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Bee Pollen as a Bio-nutrient for *Calendula officinalis.*

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ABSTRACT

The current study was performed for the sake of discovering how effective the bee pollen aqueous extract is on the vegetative growth, flowering attributes, chemical composition and essential oil and its constituents, if it is sprayed on calendula plants. Honeybee pollen was applied at 0, 0.5, 1, 2, 3, 4 and 5 g L^{-1}. Results obtained proved that bee pollen aqueous extract is of an effect upon the whole studied parameters. All concentrations led to an increase in all characters in comparison to the control. However, the highest values for the vegetative growth and flowering characters resulted from the use of the moderate rates of bee pollen (3 and 2 g L^{-1} respectively). In chemical constituents, the moderate and the highest concentrations (2, 3, 4 and 5 g L^{-1}) gave the highest results in most of the characters especially 5 g L^{-1} which produced the highest amounts of ch. a, b, c, Mn, amino acid, protein and N and 2 g L^{-1} which had the highest degrees of the essential oil, Zn, cartenoids, xanthophylls, beta carotene and flavonoids. As for the essential oil components, the highest percentage of the most important components of the essential oil; sesquiterpene hydrocarbons (α -cadinene, α-Muurolene and δ-Muurolene) and sesquiterphenols (α- cadinol, α-Cadinol (Epi) and α-Muurolol (Epi)) resulted from the application of 2 g L^{-1}.

Keywords: Bee pollen, *Calendula officinalis*, Foliar application, Essential oils, Flowers pigments, Cartenoids, Xanthophylls, Beta carotene and flavonoids.

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INTRODUCTION

Calendula officinalis L. (Asteraceae) is native to the Mediterranean region and an annual herb with yellow to orange flowers [1]. It was used medicinally in Europe, China and India amongst several places in the world. As well, it is famous as "African marigold" and has been undergone in several chemical and pharmacological studies chemical studies which assured the existenceof several classes of compounds, the main being triterpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids. It is potentially an important medicinal plant for mankind [2].

The extracts obtained from the Calendula flowers contained a high quantity of polyphenolic compounds and exhibited good antioxidant activities [3].

Therefore, *C. officinalis* has an economic value as a result of using it widely in cosmetics, perfumes, pharmaceutical preparations and food. Among such uses, Calendula was used in traditional medicine, especially for wound healing, jaundice, blood purification and as an antispasmodic. The extract of this plant as well as pure compounds isolated from it have been demonstrated to be cytotoxic, hepatoprotective, anti-HIV, anti inflammatory, spasmogenic, spasmolytic and insecticidal [2]. Also, Calendula was taken internally to treat fevers and cancer and promote menstruation [4].

The importance of bees for the pollination and multiplication of wild plants is immense. Bees when visiting flower's blossom, touch the stamen and its body is covered with pollen dust. The honey bee uses hind legs this plate to compress the pollen into the pollen basket. The bee moistens the pollen with secretion from its mouth which helps the pollen cling together and to the basket hairs. Worker honey bees mix it with nectar and salivary substances and stored at the hive entrance [5]. This secretion contains different enzymes, e.g. amylase and catalase.

Honey is added to keep pollen quality, which is called beebread. Despite the fact that honey is the energy source of the bee colony, however, pollen is the bees' essential source of the other vital nutrients: proteins, minerals, fats and other substances. As a result, an adequate pollen supply will be necessary to guarantee the long-term survival of a colony and to maintain its productivity**.** Honey bee foragers mix freshly collected pollen with some nectar before packing it into their corbiculae. In the hive, the workers add more nectar and glandular secretions to the pollen, which then undergoes lactic acid fermentation [6]. As a matter of fact, BP is referred to as the "only perfectly complete food", as it includes all the essential amino acids needed for the human organism. Nonetheless, the composition of BP relies strongly on the plant source and geographic origin, altogether with other factors as climatic conditions, soil type, and beekeeper activities [7]. Many studies have assured the existence of vitamins [8], Minerals [9] and other main components [9] in pollen composition.

Modern science has made it possible to classify its valuable antimicrobial [10], antifungal [11], antioxidant [12], anti-radiation [13], hepatoprotective [14], chemopreventive [15], anticancer [16] and anti-inflammatory activities [17]. Also it has anti-osteporosis, anti-diarroe, immunomodulating, Probiotic and prebeiotic, antiaging, anti-depressant characteristics as reported by Bogdanov, 2015[18].

MATERIALS AND METHODS

A field experiment was conducted during two successive seasons (2014/2015 and 2015/2016) at the Experimental farm in the Faculty of Agriculture, Fayoum University, Egypt to investigate the effect of aqueous extract of bee pollen on the growth and flowering characters, some chemical composition and essential oil and its constituents of Calendula plants. Prior to any practices, a composite soil sample was taken from the soil surface (0-30 cm) of the experimental site (Table 1).

Table 1: Some characteristics of the experimental site

Years	Mechanical analysis				Hydraulic conductivity (cm3/hr)
	Sand %	Silt %	Clay %	Texture class	
2014	31.22	31.28	35.73	Sandy clay	0.028
2015	32.15	31.77	37.17	Sandy clay	0.027

Years	Chemical properties										
	N	P	K	Fe	Zn	Mg	Mn	EC dSm^{-1}	pH	CaCo3 %	Organic matter%
2014	17.78	22.37	96.48	3.37	0.67	0.27	8.57	2.78	7.55	4.67	1.25
2015	18.73	.2287	.9722	3.78	0.75	0.33	8.78	2.82	7.68	4.81	1.27

After obtaining the seeds of Calendula plants from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt, they were sown in nursery on 15thAugust at the seasons of 2014 and 2015. 45 days after from being sown on 1st Oct, unified seedlings were transplanted. Necessary agricultural practices for seedlings production were achieved. The experimental design was a complete randomized blocks with three replications for each treatment. The plot area was (4 × 1.80) = 7.20 m^2 and included three ridges; each ridge was 60 cm apart and 4m in length. The seedlings of Calendula were transplanted at a distance of 30 cm between seedlings.

Bee pollen extract was prepared from bee pollen grains obtained from the Egyptian Agriculture Research Center , with some modifications by mixing bee pollen in a blender with water (1:2, w/v) at temperature of 70°C for 30 min with constant agitation, the extract was further filtered to obtain a concentrated liquid extract. That extract was then re-dissolved in distilled water to make up different concentrations (0, 0.5, 1, 2, 3, 4 and 5 gL^{-1}) which, then, were applied as a foliar spray thrice by 15 day intervals, beginning from 30 days after transplanting. Few drops of Triton B were added to the spray solution to serve as a wetting agent. Sprays were applied in the morning (8-10 a.m.) using a hand pressure sprayer. The control plants were sprayed with distilled water. The volume of the spraying solution was maintained just to cover completely the plant foliage until drip. All the plants received normal agriculture practices whenever they needed. In addition, they received uniform treatments of manure 15 m^3/feddan., irrigation and fertilization at the field according to common practices i.e. Calendula plants were fertilized with 300 kg/feddan ammonium sulphate (20.5%N), 300 kg/feddan calcium super phosphate (18% P_2O_2) and 100 kg/ feddan potassium sulphate (48%K_2O). Half of the N. and K. rates were added after 30 days from transplanting and the second application was done after 30 days from the first application. Application of calcium super phosphate was done as one dose during the preparation of the soil. All agriculture practices operations other than experimental treatments necessary for growth and development as cultivation, irrigation and pest control were followed whenever it was necessary and were done according to the recommendations of Ministry of Agriculture, Egypt.

Data recorded:

Studied Characters: after 90 days from transplanting, at full blooming, nine plants were chosen from each treatment to determine the studied characters:

Morphological and flowering yield characters; plant height (cm), leaves number $plant^{-1}$, branches number $plant^{-1}$, fresh and dry matter of leaves $plant^{-1}$ (g), fresh and dry matter of shoots $plant^{-1}$ (g), flowers number $plant^{-1}$, inflorescences diameter $plant^{-1}$, inflorescences fresh and dry matter $plant^{-1}$ (g), fresh and dry matter of ray flowers $plant^{-1}$ (g), dry matter of ray flowers $feddan^{-1}$ (kg) and seed mass $plant^{-1}$ (g) and $feddan^{-1}$ (kg).

Gas Chromatography-Mass Spectrophotometric (GC-MS) analysis; the components of essential oil extracted from Dry flower-head of calendula were determined according to Adams, 1995[19].

Chemical constituents;

Extraction of essential oil: flower head at flowering stage (50g) was subjected to Hydro distillation for 3h using a Clevenger type Hydro distillation for 3h using a Clevenger type [20].

The determination of Chlorophyll a, b and total carotenoids in leaves was done according to the method of Lichtenthaler, 1987 [21].

Pigments in dry flowers (Beta-carotene and xanthophylls) mg g^{-1}: the pigments were determined at last collection as described by AOAC, 1970 [22].

Xanthophyll content was determined at wavelength of 470 and 485 nm according to Bacot, 1954 [23].

Total flavonoids (mg g^{-1} D. M.) were estimated in dried flower-head according to Zhishen *et al.,* 1999 [24].

Total carotenoids in dry ray flowers (mg g^{-1} D. M.) were determined in dried flower head according to Britton *et al.,* 1995 [25].

Total carbohydrate percentage in stems and leaves (%): in each treatment total carbohydrates% in dry matter of herb were colorimetrically determined using phenol-sulphoric acid reagent method as outlined by Dubois *et al.,* 1956 [26].

Total N (a factor of 6.25 was used for the conversion of N% to protein% [27], P and K, in herb and seeds were determined according to the methods of Cottenie *et al.,* 1982[28]. Zinc, manganese and iron contents in herb and seeds were determined using atomic absorption spectrophotometer according to the method described by Cottenie *et al.,* 1982 [28].

The percentage of total free amino acids (%): dry matter of herb were extracted by using ethanol 80% then determined using ninhydrin reagent method as outlined by Jayarman, 1981[29].

Statistical analysis:-

All data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the Completely Randomized Design (CRD) using MSTATC computer software package according to Gomez and Gomez, 1984 [30]. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of significance.

RESULTS AND DISCUSSION

Effect of foliar application of bee pollen aqueous extract

On vegetative growth and flowering parameters of Calendula plants:

In comparing and contrasting the obtained results from the application of the six treatments (0.5, 1, 2, 3, 4, and 5 gL^{-1}) with the control, it was assured that the foliar application of bee pollen caused an increase in all investigated vegetative growth and flowering attributes in both seasons (Table 2). That increase was notably clear with 2 and 3 gL^{-1} but the highest records, concerning the vegetative growth characters, were especially given by the foliar spray with 3 gL^{-1} which result in insignificant differences with all characters except for the leaves number and leaves fresh and dry weight $plant^{-1}$ in both seasons and the shoots dry weight $plant^{-1}$ and number of branches $plant^{-1}$ in the first season. While the highest results, regarding the flowering attributes were significantly obtained by the 2 gL^{-1} except for the values of flowers number $plant^{-1}$and inflorescences diameter, seeds weight $plant^{-1}$ and seeds yield $faddan^{-1}$ in the first season which had insignificant differences between them. This enhancement from the part of bee pollen aqueous extract on vegetative growth and flowering parameters is thought to be associated with the highly nutritional components that bee pollen contains. As bee pollen includes proteins, lipids, total carbohydrates and crude fibers [31, 32]. In addition, it has dietary fiber (Pectin), ash and vitamins [5].

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Table 2: Effect of foliar application of bee pollen aqueous extract on vegetative growth and flowering yield characters of Calendula plants at two successive seasons (2014\2015) (2015\2016).

Seasons	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
characters / Conc.	Plant height (cm)		No. of branches plant^{-1}		No. of leaves plant^{-1}		Leaves F. w.plant^{-1} (g)	
0 gL^{-1}	69.83	76.65	3.77	4.92	108.45	122.19	69.69	78.47
0.5 gL^{-1}	71.19	76.76	4.32	4.93	114.76	136.56	74.21	82.48
1 gL^{-1}	71.80	78.26	4.39	5.10	136.00	159.14	78.71	89.03
2 gL^{-1}	75.97	81.46	6.95	5.44	154.36	168.90	83.32	91.85
3 gL^{-1}	76.07	81.96	7.11	5.83	160.59	174.62	93.68	100.34
4 gL^{-1}	73.69	79.40	5.46	5.21	150.78	165.34	82.38	94.62
5 gL^{-1}	72.10	79.38	4.66	5.16	144.98	159.40	77.21	90.03
L.S.D 5%	n.s.	n.s.	0.43	n.s.	11.90	10.11	10.71	10.31
	leaves D w plant^{-1} (g)		shoots F w plant^{-1} (g)		shoots D w plant^{-1} (g)		Flowers no. plant^{-1}	
0 gL^{-1}	8.31	11.29	68.91	84.87	7.90	10.28	25.73	22.89
0.5 gL^{-1}	8.62	11.56	70.01	87.90	8.28	10.74	27.77	24.03
1 gL^{-1}	9.83	13.29	73.08	89.84	8.70	11.32	32.07	25.05
2 gL^{-1}	12.59	14.05	84.03	96.16	9.05	12.84	33.09	30.83
3 gL^{-1}	14.45	16.90	84.13	98.31	11.43	13.46	31.85	29.24
4 gL^{-1}	11.75	15.14	80.24	94.41	11.24	10.86	28.79	29.81
5 gL^{-1}	11.13	12.46	74.17	93.54	8.49	10.93	28.33	27.54
L.S.D 5%	0.98	1.59	n.s.	n.s.	1.96	n.s.	n.s.	3.08
	inflorescences diameter (cm)		inflorescences FW plant^{-1} (g)		inflorescences D w plant^{-1} (g)		F w of ray flowers plant^{-1} (g)	
0 gL^{-1}	8.12	6.21	65.87	66.70	16.78	15.29	66.23	69.84
0.5 gL^{-1}	8.20	6.35	69.81	67.72	17.36	15.77	68.76	74.16
1 gL^{-1}	8.35	7.28	72.97	71.38	17.95	16.30	71.89	76.41
2 gL^{-1}	9.03	7.75	76.71	79.77	19.22	17.46	81.80	86.94
3 gL^{-1}	8.48	7.36	74.72	76.89	18.46	17.00	77.25	85.18
4 gL^{-1}	8.27	6.96	68.47	69.46	18.24	15.89	76.56	81.61
5 gL^{-1}	8.61	7.28	70.12	71.39	18.40	15.94	71.48	79.33
L.S.D 5%	n.s.	0.47	5.90	6.85	1.10	1.25	6.20	5.26
	D w of ray flowers plant^{-1} (g)		D yield of ray flowers faddan^{-1} (kg)		Seeds wt. plant^{-1} (g)		Seeds yield faddan^{-1} (kg)	
0 gL^{-1}	7.13	7.97	213.90	239.10	10.80	11.41	324.00	342.30
0.5 gL^{-1}	7.24	8.95	217.20	268.50	11.02	12.21	330.60	366.30
1 gL^{-1}	7.45	8.26	223.50	247.80	12.47	14.01	374.10	420.30
2 gL^{-1}	9.05	10.25	271.50	307.50	13.29	14.51	398.70	435.30
3 gL^{-1}	8.55	10.00	256.50	300.00	12.10	12.95	363.00	388.50
4 gL^{-1}	8.48	9.43	254.40	282.90	11.46	12.89	343.80	386.70
5 gL^{-1}	7.98	8.54	239.40	256.20	11.30	12.28	339.00	368.40
L.S.D 5%	0.59	0.78	17.7	23.4	n.s.	1.71	n.s.	51.3

Among those vitamins is B^6 which is involved in amino acid synthesis and also is an important growth factor in plants, Folic acid which is a very important coenzyme in amino acid metabolism and also in the synthesis of nitrogen bases required for the nucleotides, Niacin (B^3) is required for the development of roots and involved in respiration, photosyenthesis and amino acid synthesis and Biotine which acts as the coenzyme covantly bound to some carboxylase enzymes involved in gluconeogenesis. Also the amount of zinc, iron, phosphorus and Magnesium that bee pollen has may have a role in enhancing the growth as zinc aids plants growth hormones, enzyme system and seed formation while phosphorus involved in cell division and enlargement, the same as iron does, and photosynthesis, promotes early root formation and growth and hastens maturity also Magnesium influences earliness and uniformity of maturity. Moreover, bee pollen grains extract includes auxins, IBA and cytokinin [33, 34]. Such constituents may play an important role in improving the plant vegetative growth which in turn stimulates the flowering attributes. As well, phenolic compounds which are fundamental for plant physiology owing to its participation in the plant morphological form and structure, also, in plant growth and reproduction process they are involved [35]. These results are close to those obtained by Shafeek *et al.,* 2015 [36], on onion plants, who stated that bee pollen was among the applied bio stimulants which caused an increase in the vegetative growth characters.

Gas Chromatography-Mass Spectrophotometric (GC-MS) analysis the components of essential oil extracted from dry flower-head of calendula;

The whole constituents of plant essential oil detected by GC-MS (Table 3) were affected by all concentrations of bee pollen. The highest percentage of the most important components of the essential oil; sesquiterpene hydrocarbons (□-cadinene, α-Muurolene and □-Muurolene) and sesquiterphenols (α- cadinol, α-Cadinol (Epi) and α-Muurolol (Epi)) were obtained by 2 g L^{-1}. The majority of the sesquiterpene hydrocarbons reached their optimum degrees due to the application of 1 gL^{-1} including (Sabenene, α-Humulene, β- cymene, aromadendrene (allo), β-Patchoulene,β-Calacorene, α-Pinene, β-Pinene and □-Gurjunene). Also, the highest values of five of Sesquiterphenols were produced by 1 gL^{-1} (Nerolidol (E-), α-Bisabolol, α-terpeneol, Geraniol and Carvacrol). Whereas, 4 gL^{-1} resulted in the highest rates of α-terpeneol, 1,8 cineol and α-Eudensmol as well as α- phellandrene and Limonene. The promoting effect of bee pollen extract in oil components may be a result of the minerals and trace elements, vitamins and carotenoids, phenolic compounds, flavonoids, sterols and terpenes [8, 32].

Table 3: Effect of bee pollen aqueous extract on the chemical composition of the main constituents of the essential oil extracted from dry ray flowers of Calendula plant.

Treatments	0 gL^{-1}	0.5 gL^{-1}	1 gL^{-1}	2 gL^{-1}	3 gL^{-1}	4 gL^{-1}	5 gL^{-1}
Components (%)	sesquiterpene hydrocarbons						
□-cadinene	24.92	25.44	25.93	31.01	30.81	28.85	27.88
α-Muurolene	0.56	0.60	0.60	0.69	0.69	0.67	0.66
□-Muurolene	0.36	0.45	0.45	0.68	0.56	0.45	0.52
α-Pinene	0.27	0.31	0.35	0.34	0.34	0.34	0.31
β-Pinene	0.09	0.14	0.17	0.11	0.11	0.12	0.10
Sabenene	0.22	0.32	0.56	0.27	0.27	0.52	0.23
α- phellandrene	0.10	0.16	0.18	0.13	0.13	0.23	0.19
α-Humulene	0.38	0.36	0.47	0.47	0.46	0.44	0.45
Limonene	0.14	0.18	0.19	0.18	0.18	0.22	0.19
β- cymene	0.43	0.53	0.73	0.53	0.53	0.62	0.62
aromadendrene (allo)	0.23	0.25	0.54	0.29	0.29	0.43	0.30
□-Gurjunene	0.47	0.63	0.84	0.58	0.58	0.78	0.60
β-Patchoulene	2.46	2.67	3.45	3.06	3.04	3.24	2.63
β-Calacorene	0.49	0.60	0.73	0.61	0.61	0.55	0.56
Components (%)	Sesquiterphenols						
α- cadinol	23.10	24.16	26.32	28.73	28.55	26.23	27.38
α-Cadinol (Epi)	1.65	1.76	1.98	2.11	2.09	2.03	2.05
α-Muurolol (Epi)	10.54	10.83	10.93	13.12	13.04	11.44	11.18
α-Eudensmol	4.83	5.87	5.74	6.01	5.97	6.73	5.52
α-Eudesmol (7-epi)	1.00	1.52	1.11	1.24	1.23	1.28	1.48
Nerolidol (E-)	0.70	0.78	1.13	0.87	0.86	1.00	0.83
1,8 cineol	0.47	0.48	0.83	0.58	0.58	0.78	0.48
α-Bisabolol	0.69	1.08	1.42	0.85	0.85	0.99	1.26
α-terpeneol	1.00	0.93	1.28	1.24	1.23	1.28	1.05
Geraniol	0.49	0.52	0.87	0.61	0.61	0.80	0.60
Carvacrol	0.69	0.80	1.15	0.85	0.85	0.99	1.01

Chemical constituents:

Although an increase occurred due to the foliar spray with all concentrations of bee pollen, over the control, upon the chemical constituents studied (Table 4), the highest values ranged between the six treatments. For instance, 5 gL^{-1} significantly had the highest result of Chl. b and c, Mn in seeds, N in herb in both seasons, N in seeds in the second season and protein in the first one but insignificantly with amino acid in herb in both seasons, protein in the second season and N in seeds in the first season. While, 2 gL^{-1}, significantly, in both seasons, resulted in the highest rates of the essential oil $plant^{-1}$, Zn in herb and seeds, total carotenoids and xanthophyll, beta-carotene and total flavonoides which show insignificant differences only in the first season. Whereas, 3 gL^{-1} significantly produced the highest percentage of p, in both seasons, in herb, P in seeds in the first season, and k in herb and in seeds in the second season but insignificantly for the percentage of P in seeds in the second season and K in seeds and in herb in the first season. On the other hand, 4 gL^{-1} significantly gave the highest amounts of carbohydrates in leaves in the two seasons, Fe in herb in the first season and Fe in seeds in the second one but insignificantly for Fe in herb in the second season and Fe in seeds in the first season. Also, the highest records of carbohydrates in stems, significantly, and Mn in herb, insignificantly, were resulted from the application of 1 gL^{-1}. Such moderate effect of bee pollen on chemical constituents may be attributed to the minerals and trace elements does it contain. Such elements include Potassium, Magnesium, Calcium, Phosphorus, Iron, Zink, Copper and Manganese [5]. Besides, it also possesses carotenoides, phenolic compounds, flavonoids, sterols and terpenes [32] and cinnamic acid derivatives, which are highly capable of neutralizing the active oxygen species [37, 38].

Table 4: Effect of foliar application of bee pollen aqueous extract on chemical constituents of Calendula plants at two successive seasons (2014\2015) (2015\2016).

Seasons	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Characters / Conc.	essential oil $plant^{-1}$ (ml)		Chl. a (mg g^{-1} F.M.)		Chl. b (mg g^{-1} F. M.)		Carotenoids (mg g^{-1} F. M.)	
0 gL^{-1}	0.065	0.068	0.78	0.85	0.59	0.62	0.43	0.45
0.5 gL^{-1}	0.067	0.072	0.86	0.90	0.63	0.65	0.44	0.47
1 gL^{-1}	0.071	0.070	0.86	0.94	0.62	0.66	0.47	0.49
2 gL^{-1}	0.081	0.081	0.87	0.95	0.64	0.67	0.49	0.51
3 gL^{-1}	0.075	0.077	0.89	0.98	0.63	0.70	0.47	0.49
4 gL^{-1}	0.074	0.077	0.95	1.01	0.66	0.70	0.49	0.48
5 gL^{-1}	0.074	0.079	0.96	1.06	0.72	0.75	0.52	0.53
L.S.D 5%	0.007	0.008	0.05	0.06	0.04	0.04	0.04	0.03
	Total flavonoides (mg g^{-1} D. M.)		Total crotenoides (mg g^{-1} D. M.)		Xanthophyll (mg g^{-1} D. M.)		Beta-carotene (mg g^{-1} D. M.)	
0 gL^{-1}	15.85	13.99	2.08	2.00	1.63	1.36	1.79	1.44
0.5 gL^{-1}	16.82	16.56	2.13	2.07	1.68	1.43	1.89	1.66
1 gL^{-1}	17.15	16.78	2.13	2.07	1.73	1.44	1.89	1.77
2 gL^{-1}	17.91	18.14	2.28	2.26	1.84	1.59	1.99	1.88
3 gL^{-1}	17.76	16.89	2.21	2.16	1.76	1.56	1.96	1.83
4 gL^{-1}	16.26	14.14	2.15	2.06	1.73	1.52	1.97	1.75
5 gL^{-1}	16.96	15.15	2.17	2.13	1.74	1.51	1.90	1.46
L.S.D 5%	n.s.	1.74	0.07	0.09	n.s.	0.10	n.s.	0.17
	carbohydrate (%) stems		carbohydrate (%) leaves		N % Herb		N % Seeds	
0 gL^{-1}	25.45	23.71	20.05	16.32	1.45	1.46	2.20	2.13
0.5 gL^{-1}	29.16	27.46	23.07	20.89	1.50	1.68	2.30	2.36
1 gL^{-1}	33.04	31.30	24.55	17.58	1.65	1.74	2.35	2.48
2 gL^{-1}	30.15	28.36	22.66	20.16	1.62	1.76	2.56	2.50
3 gL^{-1}	27.82	26.15	25.68	22.39	1.75	1.81	2.62	2.62
4 gL^{-1}	29.17	26.84	26.11	23.52	1.80	1.89	2.61	2.64
5 gL^{-1}	31.70	29.35	20.26	22.08	1.81	1.96	2.65	2.89

L.S.D 5%	2.00	1.53	1.68	1.89	0.13	0.16	n.s.	0.33
	P % Herb		P % Seeds		K % Seeds		K % Herb	
0 gL^{-1}	0.35	0.34	0.50	0.52	1.50	1.56	2.37	2.13
0.5 gL^{-1}	0.36	0.38	0.54	0.54	1.56	1.66	2.44	2.44
1 gL^{-1}	0.40	0.40	0.52	0.55	1.64	1.70	2.50	2.38
2 gL^{-1}	0.39	0.40	0.54	0.56	1.63	1.64	2.41	2.44
3 gL^{-1}	0.42	0.43	0.61	0.64	1.66	1.78	2.62	2.68
4 gL^{-1}	0.39	0.41	0.52	0.53	1.56	1.77	2.58	2.57
5 gL^{-1}	0.42	0.43	0.53	0.56	1.54	1.77	2.54	2.59
L.S.D 5%	0.04	0.03	0.06	n.s.	n.s.	0.12	n.s.	0.24
	Fe (ppm) Seeds		Fe (ppm) Herb		Zn (ppm) Herb		Zn (ppm) Seeds	
0 gL^{-1}	503.69	492.13	237.95	255.12	52.05	47.11	57.76	56.07
0.5 gL^{-1}	515.37	501.64	256.59	257.77	55.89	54.25	59.37	56.30
1 gL^{-1}	528.95	523.44	265.56	281.93	54.22	54.06	62.30	59.85
2 gL^{-1}	541.82	527.42	258.50	281.67	62.42	57.00	63.68	62.70
3 gL^{-1}	541.41	551.19	273.61	289.89	52.62	50.48	60.67	58.09
4 gL^{-1}	545.12	568.74	295.73	293.90	52.62	47.43	57.99	57.12
5 gL^{-1}	535.17	535.27	288.12	287.91	55.27	48.84	62.72	60.26
L.S.D 5%	n.s.	44.52	11.32	n.s.	6.24	5.01	3.70	2.02
	Mn (ppm) Herb		Mn (ppm) Seeds		Protein (%) Herb		amino acids (%) Herb	
0 gL^{-1}	55.22	55.90	98.06	97.80	9.06	9.13	0.27	0.30
0.5 gL^{-1}	60.14	59.74	98.94	98.28	9.38	10.50	0.29	0.32
1 gL^{-1}	60.65	60.82	102.85	100.06	10.31	10.88	0.31	0.32
2 gL^{-1}	58.43	58.43	106.39	106.74	10.13	11.00	0.32	0.34
3 gL^{-1}	58.81	58.30	105.66	100.58	10.94	11.31	0.31	0.32
4 gL^{-1}	58.84	57.90	100.05	102.03	11.25	11.81	0.31	0.33
5 gL^{-1}	57.08	57.01	114.17	112.01	11.31	12.25	0.32	0.35
L.S.D 5%	n.s.	n.s.	8.08	7.88	1.32	n.s.	n.s.	n.s.

Moreover, the richness of bee pollen with amino acid, carbohydrates, protein and vitamins which may help in enhancing the vegetative growth attributes and improving the flowering characters is the factor that may have a role in increasing the rate of plant photosynthesis which increases the leaves pigments i.e., Chl. a, b and total carotenoides and the accumulation of carbohydrates increases as well. Consequently, the carbohydrates accumulation and metabolism may lead to an increase in the different flowers pigments; carotenoides, xanthophyll and beta-carotene. Besides, the enhancement occurred with carbohydrates may be because of the existence of potassium, Sodium (Na) and zinc which are necessary in carbohydrate metabolism. As well, containing constituents like iron, manganese, zinc and magnesium promotes chlorophyll production and synthesis. As well, having trace elements also could stand behind the increase in the amount of other elements in the plant such as the increase in P which may go back to the existence of manganese, which increases the availability of P and magnesium that improves utilization and mobility of phosphorus. Also, the increment occurred in protein could be interpreted by the fact that having potassium is essential to protein synthesis. In addition some vitamins like B^3, B^6 and folic acid may be responsible for the increase took place in the amount of amino acid in plant. B^2 is involved in electron transport and transfer of hydrogen groups, also B^3 is required for the development of fatty acid metabolism, glucose monophosphate shunt. Furthermore, there are some minerals as manganese, zinc and magnesium that function as a part of many enzymes systems in plants which in turn may cause an improvement in chemical constituents. These results are in correspondence with those obtained by Shafeek *et al.,* 2015 [36], on onion plants.

CONCLUSION

It could be concluded that bee pollen could be considered as a good bio stimulant that has multiple beneficial natural substances (some micro and macro minerals, vitamins, protein, dietary fibers and carbohydrates) which could help in enriching and enhancing the vegetative growth, flowering attributes, chemical and oil constituents of calendula plants especially when it is applied at moderate concentrations (2 and 3 gL^{-1}).

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