

INFLUENCE OF SUPPLYING SOME SAFE NATURAL HONEY BEE PRODUCTS ON FENNEL PLANTS GROWTH, SEEDS YIELD, OIL PRODUCTION AND ITS COMPONENTS

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ABSTRACT: Honeybee products as propolis (Pp) and royal jelly (RJ) are natural mixtures and powerful source of safe nutrients that could be safely used in agriculture as substitution of poisonous and dangerous chemical fertilizers. The impact of using them either solely or in combination as foliar application on some fennel morphological traits, fruit and oil yield and its essential components and chemical components was studied. RJ and Pp were foliar sprayed at three rates i.e. 0.0, 0.2, 0.4% and 0, 3, 6 g l⁻¹ respectively in sole and combination treatments. All records assured that the application of both materials (RJ, Pp) have a positive useful impact on all traits studied either used individually or in combination. The moderate concentrations of both materials individually or in combination (0.2 % RJ, 3 g l⁻¹ Pp) gave the highest records of growth and yield characters as well as anethole in the oil compared with other treatments with more superiority of RJ results over those of Pp in sole treatments even though RJ concentration is less than that of Pp especially in oil percentage and umbels number. On the other side, the highest concentration of either RJ (0.4%) or moderate one of Pp (3 g l⁻¹) as sole treatment or in combination gave the highest records of most chemical composition. Also 0.4% RJ with 6 g l⁻¹ Pp produced the highest percentage of estragole. Hence, fennel plants can be safely grown and highly produced by these safe natural materials without the help of the chemical fertilizers.

Key words: *Foeniculum vulgare*, propolis, Royal jelly, estragole, anethole and essential oil.

INTRODUCTION

Fennel, (*Foeniculum vulgare* Mill.), the highly aromatic herb and characterized by aniseed flavour is native to the Mediterranean regions that has become widespread in different parts in the world (Barros *et al.*, 2010). Fennel is one of the vital medicinal and economic plants which is used as flavoring agent in many food products such as bread, liqueurs, pickles, pastries and cheese (Zoubiri *et al.*, 2014). Also, fennel fruits have anticancer (Anand

et al., 2008) antioxidant, antimicrobial (Ruberto *et al.*, 2000), hepatoprotective (Ozbek *et al.*, 2004) and antihypertensive activities (Ono *et al.*, 1996). Moreover, effectiveness of fennel essential oil as acaricidal (Lee, 2004), antifungal (Singh *et al.*, 2006), insecticidal and repellent against insects (Bertoli *et al.*, 2012) emmenagogue and galactagogue (Babu *et al.*, 2010) and in reducing infantile colic (Alexandrovich *et al.*, 2003) has been evaluated as well. All these marvelous attributes may turn back to its richness in carbohydrates, including

sugars (Cataldi *et al.*, 1998), minerals (Ozcan *et al.*, 2008) and essential fatty acids (Vardavas *et al.*, 2006), protein and fiber (Boori *et al.*, 2017). It is also known as a spice due to terpenic compounds isolated from volatile oil of its fruit (Abdallah *et al.*, 1978). Antioxidant activity of sweet fennel cultivars is attributed to presence of flavonoids, phenolics, ascorbic acid and other natural active components (Salama *et al.*, 2015). *F. vulgare* as an excellent flavouring agent is due to its essential oil that is characterized by anise odor. Major essential oil components of *F. vulgare* seed were identified to be transanethole, fenchone, estragol (methyl chavicol), and α -phellandrene, (Rather *et al.*, 2016). The relative concentration of these compounds are various based on origin of the fennel and phonological state (Diaaz-Maroto *et al.*, 2006). The geographical origin and method of extraction may also change the composition of *F. vulgare* essential oil (Rather *et al.*, 2016).

Due to people awareness and eagerness towards healthy products, the use of alternate natural materials as honeybee products in agriculture has paid the attention recently. Honeybee products such as propolis and royal jelly (RJ) are natural mixture and powerful source of safe nutrients that could be used safely in dispense of poisonous and dangerous chemical fertilizers. Propolis (bee glue) is a natural resinous hive product collected from leaf buds and flowers by bees to protect and save the hive from the natural phenomena and intruders (Babaei *et al.*, 2016). The importance of propolis goes back to what it contains; polyphenols such as flavonoid aglycones, phenolic acids and their esters, alcohols, phenolic aldehydes and ketones. In addition, it contains steroids, terpenoids, inorganic compounds and amino acids (Bankova, 2005). The Chemical composition of propolis differs widely due to climate and geographical conditions based on its floral origin (Seidel *et al.*, 2008). These components may be related to the various biological properties of propolis such as antifungal, antibacterial, antioxidant,

antiviral, hepatoprotective and immunostimulating activities (Piccinelli *et al.*, 2011)

Royal jelly (RJ) is nowadays used in several manufacturing sectors such as pharmaceutical, food industries and cosmetic. Also, RJ is whity material with a gelatinous consistency, often not homogenous owing to the presence of various undissolved granules size and has a clearly sharp smell and taste (Sabatini *et al.*, 2009). The main components of RJ are carbohydrates, proteins and lipids (Garcia-Amoedo and Almeida-Muradian, 2007). It is processed by mixing of water, honey and pollens with hormones, vitamins and saliva (Nassef and El-Aref 2016). Therefore, RJ is considered as a good source of vitamins particularly B vitamin, pheromones and minerals. RJ as a special food produced by bee queen was used for human health and traditional and folkloristic medicine, particularly in Asia since ancient times. RJ also has a high activity against Gram-positive bacteria as well as having many activities such as immunomodulatory, inflammatory, metabolic syndrome preventing, neuromodulatory, antiaging activities, antioxidant, antibacterial, antiviral and antifungal (Guo *et al.*, 2008; Viuda-Martos *et al.*, 2008; Cornara *et al.*, 2017).

Based on the review of literature there is no publication available for utilization of honeybee products as propolis (Pp) and/or royal jelly (RJ) or natural mixture of them as powerful source of safe nutrients in agriculture safely as substitution of poisonous and dangerous chemical fertilizers. Therefore, the main objective of this study is to investigate the influence of using them either solely or in combination as foliar application on some morphological traits, seed yield and oil productivity and its main components as well as chemical components of fennel plants.

MATERIALS AND METHODS

Plant material and growing conditions:

The present study was done at the Experimental Farm in Faculty of

Agriculture, Fayoum University, Egypt, within two successive seasons (2017/2018 and 2018/2019) with the aim of investigating the individual and combined effect of both Royal jelly queens (RJ) and propolis (Pp) aqueous extract on the vegetative characters, seed and oil yield and its quality components and chemical constituents of fennel plants.

Fennel seeds were given by the Department of Medicinal Plants Production, National Research Center, Egypt and sown on 9th and 8th of Oct. (for the two seasons, respectively). Five seeds were sown in each hill at 35 cm apart and then were thinned (at the age of 30 days) to two plants with receiving the normal needed agricultural practices.

The experiment layout used was factorial experiment in complete randomized block design system with three replicates containing 3 plots with four rows. The plot area was $2.8 \times 2.4 = 6.72 \text{ m}^2$ including 4 rows with 60 cm apart and 2.8 m in length.

In addition, the treatments of manure applied on plants were $25 \text{ m}^3/\text{fed}$, and (200: 200: 100) kg/fed/season of NPK fertilization. The NPK composition was ammonium sulphate (20.6% N), calcium super/phosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) according to Mohamed (2009). Calcium superphosphate was applied as one dose during the soil preparation. half of the N and K rates were added after 50 days from sowing with 30 days interval between it and second addition. Before any practices to be done, the physical and chemical analyses of the soil were determined according to Jackson (1973) and Black *et al.* (1982), respectively as shown Table (1).

Preparation and analysis of chemical constituents of RJ and Pp:

RJ extract and Pp were added thrice/season as foliar application at 0.0, 0.2, 0.4% and 0, 3, 6 g l^{-1} respectively. Triton B was used as a wetting agent to the spray solution at 0.1%. The first spray was applied 50 days after sowing with 3 and 6 weeks as

an interval between it and the second and the third sprays, respectively.

RJ treatments were obtained from the Experimental Farm Apiary (bee yard) in the Faculty of Agriculture, Fayoum Univ. on March 2017 and 2018 and stored in the freezer till it was applied as foliar application. Phytochemical composition of RJ characteristics is shown in Table (2).

Propolis sample (5 g) was brought by scarping the cover and entrances of the hives by a stainless-steel spatula. and kept at $10 \text{ }^\circ\text{C}$ in glass bottles and then extracted by distilled water of 250 ml, sonicated for 1 h, and left to stand for 24 h at $25 \text{ }^\circ\text{C}$. The extract was filtered and evaporated to dry under reduced pressure for a brown powder (Chen *et al.*, 2004) to be obtained. The dry residue was re-dissolved in distilled water for making up different concentrations (0, 3 and 6 g l^{-1}). Propolis chemical characteristics were determined by GC/MS as presented in Table (3).

Pp was applied twice on plants once at the vegetative stage and the other at the beginning of the flowering stage. The control plants were sprayed with a distilled water. The spraying solution volume was maintained just for covering completely the plant foliage until drip. The agricultural practices operations other than experimental treatments needed for the growth and development of plants such as cultivation, fertilization, irrigation and pest control were undertaken and done whenever it was necessary.

Growth and fennel yield measurements:

When plants reached 5 months and half old (within vegetative stage), the first and the fourth rows of each plot were taken and cut off at ground level from each experimental unit for submitting the next determinations: plant height (cm), branches number plant^{-1} , fresh (fw) and dry weight (dw) plant^{-1} (g) and weight (g) and length (cm) of the longest root plant^{-1} .

Table 1. Some initial physico-chemical characteristics of the studied soils.

Years	Mechanical analysis				Hydraulic conductivity (cm ³ hr ⁻¹)
	Sand %	Silt %	Clay %	Texture class	
2017	10	20	70	clayey	0.028
2018	9	21	70	clayey	0.029

Years	Chemical properties							EC (dS m ⁻¹)	pH	CaCO ₃ (%)	Organic matter (%)
	Mineral elements (mg kg ⁻¹ soil)										
	N	P	K	Fe	Zn	Mg	Mn				
2017	17.57	223.7	696.6	3.73	0.89	0.37	8.73	1.91	7.52	3.87	1.11
2018	18.67	229.8	699.9	3.66	0.87	0.39	8.78	1.89	7.49	3.91	1.15

Table 2. Phytochemical composition of (RJ) assessed by GC-MS.

Parameter	DPPH	ABTS	FRAB
IC50	0.320	0.16	0.13
TEAC (μmol Trolox/g)	271.17	553.38	674.54
Vit C (μmol Vit C/g)	256.55	523.56	638.19
Vit E (μmol Vit E/g)	226.01	461.23	562.22
TEAC (g Trolox/g)	0.0679	0.139	0.169
Vit C (g Vit C/g)	0.0452	0.092	0.112
Vit E (g Vit E/g)	0.0973	0.199	0.242
Antioxidant capacity	EC50	0.320	0.13
	ARP	3.125	7.77

Table 3. Chemical composition of propolis (Pp) and extracts identified by GC/MS.

Parameter (units)	Propolis	Parameter (units)	Propolis
Total terpenoids (%)	2.21	Potassium (ppm)	160.00
Total flavonoids (%)	0.22	Magnesium (ppm)	49.00
Phenolic acids (%)	0.33	Calcium (ppm)	63.00
Total sugars (%)	1.32	Iron (ppm)	21.00
Total amino acids (%)	0.23	Mn (ppm)	11.00
Ascorbic acid; vitamin C (ppm)	94.00	Iodine (ppm)	9.00
Total B-group vitamins (ppm)	155.00	Zn (ppm)	8.00
Vitamin E (ppm)	62.00	Cu (ppm)	5.00

Oil production and yield attributes:

With reaching 190 days old, the central rows were chosen from each experimental unit for estimating the following yield attributes: umbels number plant⁻¹, fruit yield plant⁻¹ (g), essential oil (%) in fruits (seeds) determined using water distillation methods and volatile oil yield plant⁻¹ (ml) calculated in proportion to fruit weight as following:

$$\text{Oil yield plant}^{-1} \text{ (ml)} = \frac{\text{fruit yield} \times \text{oil content (\%)}}{100}$$

Essential oils distillation (extraction):

Essential oil was quantified gravimetrically by hydro-distillation according to the European Pharmacopoeia (EP) (C.O.E., 1997). Each plot fruit samples were hydro-distilled for 2 h by a Clevenger-type apparatus. The essential oil content was

quantified gravimetrically. Each sample was analyzed in three replications and the average was used for statistical analyses. The volatile oil concentration was calculated as the amount (g) of dry fennel fruits oil weight (g), while the oil yield/area was calculated from the area of fruit yields and oil content of every fennel accession and replicate. For determining the essential oil content of seeds (v/w%), 100 g of powdered fennel samples in 500 ml of water were extracted from each plant population.

The oil was dried using anhydrous sodium sulphate Na₂SO₄. The essential oil obtained was kept at 4 °C in dark glass containers for further lab analyses, 1.0 ml (density = 1.04 g ml⁻¹) (Cheronis and Entrikin, 1963).

Gas chromatography-mass spectrometry analysis of essential oil (GC-MS Conditions):

Gas chromatography–mass spectrometry analysis (GC-MS) was done by the use of Agilent auto system 7890B GC-MS and equipped with HB-5MS capillary column (5% phenyl–95% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 µm). Helium with flowrate of 1 ml/min was used as the carrier gas. Heat temperature was kept at 50 °C for 5 min and programmed to 250 °C at 5°C/min which was then fixed at 250 °C for 10 min.

The injector, GC–MS interface, ion source and mass detector temperature was maintained at 230, 270, 200 and 150 °C, respectively. Mass spectra were taken at 70 eV. Scan duration lasted for 0.25 sec and mass rang was 50–500 Da. The injection of sample 1µl was at a split ratio of 1:50 with the ionization performed in the EI ion source (70 eV) and the acquisition mass range was set at 35-500 amu.

Identification of components was based on comparison of their mass spectra (using molecular ion (M⁺) peak and the m/z values) with those provided in mass spectra library and in literature. The percentages of the relative peak area were the point to report the abundance of a compound in the extracts.

Chlorophyll fluorescence measurements:

The content of pigments in leaves was estimated in fresh chosen samples of leaves and were extracted by 80% of acetone and colorimetrically determined by Metzner *et al.* (1965).

The content of chlorophyll, calculated as mg/g fresh weight of leaves, was spectrophotometrically analyzed in a UV visible spectrophotometer (Optizen Pop, Mecasys-Korea) by 3 ml sealed quartz-glass cuvettes with 1 cm path length and chlorophyll a, b and carotenoids wave length of 663 µm, 644 µm and 452.5 µm, respectively.

Determination of macronutrient concentrations, total carbohydrates and Total soluble sugar:

The content of total macro minerals (N, P and K) in fennel leaves were estimated after being ground and wet washing. Total nitrogen was estimated using semi-micro Kjeldahle method according to Black *et al.* (1982). Spectrophotometer was used for determining total phosphorus and a flame photometer was used for estimating the potassium leaf content photometrically as described by (Jackson, 1973).

The total carbohydrates (%) was determined in powdered dry matter of fennel herb determined color-metrically at the early flowering stage according to Herbert *et al.* (1971).

Total soluble sugars concentration of fennel fruit was estimated according to Irigoyen *et al.* (1992), by a UV-160A UV Visible Recording Spectrometer, Shimadzu, Japan.

Antioxidant enzyme assays:

According to Mukherjee and Choudhuri, (1983) the plant samples were prepared as a fresh sample (250 mg) was frozen in liquid nitrogen and finely ground by pestle in a chilled motor which then was added to 10 ml of 100 mM phosphate buffer (KH₂PO₄ /K₂HPO₄, pH 7.0) containing 0.1 mM Na₂EDTA and 0.1g of polyvinyl pyrrolidone (PVP). The homogenate was filtered by cheesecloth then centrifuged at 15000 g for 10 min. The supernatant was recentrifuged at 18000 g for 10 min and then collected and stored at 4 °C for assay of catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX).

CAT (EC 1.11.1.6) activity: CAT activity is assessed by the decrease of absorbance at 240 nm as a consequence of H₂O₂ consumption and was expressed according to (Havir and Mellate, 1987).

POD (EC 1.11.1.7) activity: POD activity is estimated according to (Chance and Maehly, 1955).

APX (EC 1.11.1.11) activity: APX activity is measured from the decrease in absorbance ascorbic at 290 nm as ascorbic acid oxidized (Asada and Chen, 1992).

Malondialdehyde (Lipid peroxidation MDA) measurements:

A level of lipid peroxidation was determined through malondialdehyde (MDA) content by Heath and Packer (1968) method

Statistical analysis:

Appropriate analysis of variance was performed on gained data. Comparisons among means of treatments were performed using the Revised Least Significant Difference procedure at $P=0.05$ level as illustrated by (Waller and Duncan, 1969).

RESULTS

Effects of Pp and RJ on morphological traits and yield components as a sole treatment:

As Table (4) indicates, there was increment in all growth characters over the control because of the use of RJ or Pp as sole treatment in both seasons. The most rate to give the highest result was the moderate one of either substance (0.2% RJ, 3 g l⁻¹ Pp) with significant differences between results in all traits of vegetative growth except for Pp results of plant height which show insignificant differences.

The same trend was found in yield components as 0.2% of RJ fruited significantly with the highest fruit weight plant⁻¹, oil content plant⁻¹, umbels number and oil percentage in both seasons. Concerning the effect of Pp on oil yield, 3 g l⁻¹ of Pp significantly resulted in the highest fruit weight plant⁻¹, oil content plant⁻¹ while the highest concentration of Pp significantly produced the highest values of umbels number and oil percentage in both seasons.

Influence of the interaction between Pp and RJ on morphological traits and yield components:

The interaction between RJ and Pp was of obvious positive significant effect on all growth and yield traits over the control. The mixture between the moderate rates of both materials together (0.2% RJ and 3 g l⁻¹ Pp) gave the best results of most growth and yield characters; fw and dw plant⁻¹, root length and root weight plant⁻¹, fruit weight and oil percentage and content. RJ at 0.4% with Pp at 3 g l⁻¹ gave significantly the tallest plants in both seasons. However, the highest rates of both materials (RJ and Pp) when sprayed together gave greatly and significantly the highest number of umbels in the two successive seasons of study. The highest number of branches ranged between 0.2% RJ with 6 or 4 g l⁻¹ of Pp in the 1st and 2nd seasons respectively (Table, 4).

Effects of Pp and RJ on chemical constituents and anti-oxidant enzyme assays:

The use of either material (RJ and Pp) solely showed positive significant effect on all chemical components studied (Table, 5) over the control in both seasons. The most effective rate in this regard was 0.4% of RJ which gave the highest values of ch. a, b, carotenoids, total carbohydrates and content of N and P, ascorbate peroxidase (APX), catalase (EU mg⁻¹) protein and peroxidase (POD). However, the moderate rate of RJ (0.2%) had the highest results of percentage of K (%) and total soluble sugars of fennel seed. On the other side, most of the chemical traits were highly increased by the moderate concentration of Pp (3 g l⁻¹). But this was not true for the K (%), total soluble sugars of fennel seed, Catalase EU mg⁻¹ protein and peroxidase (POD) which showed increment with the highest rate of Pp (6 g l⁻¹) in both seasons. There were not significant differences between all records of ascorbate peroxidase in the first season however these differences were not found only between values of the moderate and the highest concentration in the second season with the

Table 4. Effects of Pp and RJ aqueous extract as foliar application and their interaction on some fennel morphological traits and yield components.

Royal jelly (RJ)	Propolis (Pp)			Mean				
	0 g l ⁻¹	3 g l ⁻¹	6 g l ⁻¹	0 g l ⁻¹	3 g l ⁻¹	6 g l ⁻¹	Mean	
	1 st season (2017/2018)							
	2 nd season (2018/2019)							
	Plant height plant⁻¹ (cm)							
0.0%	87.67 d	116.67 ab	120.00 a	108.11 a	81.00 e	121.67 a-c	120.00 bc	107.56 a
0.2%	96.67 cd	123.33 a	116.67 ab	112.22 a	96.67 d	126.67 ab	116.67 bc	113.33 a
0.4%	106.67 bc	126.67 a	93.33 d	108.89 a	110.00 c	133.33 a	96.67 d	113.33 a
Mean	97.00 c	122.22 a	110.00 b		95.89c	127.22 a	111.11 b	
	Branches number plant⁻¹							
0.0%	3.33 c	4.00 a-c	3.33 c	3.56 b	3.33 d	4.33 bc	4.67 ab	4.11 b
0.2%	4.33 ab	4.33 ab	4.67 a	4.44 a	5.33 a	5.33 a	4.67 ab	5.11 a
0.4%	3.33 c	4.00 a-c	3.67 bc	3.67 b	3.67 cd	4.33 bc	4.33 bc	4.11 b
Mean	3.67 b	4.11 a	3.89 ab		4.11 a	4.67 a	4.56 a	
	Fresh weight plant⁻¹ (g)							
0.0%	190.33 e	401.33 bc	239.67 de	277.11 b	194.67 e	403.67 bc	237.33 de	278.56 b
0.2%	449.00 b	515.00 a	420.00 b	461.33 a	459.33 ab	522.33 a	424.67 bc	468.78 a
0.4%	281.67 d	356.33 c	274.00 d	304.00 b	286.00 d	377.33 c	276.33 d	313.22 b
Mean	307.00 b	424.22 a	311.22 b		313.33 b	434.44 a	312.78 b	
	Dry weight plant⁻¹ (g)							
0.0%	45.92 f	96.17 bc	58.25 e	66.78 c	50.33 e	101.75 bc	61.00 de	71.03 b
0.2%	73.92 d	129.58 a	101.67 b	101.72 a	96.50 bc	129.75 a	106.17 b	110.81 a
0.4%	67.25 de	89.75 c	69.33 d	75.44 b	71.33 d	90.33 c	71.58 d	77.75 b
Mean	62.36 c	105.17 a	76.42 b		72.72 b	107.28 a	79.58 b	
	Root length plant⁻¹ (cm)							
0.0%	9.72 d	14.85 b	16.61 ab	13.73 c	9.26 f	13.10 d	14.23 cd	12.20 c
0.2%	16.19 ab	17.25 a	17.05 a	16.83 a	15.47 ab	16.53 a	16.48 a	16.16 a
0.4%	17.24 a	15.84 ab	12.02 c	15.03 b	16.52 a	15.02 bc	10.53 e	14.02 b
Mean	14.39 b	15.98 a	15.23 ab		13.75 b	14.88 a	13.75 b	
	Root weight plant⁻¹ (g)							
0.0%	2.45 e	4.48 d	3.07 de	3.33 b	2.91 e	4.24 d	2.36 e	3.17 c
0.2%	8.32 ab	8.93 a	6.78 bc	8.01 a	6.79 bc	8.94 a	8.21 a	7.98 a
0.4%	8.56 a	6.50 c	6.71 bc	7.26 a	6.10 c	6.06 c	7.23 b	6.46 b
Mean	6.45 b	6.64 a	5.52 b		5.27 b	6.41 a	5.93 a	
	Umbels umber plant⁻¹							
0.0%	15.00 e	23.67 cd	19.67 de	19.44 b	18.67 c	20.67 c	27.33 b	22.22 b
0.2%	22.67 cd	32.33 ab	27.33 bc	29.83 a	27.33 b	36.67 a	24.00 bc	30.34 a
0.4%	23.67 cd	19.67 de	35.33 a	26.22 a	19.67 c	24.67 bc	39.00 a	27.78 a
Mean	20.45 b	25.22 a	27.44 a		21.89 b	27.34 a	30.11 a	
	Fruit weight plant⁻¹ (g)							
0.0%	114.00 g	211.33c	141.00 fg	155.44 c	123.33 g	214.67 c	156.67 f	164.89 c
0.2%	206.00 c	286.67 a	159.67 ef	223.17 a	214.33 c	286.67 a	165.00 ef	225.84 a
0.4%	200.00 cd	171.67 de	251.00 b	207.56 b	206.67 cd	186.67 de	254.33 b	215.84 b
Mean	173.33 b	223.22 a	183.89 b		181.44 b	229.33 a	192.00 b	
	Oil percentage %							
0.0%	0.65 d	0.79 c	0.85 c	0.76 c	0.66 d	0.83 b	0.84 b	0.75 b
0.2%	0.93 b	1.05 a	1.03 a	1.00 a	0.88 b	1.02 a	1.01 a	0.97 a
0.4%	0.83 c	1.04 a	1.01 a	0.96 b	0.75 c	1.01 a	0.99 a	0.95 a
Mean	0.80 b	0.96 a	0.96 a		0.79 b	0.93 a	0.95 a	
	Oil content plant⁻¹ (ml)							
0.0%	0.74 f	1.67 cd	1.20 e	1.20 c	0.81 f	1.78 c	1.32 e	1.30 c
0.2%	1.92 c	3.01 a	1.64 cd	2.19 a	1.89 c	2.92 a	1.67 c	2.16 a
0.4%	1.66 cd	1.79 cd	2.54 b	1.99 b	1.55 cd	1.89 c	2.52 b	1.98 b
Mean	1.44 c	2.15 a	1.79 b		1.42 c	2.20 a	1.83 b	

Means with a common having the same letters are not significantly different according to Duncan's multiple range test (DMRT) (p > 0.05)

Table 5. Effects of Pp and RJ and their interaction aqueous extract as foliar application on some fennel chemical constituents, anti-oxidant enzyme assays.

Royal jelly (RJ)	Propolis (Pp)			Mean				
	0 g l ⁻¹	3 g l ⁻¹	6 g l ⁻¹	0 g l ⁻¹	3 g l ⁻¹	6 g l ⁻¹	Mean	
	1 st season (2017/2018)			2 nd season (2018/2019)				
Ch. a (mg g⁻¹ fw)								
0.0%	1.25 f	1.43 de	1.38 e	1.36 c	1.24 f	1.42 de	1.38 e	1.34 c
0.2%	1.50 cd	1.55 bc	1.53 cd	1.53 b	1.50 cd	1.56 bc	1.55 bc	1.54 b
0.4%	1.51 cd	1.68 a	1.63 ab	1.61 a	1.52 cd	1.70 a	1.64 ab	1.62 a
Mean	1.42 b	1.56 a	1.52 a		1.42 b	1.56 a	1.52 a	
Ch. b (mg g⁻¹ fw)								
0.0%	0.50 f	0.60 de	0.55 ef	0.55 c	0.52 f	0.61 de	0.57 ef	0.57 c
0.2%	0.64 cd	0.64 cd	0.65 cd	0.66 b	0.63 d	0.69 c	0.66 cd	0.66 b
0.4%	0.65 cd	0.86 a	0.76 b	0.76 a	0.65 cd	0.84 a	0.76 b	0.75 a
Mean	0.60 c	0.72 a	0.65 b		0.60 c	0.71 a	0.66 b	
Carotenoids (mg g⁻¹ fw)								
0.0%	0.46 e	0.49 c-e	0.47 de	0.48 b	0.45 c	0.52 a-c	0.46 bc	0.48 b
0.2%	0.52 bc	0.55 ab	0.54 ab	0.54 a	0.52 a-c	0.56 a	0.54 a	0.54 a
0.4%	0.52 b-d	0.58 a	0.56 ab	0.55 a	0.53 ab	0.58 a	0.57 a	0.56 a
Mean	0.50 b	0.54 a	0.53 ab		0.50 b	0.56 a	0.52 ab	
Total carbohydrates (mg g⁻¹ dw)								
0.0%	11.65 bc	12.95 a-c	12.26 a-c	12.29 b	10.96 b	13.29 ab	14.65 a	12.97 a
0.2%	13.23 ab	12.22 a-c	11.44 c	12.30 b	13.14 ab	13.01 ab	12.38 ab	12.84 a
0.4%	13.05 a-c	13.68 a	13.45 a	13.40 a	14.44 a	14.52 a	13.45 ab	14.14 a
Mean	12.64 a	12.95 a	12.38 a		12.85 a	13.61 a	13.49 a	
Percentage of N (%)								
0.0%	3.21 d	3.53 bc	3.44 cd	3.39 b	3.18 d	3.47 cd	3.51 bc	3.39 b
0.2%	3.60 a-c	3.71 a-c	3.68 a-c	3.66 a	3.63 a-c	3.71 a-c	3.71 a-c	3.68 a
0.4%	3.64 a-c	3.79 ab	3.85 a	3.76 a	3.63 a-c	3.86 a	3.76 ab	3.75 a
Mean	3.48 b	3.68 a	3.66 a		3.48 b	3.68 a	3.66 a	
Percentage of P (%)								
0.0%	0.48 c	0.54 bc	0.55 a-c	0.52 b	0.49 d	0.54 b-d	0.50 cd	0.51 b
0.2%	0.57 a-c	0.61 ab	0.61 ab	0.60 a	0.56 a-d	0.61 a-c	0.60 a-c	0.59 a
0.4%	0.58 a-c	0.67 a	0.62 ab	0.62 a	0.58 a-d	0.63 ab	0.65 a	0.62 a
Mean	0.54 a	0.60 a	0.59 a		0.54 a	0.59 a	0.58 a	
Percentage of K (%)								
0.0%	4.28 d	4.32 d	4.60 c	4.40 b	4.43 d	4.56 cd	4.56 cd	4.52 b
0.2%	4.66 bc	4.93 a	4.82 ab	4.80 a	4.65 bc	4.84 a	4.78 ab	4.76 a
0.4%	4.61 c	4.73 bc	4.76 a-c	4.70 a	4.62 bc	4.71 a-c	4.76 ab	4.70 a
Mean	4.51 b	4.66 a	4.73 a		4.57 b	4.70 a	4.70 a	
Total soluble sugars of fennel seed (mg g⁻¹ dw)								
0.0%	3.55 g	3.72 e	3.72 e	3.66 c	3.55 g	3.72 e	3.72 e	3.66 c
0.2%	3.88 b	4.08 a	4.08 a	4.02 a	3.88 b	4.08 a	4.08 a	4.02 a
0.4%	3.70 f	3.78 c	3.76 d	3.75 b	3.70 f	3.76 d	3.78 c	3.75 b
Mean	3.71 c	3.86 a	3.86 b		3.71 c	3.85 b	3.86 a	
Catalase EU mg⁻¹ protein								
0.0%	4.20 f	4.90 ef	5.30 de	4.80 c	4.33 e	4.87 de	5.40 c-e	4.87 b
0.2%	5.67 c-e	6.37 bc	6.70 ab	6.24 b	5.77 b-e	6.40 a-d	6.67 a-c	6.28 a
0.4%	6.27 b-d	7.13 ab	7.53 a	6.98 a	6.20 a-d	7.37 ab	7.67 a	7.08 a
Mean	5.38 b	6.13 a	6.51 a		5.43 b	6.21 ab	6.58 a	
Peroxidase (POD)								
0.0%	1.71 f	1.73 ef	1.82 c-e	1.75 c	1.73 f	1.7 ef	1.87 c-e	1.78 c
0.2%	1.80 d-f	1.88 cd	1.91 c	1.87 b	1.85 d-f	1.91 b-d	1.99 bc	1.92 b
0.4%	1.87 cd	2.04 b	2.19 a	2.04 a	1.87 c-e	2.00 b	2.14 a	2.00 a
Mean	1.80 c	1.88 b	1.98 a		1.81 c	1.89 b	2.00 a	
Ascorbate peroxidase (APX)								
0.0%	0.15 d	0.16 b-d	0.16 b-d	0.16 b	0.15 e	0.16 de	0.16 de	0.16 c
0.2%	0.16 cd	0.16 cd	0.17 a-c	0.16 b	0.16 de	0.17 cd	0.17 bc	0.17 b
0.4%	0.16 cd	0.18 ab	0.18 a	0.17 a	0.16 de	0.19 a	0.18 ab	0.18 a
Mean	0.17 a	0.17 a	0.17 a		0.16 b	0.17 a	0.17 a	
Malondialdehyde nmol (MDA) g⁻¹ fw								
0.0%	119.60 c	121.08 bc	120.51 c	120.39 b	119.93 b	123.70 ab	120.85 b	121.49 b
0.2%	125.88 a-c	133.95 a	130.61 a	130.14 a	127.52 ab	131.72 a	130.81 a	130.02 a
0.4%	125.73 a-c	129.39 ab	129.13 ab	128.09 a	124.12 ab	130.71 a	127.79 ab	127.54 a
Mean	123.74 a	128.14 a	126.75 a		123.86 a	128.71 a	126.48 a	

Means with a common having the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p > 0.05$).

moderate concentration having superiority in giving the highest value over other treatments. Differences between values were significant in all treatments except for percentage of K values which showed insignificant differences.

Influence of the interaction between Pp and RJ on chemical constituents and antioxidant enzyme assays:

Likewise, the interaction positively and significantly produced the best and highest results over the control in both seasons. Nevertheless, the best treatment to give the highest result for ch. a, b, carotenoids, total carbohydrates, ascorbate peroxidase (APX), peroxidase (POD) and content of N and P was 0.4% RJ with 3 g l⁻¹ Pp. The highest concentration of both materials gave significantly the highest value of catalase EU mg⁻¹ protein in both seasons, while 0.2% RJ with 3 or 6 g l⁻¹ Pp fruited with the highest results of malondialdehyde nmol (MDA), total soluble sugars of fennel seed and percentage of K (%) as mentioned in Table (5).

Chemical constituents (%) of the essential oils as affected by RJ and Pp treatments:

The interaction between the moderate concentration of both materials; 0.2% RJ and 3 g l⁻¹ Pp produced the highest percentage of α -thujone, anethole, γ -terpinene, α -thujone, camphor, anethole and thymole. However, the highest concentrations of both materials (0.4% RJ and 6 g l⁻¹ Pp) when applied together gave the highest result of D.limonene, Estragole, α .pinene, Camphene, β .Terpinene, β .myrcene, α .phellandrene, D. limonene, Eucalyptol and Cis-ocimene, as shown in Table (6) and Figures (1 and 2).

DISCUSSION

RJ is a valuable food supplement based on its biological, pharmaceutical and functional characteristics (Seven *et al.*, 2014). The significant increase by RJ in all parameters studied especially when added at moderate rate (2 g l⁻¹) may turn back to its contents of lipids, proteins, sugars, vitamins

(B1, B2, B5, B6, B8, B9 and vitamin C), minerals (K, Na, Mg, Zn, Ca, Fe, P, S, Mn and Si), amino acids (contains at least 17 amino acids), six hormones and other antioxidants (Melliou and Chinou, 2005). This may present an interpretation of the increase of N, P and K contents in the plant and leaves pigments content. Because of fatty acids and sugars existing in the RJ, this could explain the increasing rate of carbohydrates in the plant. Also, organic compounds, flavonoids, fatty acids and other active components presented in RJ led to improve the growth rate (Seven *et al.*, 2014). In addition, 10-hydroxy-trans-(2)-decanoic acid (HDA) represents about 15% of RJ, which led to growing the queen bee so large. This may explain the increase in plant growth. Antioxidant capacity of phenolic compounds led to prevent oxidation or destroy oxidizable compounds such as ascorbic acid and lipid and organize the growth of the plant. The RJ extraction way as well, may add to its effectiveness because of the high content of phenolic compounds that added to the aqueous extract more of antioxidant activity (Viuda-Martos *et al.*, 2008; Santos, 2012).

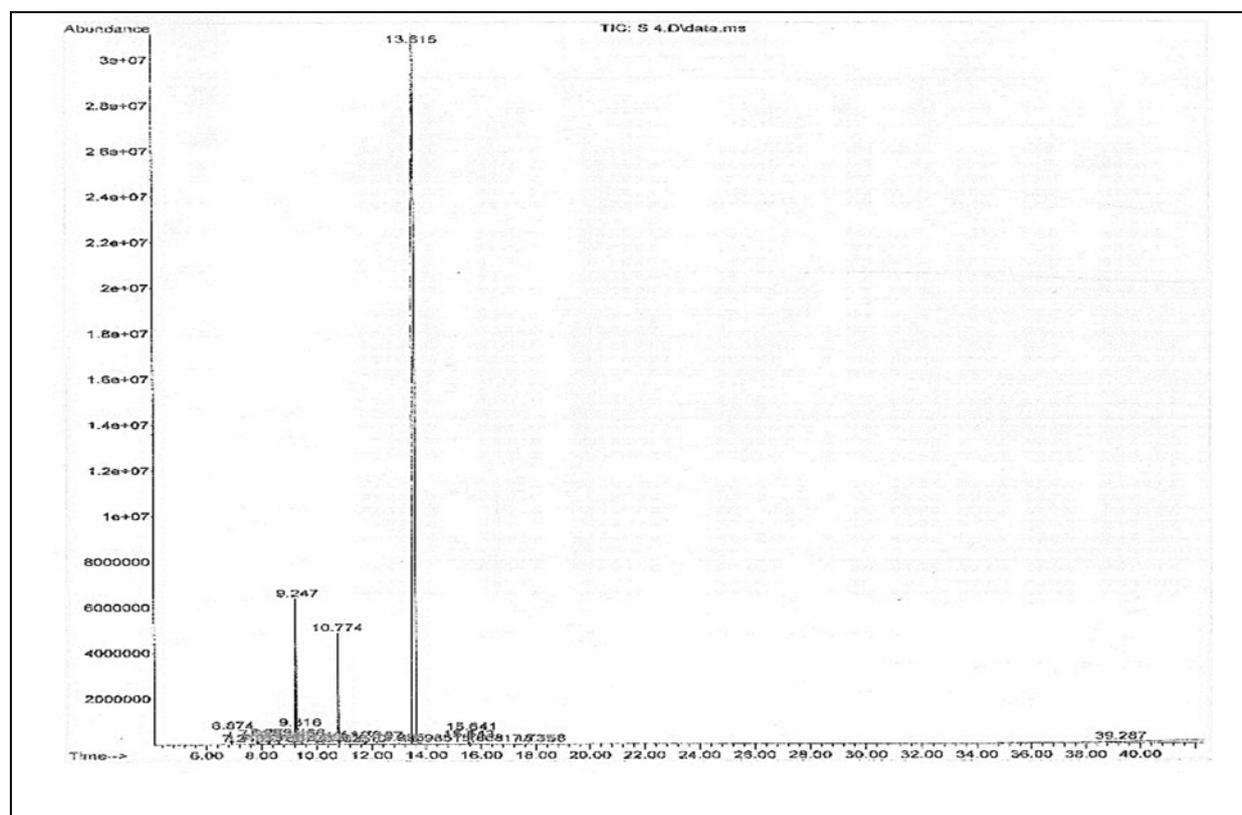
Minor components of bee products are organic acids, vitamin C, carotenes and α -tocopherol which have antioxidant characteristics. RJ is unified by having a set of C8-, C10-, and C12-hydroxy fatty acids. Also, 10 acids in bee product were identified in several combinations (Viuda-Martos *et al.*, 2008).

RJ proteins have antioxidant activity against unsaturated fatty acids peroxidation, which contain about 29 antioxidant peptides. About twelve small peptides of them had 2–4 amino acids with strong hydroxyl radical scavenging activity. Furthermore, tyrosine residues at the C-terminal presented in 3 dipeptides of them had strong hydroxyl-radical and hydrogenperoxide scavenging activity (Guo *et al.*, 2008).

Using royal jelly showing incredible advancement in nutritional status, growth

Table 6. Chemical constituents (%) of the essential oils of fennel as affected by RJ aqueous extract and Pp treatments.

Components (%)	RJ (%)	0.0			0.2			0.4		
	Pp (g l ⁻¹)	0	3	6	0	3	6	0	3	6
α pinene		0.70	0.71	0.88	0.71	0.72	1.01	0.98	1.05	1.08
Camphene		0.03	0.04	0.05	0.03	0.04	0.05	0.03	0.04	0.06
β .Terpinene		0.21	0.23	0.25	0.22	0.27	0.27	0.21	0.28	0.38
β .myrcene		0.22	0.25	0.26	0.25	0.31	0.32	0.23	0.29	0.32
α .phellandrene		0.14	0.13	0.17	0.15	0.18	0.18	0.15	0.21	0.21
D.limonene		5.55	5.89	5.87	5.09	6.92	6.79	6.22	6.73	7.09
Eucalyptol		0.67	0.66	0.71	0.70	0.72	0.73	0.66	0.82	0.86
Cis-ocimene		0.73	0.58	0.69	-	0.25	0.77	0.67	0.78	0.80
γ -Terpinene		-	0.09	0.08	0.11	0.15	0.08	-	0.09	0.08
α -Thujone		4.48	4.82	4.87	4.67	5.02	4.90	4.33	4.88	4.49
Camphor		0.12	0.12	0.11	0.10	0.15	0.12	0.08	0.11	0.13
Estragole		79.99	81.44	82.88	78.76	84.05	83.33	80.82	82.89	84.27
Anethole		0.17	0.20	0.21	0.22	0.29	0.28	0.23	0.28	0.25
Thymol		0.44	0.45	0.48	0.49	0.51	0.50	0.47	0.46	0.45

**Fig. 1. Chromatograms of essential oils extracted from *Foeniculum vulgare*, Mill fruits: 0.2% RJ aqueous extract + 3 g l⁻¹ Pp aqueous extract.**

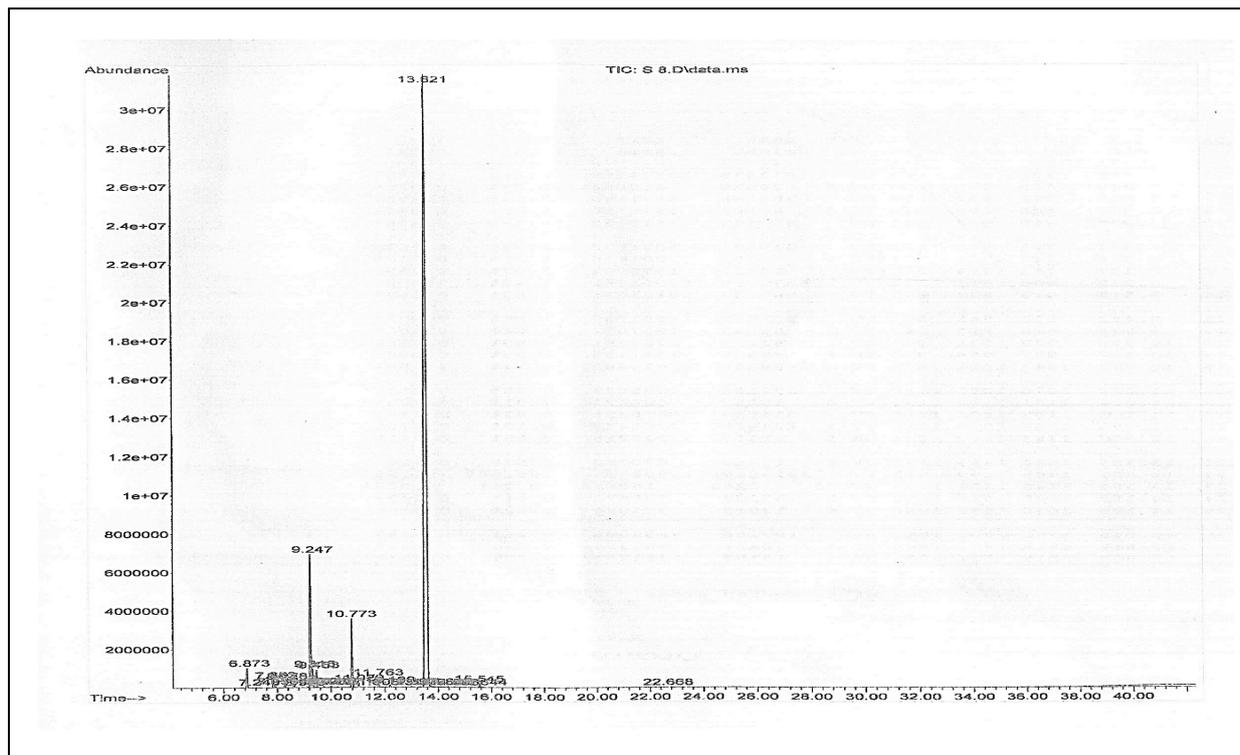


Fig. 2. Chromatograms of essential oils extracted from *Foeniculum vulgare*, Mill fruits: 0.4% RJ aqueous extract + 6 g l⁻¹ Pp aqueous extract.

characters, fruit quality and yield of horticultural crops was observed (Nassef and El-Aref, 2016).

Pp showed positive effect upon all attributes studied over the control either in sole or in combined treatments. This improving for propolis aqueous extract as foliar spray could be attributed to the valuable constituents of propolis such as organic acids and minerals that contribute in increasing their contents in the plant and concentration of pigments in leaves. Also, as results presented both amino acids, acids (nicotinic acid and pantothenic acid) and vitamins (B1, B2, B6, C and E) may contribute in raising the content of amino acid and in turn the protein content in the plant. In addition, propolis has various substances: alcohols, aliphatic esters, aldehydes, aliphatic acids, aromatic acids, aromatic esters, ethers, ketones, terpenoids and steroids which explain the increases in the volatile oil components such as sesquiterpene hydrocarbons and sesquiterphenols. On the other hand, an increase in flavonoids, hydrocarbohydrites

esters, sugars and fatty acids may contribute in increasing the carbohydrates content in the plant (Ahn *et al.*, 2007)

Phenolic compounds, flavonoids, sterols, some phenolic acids (ferulic, salicylic, benzoic acid, and coumaric), aromatic acids and diterpenic acids are concerned the major compounds in charge of the propolis biological activities (Noweer and Dawood, 2009).

Moreover, propolis extract can improve form stimulate or alter plant metabolism to rise within the leaf area, for example, terpenoids that improve the vigorous growth and/or catalyze plant metabolism to increase fresh and dry weight. These terpenoids besides the potential to enhance plant growth can provide the plants a lucid vigor in growth (Bankova *et al.*, 2000).

In this regard, Salama *et al.* (1992) reported that leaf pigments concentration was increased and this attributed to the increase in their hormones. Also, propolis extract catalyzes the absorption of minerals (such as Mn and Fe) required for chlorophyll

synthesis. The concentration of total sugars was increased due to the sweetening of photosynthesis by the effect of propolis aqueous extract. In addition, the concentration of protein was increased in the plants treated by propolis extract could be due to B group vitamins that propolis extract contains (Salama *et al.*, 1992), Which act as coenzymes and have some independent roles within the biochemical processes in the plants (El-Tayeb, 1995). Also, Gopala Rao *et al.* (1987) pointed that the increase of protein synthesis associated with the increase in B vitamins accumulation may well be by functioning at the level of protein synthesis perception.

The presence of amino acids with tryptophan as a part of propolis extracts may go back to the increment of total free amino acids. Also, propolis extract acts to prevent amino acid to incorporate into proteins. Using different propolis samples 22 minerals and 149 compounds could explain the increase in macro parts in plants treated by propolis extract (Walker and Crane, 1987).

Our results are in line with those obtained by Abou-Sreca *et al.* (2017) reporting that foliar spray with propolis extract increased all studied parameters; all vegetative growth and flowering attributes, chemical ingredients and constituents of plant essential oil of calendula plants. Similarly, propolis extract applied as a foliar application or presoaking had also positive response on other crops as reported by Noweer and Dawood (2009), Semida and Rady (2014) and Seif El-Yazal (2019) that the effectiveness of Pp extract on *Phaseolus vulgaris* L. plants as a foliar application on carotenoids, chlorophyll, carbohydrates and protein content.

CONCLUSION

Hence, it could be concluded that RJ or Pp, some of honey bee products that are natural safe effective substances, proved their positive effect on the fennel plants growth attributes, oil and fruit yield and components as well as chemical

composition. They both used together or in apartment gave superior results over the control in all characters investigated. This may be taken seriously for using them in the field of agriculture, but this still needs more investigation on other plants, as a replacement of chemical fertilizers substances in improving the plants quality with no dangerous harms upon the environment or the plants, which are the dietary source for animals and humans.

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