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Research Article

Effectiveness of Propolis Aqueous Extract on Chemical Constituents of *Calendula* Plants

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ABSTRACT

This study was performed in order to investigate how the foliar spray of propolis aqueous extract can influence the vegetative growth and flowering characters, chemical constituents and oil composition of Calendula plants. The aqueous extract was applied at four levels (0, 5, 10, 15 and 20 gL^{-1}). It was revealed that propolis was of a positive effect upon all studied parameters; there was an increase done due to the application of the aqueous extract of propolis. The highest results were obtained due to the application of 5, 10 and 15 g L^{-1} from the aqueous extract of propolis compared to 20 g L^{-1} and the control plants. 5 and 10 g L^{-1} gave the highest records of vegetative characters. While, 15 g L^{-1} had the highest records of all flowering attributes. In chemical composition optimum results ranged between 10 and 15 gL^{-1}. The highest percentage of the most important components of the essential oil; sesquiterpene hydrocarbons (cadinene, α-Muurolene and Muurolene) and sesquiter phenols (α- cadinol, α-Cadinol (Epi) and α-Muurolol (Epi)) resulted from 15 g L^{-1}. Hence, it could be suggested that propolise aqueous extract could be sprayed on calendula plants at a rate not exceeding 15 g L^{-1} to get higher results.

Keywords: Propolis, *Calendula officinalis*, foliar application, essential oils and flowers pigments.

INTRODUCTION

Calendula (*Calendula officinalis* L., Asteraceae), known as pot marigold, grows as a wild and common garden. The reason behind gaining a worldwide importance is that it is a crop with a long history of use in medicine (e.g., flos calendulae)[1], ornamental horticulture[2] and produced commercially in parts of Europe (in the northern U.S., especially the northern Corn Belt region) for pharmaceutical uses[3]. Also, due to the fact that being considered as an oilseed crop, primarily because it produces a drying oil comprised largely of a special conjugated fatty acid, calendic acid (C18:3_8trans, 10trans, 12cis) that is highly oxidative, and it can be used to replace volatile organic compounds (VOC) of which reducing its use in industrial chemicals is a major concern of the European Union and United States, as a drying agent in many industrial chemicals including paints, coatings, and adhesives[4]. Nowadays, *C. officinalis* is approved for food use in U.S.A. and appears in the Food and Drug Administration's list of GRAS (Generally Recognized as Safe) substances. *C. officinalis* exhibit a broad range of biological effects, such as antimicrobial and immunomodulatory[5], anti-inflammatory and anti-tumoral[6], antioxidant[7] a property belong mainly flavonoids and carotenoids[8], wound healing[4] and antiviral[9].

Propolis is a term which has its origin from the Greek Pro, "opposite, the entry" and polis, "city or community"[10]. It is the natural substance collected by honeybees from the buds, seedlings, flowers and cracks in the bark of various plants and other botanical sources[11,12]. This adhesive resinous product is mixed by bees with their salivary secretions (mainly β-glucosidase), wax, sugar and pollen[13]. Its colour changes from yellowish green to dark brown according to the place - savannah, tropical forests, desert, coastal and mountainous regions - where it is produced[14,15]. Characteristically, it is a lipophilic material, hard and brittle when cold, but soft, flexible and very sticky when warm. Hence the name "beeswax"[16] so it is water insoluble and semi solid in room temperature with a strong and nice odor[17]. Bees collect this material for many reasons; to exclude draughts, to protect against external invaders (such as microorganisms, fungi and bacteria) and to mummify their carcasses[11,18]. As well as to maintain internal temperature and humidity[19]. It is also used to line the comb, to allow the deposition of eggs by the queen, and to embalm small dead animals (beetles and insects) that usually bees could not take into the hive, preventing its putrefaction[10,11,16]. As for the chemical composition of propolis, it is greatly affected by many factors as the botanical origin of propolis and its time of collection[20]. Also the climate and environmental conditions have an important role in determining the ratio and concentration of the components of propolis[11,14]. All these factors influence the pharmacological properties of propolis. Pena (2008)[16] state that more than 300 chemical compounds have been identified in propolis. Such constituents include

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waxes, resins, balsams, oils and ether, pollen and organic material[21]. Resinous and balsamic compounds are the major constituents in propolis which exist at a rate of (55%). The remaining constituents are beeswax (30%), essential oils (10%), bee pollen (5%), organic compounds (5%; phenolic, esters, and flavonoids) and 5% debris of wood and earth[11,22].

Depending upon these compounds, propolis have multiple and variable uses. It has been widely used in traditional medicine[13] for many years also in dentistry and cosmetics[23]. Many studies has referred to propolis as antimicrobial[18,24], antiviral[25], antiulcer, hypotensive and immunostimulating[11], anti-inflammatory[26], antioxidant[27] and cytostatic[28] activities. Propolis, as well show antibacterial activity which could be explained by the presence of flavonoids, aromatic acids and esters in its composition; bactericidal action resulting from the presence of cinnamic acid and coumarin[29]. As for the extraction of propolis, some studies[30] demonstrated higher antioxidative and inhibitory activities of water-soluble extracts of propolis as compared to ethanolic extract. Therefore, the purpose behind this study was to determine how aqueous extract of propolis, applied as foliar spray can affect the vegetative and flowering parameters, chemical constituents and essential oil components of Calendula plants.

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 study the effect of aqueous extract of propolis on the growth, flowering and some chemical 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.

From the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt the seeds of Calendula plants were kindly obtained then sown in nursery on 17thAugust at the seasons of 2014 and 2015. After 45 days from sowing date on 3rd 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 $(4 \times 1.80) = 7.20$ 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.

For collection, propolis produced by bees in wooden hives was scarped from the cover and entrances of the hives with a stainless steel spatula. The samples were kept in glass bottles at 10 c◦ until used. Propolis samples (5g) were extracted by using distilled water 250 ml, sonicated for 1h, and left to stand for 24 h at 25 c◦, the filtered extract was evaporated to dryness under reduced pressure to yield a brown powder. Dry residue was redissolved distilled water to make up different concentrations (0, 5, 10, 15 and 20 gL^{-1}). The treatments were applied two times on the plants; the first time at 30 days after transplanting and the second is one month after the first application. Few drops of Triton B were added to the spray solution to serve as a wetting agent. The plants were sprayed twice at elongation and flowering stages of stem. 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 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 75 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), fresh and dry matter of leaves plant^{-1} (g), fresh and dry matter of shoots plant^{-1} (g) of herb, flowers number plant^{-1}, inflorescences diameter plant^{-1}, inflorescences fresh and dry matter plant^{-1}, fresh and dry matter of ray flowers plant^{-1} and seed mass plant^{-1}.

Gas Chromatography-Mass Spectrophotometric (GC-MS) analysis

the components of essential oil extracted from Dry flower-head of calendula were determined according to[31].

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[32].

The determination of Chlorophyll a, b and total carotenoids in leaves was done according to the method of[33].

Pigments in dry flowers (Beta-carotene and xanthophylls) mg g^{-1}: the pigments were determination at last collection was described according to the method of[34].

Xanthophyll content was determined at wavelength of 470 and 485 nm according to[35].

Total flavonoids (mg g^{-1} D. M.) were determined in dried flower-head according to[36].

Total carotenoids in dry ray flowers (mg g^{-1} D. M.) were determined in dried flower head according to[37].

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[38].

Total N (a factor of 6.25 was used for the conversion of N% to protein%[39], P and K, in herb and seeds were

Table 1: Some characteristics of the experimental site.

Years	Mechanical analysis				
	Sand %	Silt %	Clay %	Texture class	Hydraulic conductivity (cm3/hr)
2014	31.25	31.23	35.77	Sandy clay	0.029
2015	32.83	31.97	37.85	Sandy clay	0.027

Years	Chemical properties										
	N	P	K	Fe	Zn	Mg	Mn	EC dSm^{-1}	pH	CaCo3 %	Organic matter%
2014	17.48	23.33	96.43	3.39	0.64	0.29	8.55	2.79	7.51	4.66	1.24
2015	18.73	22.57	97.88	3.77	0.81	0.35	8.64	2.88	7.48	4.87	1.26

Table 2: Effect of foliar application of propolis 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)		Leaves F. w.plant^{-1} (g)		leaves D w plant^{-1} (g)		shoots F w plant^{-1} (g)	
0 gL^{-1}	73.00	72.00	68.70	69.80	9.80	9.80	75.40	71.20
5 gL^{-1}	76.00	80.00	109.50	114.10	15.10	15.10	83.50	84.50
10 gL^{-1}	78.00	82.00	90.80	94.60	13.40	12.40	84.00	95.20
15 gL^{-1}	77.00	80.00	80.00	81.90	10.70	10.70	80.10	82.40
20 gL^{-1}	76.00	77.00	74.60	76.30	9.60	9.30	76.40	81.10
L.S.D 5%	n.s.	n.s.	24.70	27.20	3.37	2.70	n.s.	n.s.
	shoots D w plant^{-1} (g)		Flowers no. plant^{-1}		inflorescences diameter (cm)		inflorescences FW plant^{-1} (g)	
0 gL^{-1}	9.70	9.00	33.00	28.00	6.20	6.10	59.60	60.00
5 gL^{-1}	10.20	11.40	34.00	30.00	7.90	7.90	62.60	62.50
10 gL^{-1}	9.40	10.70	37.00	36.00	7.30	7.90	75.00	75.30
15 gL^{-1}	9.50	9.90	43.00	41.00	8.30	8.70	80.20	96.00
20 gL^{-1}	9.50	9.50	35.00	37.00	6.80	6.90	61.30	61.60
L.S.D 5%	n.s.	n.s.	5.00	8.00	0.72	0.90	n.s.	n.s.
	inflorescences D w plant^{-1} (g)		F w of ray flowers plant^{-1} (g)		D w of ray flowers plant^{-1} (g)		Seeds wt. plant^{-1} (g)	
0 gL^{-1}	15.10	15.10	66.30	68.30	6.80	6.70	10.76	10.45
5 gL^{-1}	16.50	16.90	69.90	75.20	7.80	7.90	11.66	11.66
10 gL^{-1}	17.80	17.10	78.20	79.00	8.70	8.60	13.82	12.27
15 gL^{-1}	18.80	18.90	82.20	83.30	9.00	9.20	13.97	14.88
20 gL^{-1}	16.50	16.50	74.60	74.40	7.30	8.00	11.47	12.86
L.S.D 5%	1.88	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

determined according to the methods of the[40]. Zinc, manganese and iron contents in herb and seeds were determined using atomic absorption spectrophotometer according to the method described by[40].

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[41].

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. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of significance.

RESULTS

Vegetative growth and flowering yield characters

All concentrations of aqueous extract of propolis (5, 10, 15 and 20 gL^{-1}) sprayed on calendula plants raised the values of all vegetative growth and flowering attributes (Table 2) as compared to the control. Concerning vegetative growth parameters, 5 gL^{-1} caused the significant increment compared to the other three concentrations; as that increase started to abate with escalating the concentration. This was significantly resulted with the fresh and dry weights of leaves plant^{-1}, and shoots dry weight plant^{-1} but 10 gL^{-1} not significantly increased plant height and the shoots fresh weight plant^{-1} in both seasons of study respectively. Regarding flowering characters, foliar spray of propolise increased the parameters investigated but 15 gL^{-1} was the prominent to result in the highest estimations of flowers number plant^{-1}, inflorescences diameter and fresh and dry weight plant^{-1}, fresh and dry weight of ray flowers plant^{-1} and seeds weight plant^{-1}. There were no significant differences shown between mean values except for flowers

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

Treatments	0 gL^{-1}	5 gL^{-1}	10 gL^{-1}	15 gL^{-1}	20 gL^{-1}
Components (%)	sesquiterpene hydrocarbons				
-cadinene	24.43	25.43	27.33	29.74	24.94
α-Muurolene	0.55	0.58	0.58	0.69	0.65
-Muurolene	0.36	0.44	0.55	0.70	0.51
α-Pinene	0.27	0.30	0.34	0.36	0.30
β-Pinene	0.09	0.14	0.17	0.13	0.10
Sabenene	0.22	0.32	0.55	0.53	0.23
α- phellandrene	0.10	0.15	0.18	0.24	0.19
α-Humulene	0.37	0.36	0.46	0.46	0.44
Limonene	0.14	0.18	0.19	0.23	0.19
β- cymene	0.42	0.52	0.71	0.64	0.61
aromadendrene (allo)	0.23	0.24	0.53	0.44	0.29
-Gurjunene	0.46	0.62	0.83	0.80	0.58
β-Patchoulene	2.41	2.62	3.38	3.34	2.58
β-Calacorene	0.48	0.58	0.71	0.57	0.55
sesquiterphenols					
α- cadinol	22.64	23.69	25.81	27.04	26.85
α-Cadinol (Epi)	1.61	2.07	1.94	2.16	1.73
α-Muurolol (Epi)	10.34	10.96	11.21	11.26	10.62
α-Eudensmol	4.74	5.75	5.63	6.93	5.41
α-Eudesmol (7-epi)	0.98	1.49	1.09	1.32	1.45
Nerolidol (E-)	0.69	0.76	1.10	1.03	0.81
1.8 cineol	0.46	0.47	0.81	0.80	0.47
α-Bisabolol	0.67	1.05	1.40	1.02	1.23
α-terpeneol	0.98	0.91	1.26	1.32	1.03
Geraniol	0.48	0.51	0.85	0.83	0.58
Carv Carvacrol	0.67	0.79	1.13	1.02	0.99

number $plant^{-1}$ and inflorescences dry weight $plant^{-1}$ in the first season and inflorescences diameter in both seasons.

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

All concentrations of propolis led to an increase in the whole constituents of plant essential oil detected by GC-MS (Table 3) especially with regard to 10 gL^{-1} and 15 gL^{-1}. Foliar spray of propolis at 10 gL^{-1} led to having the highest percentages of Sabenene, α-Humulene, β- cymene, aromadendrene (allo), Gurjunene, Gurjunene, β-Patchoulene and β-Calacorene. Whereas the highest percentage of cadinene, α-Muurolene, Muurolene, α-Pinene, α- phellandrene, Limonene, α- cadinol, α-Cadinol (Epi), α-Muurolol (Epi), α-Eudensmol and α-terpeneol was obtained by 15 gL^{-1}.

Chemical constituents

The use of aqueous extract of propolise was of an apparent influence upon the chemical constituents of calendula plants compared to the non treated plants especially with regard to 15 gL^{-1} which had the highest records with regard to head flower essential oil $plant^{-1}$, chl. a and b, total carotenoids, total flavonoides, crotenoides, Xanthophyll and beta-carotene in ray flowers, Zn and Mn in herb and in seeds and total free amino acids in herb in both seasons (Table 4). The highest significant rates of differences between values were clearly obtained with zn content in herb and in seeds and Mn content in seeds in both seasons. Whereas total carbohydrate percentage in stems and in leaves, N, P, K and Fe in herb an in seeds and protein in herb show higher affectedness with 10 gL^{-1} especially when compared with the highest concentration of propolis (20 gL^{-1}) with which the values began to be decreased that its records were approximately equal to those of 5 gL^{-1} and even under its level in some attributes as in total carbohydrate percentage in stems, N in herb and in seeds, P in herb and Fe in seeds in both seasons, P in seeds and K in herb in the first season and total carbohydrate percentage in leaves and Fe in herb in the second season.

DISCUSSION

Propolis extract undergoes qualitative tests which proved that it contain sterols, flavonoids and phenolic compounds. As well as, few numbers of phenolic acids (coumaric, ferulic, salicylic, and benzoic acid) were also detected on TLC plates[42].

The current study clarified the enhancing and stimulatory effect of propolis aqueous extract as foliar spray on calendula plants. That it increased its vegetative growth and flowering characters, chemical constituents and the essential oil components. This actually may be interpreted by the wide range of beneficial constituents that propolis has. As stated by Marcucci (1995)[16], Burdock (1998)[11], Marcucci *et al.*, (2000)[43], Ahn *et al.,* (2007)[44] propolis includes variable organic acids, considerable amount of minerals (including, manganese, zinc, calcium, phosphorus, copper); which may contribute in raising their

Table 4: Effect of foliar application of propolise 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.	Head flower essential oil plant^{-1} (ml)		Chl. a (mg g^{-1} F.M.)		Chlorophyll b (mg g^{-1} F. M.)		Total carotenoids (mg g^{-1} F. M.)	
0 gL^{-1}	0.05	0.06	0.79	0.81	0.57	0.61	0.41	0.42
5 gL^{-1}	0.06	0.07	0.87	0.85	0.65	0.65	0.48	0.46
10 gL^{-1}	0.08	0.08	0.93	0.88	0.69	0.68	0.50	0.51
15 gL^{-1}	0.09	0.10	0.97	0.98	0.78	0.79	0.58	0.59
20 gL^{-1}	0.08	0.07	0.88	0.86	0.63	0.62	0.49	0.48
L.S.D 5%	0.02	0.02	n.s.	n.s.	0.06	0.10	0.09	0.11
	Total flavonoides in ray flowers (mg g^{-1} D. M.)		Total crotenoides in ray flowers (mg g^{-1} D. M.)		Xanthophyll in ray flowers (mg g^{-1} D. M.)		Beta-carotene in ray flowers (mg g^{-1} D. M.)	
0 gL^{-1}	15.50	14.40	1.98	1.94	1.47	1.45	1.52	1.57
5 gL^{-1}	16.80	15.60	2.10	2.04	1.53	1.51	1.63	1.72
10 gL^{-1}	17.20	17.00	2.28	2.13	1.65	1.62	1.70	1.85
15 gL^{-1}	18.80	19.80	2.53	2.67	1.77	1.75	1.81	1.93
20 gL^{-1}	16.80	16.90	2.06	2.06	1.54	1.59	1.73	1.79
L.S.D 5%	n.s.	2.60	0.31	0.24	n.s.	n.s.	n.s.	n.s.
	Total carbohydrate percentage in stems (%)		Total carbohydrate percentage in leaves (%)		N % Herb		N % Seeds	
0 gL^{-1}	24.40	24.50	18.50	19.00	1.55	1.46	2.20	2.10
5 gL^{-1}	27.40	27.10	20.00	21.30	1.79	1.58	2.60	2.60
10 gL^{-1}	29.60	29.10	22.80	24.30	1.90	1.92	2.80	2.80
15 gL^{-1}	28.50	28.30	20.80	21.10	1.86	1.77	2.50	2.40
20 gL^{-1}	26.50	25.80	20.10	20.50	1.61	1.50	2.40	2.30
L.S.D 5%	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	P % Herb		P % Seeds		K % Seeds		K % Herb	
0 gL^{-1}	0.37	0.39	0.49	0.48	1.53	1.54	2.07	2.33
5 gL^{-1}	0.40	0.42	0.55	0.49	1.59	1.58	2.42	2.36
10 gL^{-1}	0.48	0.49	0.78	0.79	1.71	1.78	2.83	3.25
15 gL^{-1}	0.43	0.45	0.61	0.65	1.61	1.63	2.62	2.62
20 gL^{-1}	0.40	0.39	0.55	0.57	1.64	1.63	2.36	2.40
L.S.D 5%	0.05	0.06	0.13	0.18	n.s.	n.s.	0.27	0.54
	Fe (ppm) Seeds		Fe (ppm) Herb		Zn (ppm) Herb		Zn (ppm) Seeds	
0 gL^{-1}	497.00	490.00	235.00	248.00	47.10	46.70	49.20	51.10
5 gL^{-1}	519.00	492.00	265.00	262.00	54.90	49.10	56.60	58.00
10 gL^{-1}	585.00	616.00	294.00	300.00	63.80	62.30	65.60	62.40
15 gL^{-1}	525.00	523.00	277.00	274.00	69.40	72.50	68.90	71.40
20 gL^{-1}	500.00	468.00	268.00	261.00	50.80	50.40	58.20	56.60
L.S.D 5%	55.00	n.s.	n.s.	n.s.	12.50	11.20	11.50	6.80
	Mn (ppm) Herb		Mn (ppm) Seeds		Protein (%) Herb		Total free amino acids (%) Herb	
0 gL^{-1}	49.70	49.80	87.60	89.50	9.71	9.15	0.28	0.29
5 gL^{-1}	56.70	55.40	102.10	94.10	11.17	9.90	0.31	0.32
10 gL^{-1}	59.00	59.80	110.90	110.20	11.90	12.02	0.34	0.35
15 gL^{-1}	61.20	61.30	124.80	129.30	11.63	11.04	0.38	0.39
20 gL^{-1}	58.00	56.50	99.30	99.60	10.08	9.35	0.32	0.32
L.S.D 5%	n.s.	n.s.	20.60	16.50	n.s.	n.s.	0.05	0.04

concentration in the plant and the pigments in leaves and ray flowers; vitamins B1, B2, B6, C and E, acids (nicotinic acid and pantothenic acid) and amino acids which could played a role in increasing the amount of amino acid and the protein in the plant. According to Manara *et al.*, (1999)[29], nowadays there are multiple substances known in propolis with distinct chemical structures from following classes: alcohols, aldehydes, aliphatic acids, aliphatic esters, aromatic acids, aromatic esters, ethers, ketones, terpenoids and steroids; that could stand behind the increase in the essential oil components such as sesquiterpene hydrocarbons and sesquiterphenols;

flavonoids, hydrocarbohydrates esters, fatty acids and sugars that may have a share in the increment in the carbohydrates amount in the plant. Flavonoids, aromatic acids, diterpenic acids and phenolic compounds are considered the principal components that stand behind the biological activities of propolis[45]. Moreover, the powerful antioxidant compounds in propolis are due to a large extent to flavonoids that protect lipid and other compounds such as vitamine C from being oxidized or destroyed[46] and regulate plant growth[47]. In addition, the apparent effectiveness of propolis may be due to the way it was extracted; as Santos (2012)[21] reported, the aqueous extract has good antioxidant activity, associated with high content of phenolic compounds.

The results obtained in this study is in harmony with those obtained by Noweer and Mona (2009)[42] who stated the efficacy of propolis extract on faba bean plants as foliar application on chlorophyll a and b, cartotenoids, protein and carbohydrates content and shot dry weight plant^{-1}.

CONCLUSION

It can be concluded that propolis is that natural material which proved high benefit for Calendula plants growth and flowering attributes, chemical and essential oil constituents especially when used at 10 and 15 gL^{-1}.

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