضوي والحيوي حسنت خصائص النمو الخضري المختلفة وعززت معاملات التزهير وحفزت المكونات الكيميائية المختلفة (كلوروفيل أ،ب)والكاروتينات وايضا زيادة في نسبة الكربوهيدرات والعناصر المعدنية وحمض الأولونوليك .ومن هنا يمكن إستخدام الأسمدة العضوية والحيوية كبديل للتسميد الكيميائي مما يقلل من التكاليف الإنتاجية وتلوث البيئة الزراعية وأيضا يؤدي إلي تحسين الخواص الطبيعية والكيميائية.