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EFFECT OF THE EGYPTIAN PROPOLIS ON THE BIOACTIVE COMPOUNDS CONTENT IN TOMATO PLANTS

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ABSTRACT: Propolis is an organic substance that considered a biostimulant agent and plays a vital role in increasing bioactive compounds content in plants. The study investigated the properties of two different kinds of propolis *i.e.*, Egyptian and Chinese propolis. The physical and chemical analyses results of the Egyptian propolis showed that it contain 253.703 mgGAE/g total phenolic compound, 76.766 mgQE/g total flavonoid compound and 5.417 g/100g total alkaloid. Also, the effect of five concentrations of aqueous extract of the Egyptian propolis was studied as a foliar spray on tomato plants, which were (1, 2, 10, 20, 100 mg propolis ml^{-1}). Tomato plants treated with propolis (100 mg/ml) showed a significant effect in antioxidant content and other bioactive compounds compared to control plants.

Key words: Tomato plants, Egyptian and Chinese propolis, physical and chemical properties, antioxidant and bioactive compounds.

INTRODUCTION

Egypt ranked as the fifth largest producer of tomatoes with a cultivated area of 182.444 ha and a productivity of 40 t ha^{-1} (**FAO, 2017**).

Tomato plants considered as a very important crop in the world as it contains a lot of bioactive compounds necessary for our health (**Chaudhary *et al*., 2018**). High temperatures especially in arid countries cause morphological, physiological and biochemical changes, which affect the growth and development of plants (**Ohama *et al*., 2017; Deligios *et al*., 2019**).

Tomato is very sensitive to high temperature, this lead to reduce the quantity and quality of the fruits, also the high temperature may affect the bioactive compounds of tomato (**El-Saka, 2016; Driedonks *et al*., 2018**).

Propolis is a natural product derived from plant resins and collected by honeybees to seal the walls and entrance of the hive and contributes to protect the colony against different pathogens (**Ghisalberti, 1979; El Sohaimy and Masry, 2014; Anjum *et al*., 2018**).

Propolis considered a powerful defense agent as an antibacterial, antifungal and antiviral (**Nieva *et al*., 1999; Santos *et al*., 2003**).

In this study, a new foliar spray methodology was applied on tomato plants in field conditions by using different concentrations of an organic material, which called propolis. Application of natural biostimulants (**Grabowska *et al*., 2015**) and hormonal compounds (**Jahan *et al*., 2019**) could improve the tolerance of tomato towards stress conditions.

The aim of this study was to investigate the physical and chemical properties of the Egyptian propolis compared to the Chinese propolis and study the effect of the water extract of the Egyptian propolis as a foliar spray on tomato plants.

MATERIALS AND METHODS

Propolis Samples

Two different kinds of propolis were used, Egyptian propolis obtained from the Apiary of

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Beekeeping Research Section, Plant Protection Research Institute, Agriculture Research Center at Dokki, Giza, Egypt. The Chinese Propolis was Obtained from Local Markets, Giza, Egypt.

Plant Material

Tomato plants (*Solanum lycopersicum* Mill.) hybrid Al-Quds E448 (Ministry of Agriculture-Tadress Lyon company, Cairo, Egypt), was cultivated in a field conditions in the New Salhia, Sharkia Governorate (72° 32' E; 23° 3' N), Egypt during October 2018-2019 growing season.

Chemicals

All solvents used throughout the present work were obtained from different companies. Gallic acid, Quercetin, DPPH and substrates were purchased from Sigma chemical Co., UK.

Methods

Determination of Physical and Chemical Properties of Egyptian and Chinese Propolis

Physical Properties

The appearance, form, color and smell of the Egyptian and Chinese propolis were described according to **Kosalec *et al.* (2004)**.

Chemical properties

Determination of moisture, crude proteins, fats, crude fibers, carbohydrates and ash were determined according to the method described in **AOAC (2005)**.

The resin percentage, volatile substances percentage and total insoluble solids were determined according to **Bankova (2005)**.

Total phenolic determination

Total phenolic compounds of Egyptian and Chinese propolis were determined according to the method investigated by **Ghasemzadeh *et al.* (2010)**.

Total flavonoids determination

Total flavonoids compounds of Egyptian and Chinese propolis were determined according to the method investigated by **Ahn *et al.* (2007)**.

Determination of total alkaloids

Total alkaloids compounds of Egyptian and Chinese propolis were determined according to the method described by **Adham (2015)**.

Antioxidant Activity of Egyptian and Chinese Propolis

Free radical scavenging activity (RSA) DPPH assay

The RSA of the ethanolic extract of Egyptian and Chinese propolis was assessed by the discoloration ethanolic solution of 1,1-diphenyl-2picryl hydrazyl (DPPH) radical 0.2mM aromatic in ethanol by using four concentrations (40, 100, 150, 200 µg/ml) according to **Elslimani *et al.* (2013)**.

Determination of chemical composition of plant sample

Determination of moisture, crude protein and ash were determined according to **AOAC (2005)**.

The percentage of nitrogen, phosphorus and potassium were determined according to **Zhai *et al.* (2013)**.

Field experiment and preparing aqueous extract of Egyptian propolis

50 g of propolis was freeze dried for 3 hr., suspended and extracted with 50 ml of ethanol (70%), and kept at 26°C on a shaker at 150 rpm for 2 days. The extract was centrifuged at 28000 *g* for 30 min, and the supernatant was pooled and evaporated at room temperature (25°C) for 3 days; then, the remaining resin was collected to use in subsequent test. Dilutions of 1:10, 1:50, 1:100, 1:500 and 1:1000 were prepared with the final concentrations of 1 (P1 treatment), 2 (P2 treatment), 10 (P3 treatment), 20 (P4 treatment), and 100 (P5 treatment) mg propolis ml^{-1} distilled water, respectively, and then stored temporarily at room temperature (**Abo-Elyousr *et al.*, 2017**). The initial foliar propolis treatment occurred after 20 days when the seedlings had 2–3 true leaves. The propolis was sprayed with the solutions until dripping, with a held atomizer.

Plants sprayed with water only served as the control (Control treatment).

Determination of the Effect of Different Concentrations of the Egyptian Propolis as Foliar Sprays on Tomato Plants

Antioxidant activity

Free radical scavenging activity of tomato leaves which treated with different concentrations of the Egyptian propolis was assayed with 1,1-diphenyl-2picrylhydrazyl (DPPH) radicals dissolved in ethanol according to the method of **Lee *et al*. (2002)**.

Total carbohydrates

Total carbohydrate was estimated colorimetrically by the Nelson's reagent as reported by **Cherry (1973)**. Total carbohydrate was extracted from dry treated leaves with 1N HCl for 6 hours in boiling water bath under reflex condenser.

Total Protein

Total protein was calculated by multiplying the total nitrogen by 6.25. The total nitrogen was determined by using microkyeldahl method according to **AOAC (1990)**.

RESULTS AND DISCUSSION

Physical Properties

Results in Table 1 show some of physical properties of Egyptian and Chinese propolis, which presented common variations in appearance, color, smell and formation. It may be due to the geographic location of Egyptian and Chinese propolis as the color of propolis depends on its origin. The odor can vary from sample to sample with some being odorless (**Kosalec *et al*., 2004**).

Chemical Properties

Chemical composition of Egyptian and Chinese propolis

In Table 2, results showed that the Egyptian propolis was higher than the Chinese one in the percentage of crude protein (11.03%) and fibers (51.02%), while the Chinese propolis was higher in fat concentration (52.10%), moisture (9.27%), carbohydrates (8.91%) and ash (5.21%).

The resin, volatile substances and insoluble solids percentage

Results in Table 3 show that there was a significant different between the Egyptian and Chinese propolis. The highest value was observed in each of percentage of resin and insoluble matter in each of Egyptian propolis that valued 57.92% and 40.91% respectively, on the other hand the percentage of volatile substances was the highest in the Chinese propolis (3.98%).

Raw propolis is composed of approximately 50% resin (poly phenolic fraction), 30%wax, 10% essential oils, 5% pollen and 5% various organic and inorganic compounds (**Burdock 1998; Bankova *et al*., 2000**).

Content of wax varied according to propolis samples between 20-49% (**Laura, 2007**).

Total active compounds in propolis

As observed in Table 4 the highest value for each of total phenolic compound, total flavonoid compound and total alkaloid was 253.703 mg GAE/g DW, 76.766 mg QE/g DW, 5.417 g/100g FW, respectively for the Egyptian propolis. Values were 134.976 mg GAE/g DW, 20.062 mg QE/g DW and 1.129 g/100g FW for Chinese propolis.

This reflects the enrichment of Egyptian propolis with bioactive compounds. As investigated by **Bankova *et al*. (1988) and Scheller *et al*. (1990)** the natural antioxidants such as phenolics and flavonoids have bioactive and pharmacological effects to protect organisms from diseases.

Radical Scavenging Activity (RSA) of Propolis

Antioxidants are known for their ability to protect human body cells from damage as a result of free radical exposure. As the free radical is a chemical species that has unpaired electrons (**Pryor *et al*., 2006**). Propolis has a lot of bioactive compounds that make it strong antioxidant biostimulant and as observed in Table 5 there is a continuous increase in RSA by increasing the propolis concentration in both Egyptian and Chinese propolis, the highest value was 91.684% in the Egyptian propolis and 74.745% in the Chinese propolis.

Determination of Chemical Composition of Plant Sample

Table 6 show the approximate composition of the plant before treatments (As control).

The highest percentage was the protein content (26.31%), and the lowest was the phosphorus content (1.01%).

Table 1. The physical properties of crude Egyptian and Chinese propolis

	Egyptian propolis	Chinese propolis
Appearance	Waxy	Dry
Color	Dark brown	Brown
Smell	Not aromatic	Aromatic
Form	Sticky	Powder

Table 2. The proximate composition of studied propolis sample treatment (g/100g DW)

Percentage of parameter	Egyptian propolis	Chinese propolis
Moisture (%)	7.05%	9.27% (FW)
Proteins (%)	11.03%	10.74% (DW)
Fats (%)	23.12%	52.10% (DW)
Fibers (%)	51.02%	13.77% (DW)
Carbohydrates (%)	6.02%	8.91% (DW)
Ash (%)	2.11%	5.21% (DW)

FW: Fresh weight DW: Dried weight

Table 3. The resin, insoluble matter and volatile substances percentage of Egyptian and Chinese propolis

Percentage of parameter	Egyptian propolis	Chinese propolis
Resin (%)	57.92%	46.21%
Insoluble matter (%)	40.91%	38.92%
Volatile substances (%)	3.33%	3.98%

Table 4. The total phenolic, total flavonoid and total alkaloid contents of Egyptian and Chinese propolis

	Total phenolic content (mg GAE/g sample DW)	Total flavonoid content (mg quercetin/g sample DW)	Total alkaloid (g/100 g FW)
Egyptian propolis	253.703	76.766	5.417
Chinese Propolis	134.976	20.062	1.129

Table 5. DPPH scavenging activity of Egyptian and Chinese propolis as affected by propolis concentration

Propolis concentration	40 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)
	Free radical scavenging activity "DPPH" (%)			
Egyptian propolis	60.561%	75.510%	87.551%	91.684%
Chinese Propolis	56.071%	64.464%	69.362%	74.745%

Table 6. The proximate composition and mineral content of the plant before treatments (%)

Parameter	Plant as control
Moisture	22.50%
Protein	26.31%
Ash	22.14%
Nitrogen	4.20%
Potassium	2.82%
Phosphorus	1.01%

The Effect of Different Concentrations of the Egyptian Propolis as Foliar Sprays on some Bioactive Compounds

As observed from the previous results, the Egyptian propolis was the highest in bioactive compounds compared to the Chinese propolis so it was used as a foliar spray on the leaves of tomato plants.

Free Radical Scavenging Activity (RSA) of the Tomato Plant Leaves Treated with Different Concentration of Egyptian Propolis

As indicated in Table 7, all samples showed an increase in antioxidant activity by increasing concentrations of propolis, therefore exhibiting a concentration dependent pattern of free radical scavenging ability. As showed by **Shahwar *et al.* (2010)** there is a great association between antioxidant activity and phenolic compound concentration.

Determination of Protein and Carbohydrates Contents in the Treated Tomato Samples

Results in Table 8 show a continuous increase in the concentration of carbohydrate and protein in the tomato treated leaves by increasing the concentration of propolis compared to the control. **Noweer and Dawood (2009)** reported that, the foliar application of propolis extracts caused an increasing in protein and carbohydrate contents and this increase occurred by increasing the propolis concentration.

Conclusion

The results of this study indicated that, the Egyptian propolis has an effective role as a vital biostimulant on bioactive compounds especially the antioxidant compounds that lead to activate the biological compounds in the leaves of tomato plants and preserve the quality and productivity of the plant.

Table 7. DPPH scavenging activity of different concentrations of Egyptian propolis treatment on Tomato leaves

Propolis treatment	1 (mg propolis ml^{-1})	2 (mg propolis ml^{-1})	10 (mg propolis ml^{-1})	20 (mg propolis ml^{-1})	100 (mg propolis ml^{-1})	Control
	Free radical scavenging activity "DPPH" (%)					
HPPH (%)	62.739%	64.249%	69.085%	72.708%	76.037%	61.631%

Table 8. Carbohydrate and protein contents (mg/g FW) of different concentrations of Egyptian propolis treatment on Tomato leaves

Treatment	1 (mg propolis ml^{-1})	2 (mg propolis ml^{-1})	10 (mg propolis ml^{-1})	20 (mg propolis ml^{-1})	100 (mg propolis ml^{-1})	Control
Carbohydrate	15.135	16.544	19.942	26.654	29.321	14.125
Protein	47.494	49.866	54.774	56.910	60.187	48.913

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تأثير البروبوليس المصري على محتوى نبات الطماطم من المركبات الحيوية النشطة

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البروبوليس مادة عضوية تعتبر عامل منشط حيوي وتلعب دوراً كبيراً في زيادة محتوي المركبات النشطة بيولوجيا في النباتات، قارنت تلك الدراسة بين نوعين مختلفين من البروبوليس: البروبوليس المصري والصيني، لإختيار النوع الأكثر فعالية من ناحية الخواص الفيزيائية والكيميائية، حيث أظهرت النتائج احتواء البروبوليس المصري على كمية كبيرة من المركبات الفينولية (253.703 mg GAE/g) وكمية من المركبات الفلافونويدية (76.766 mg QE/g) والمركبات القلويدية (5.417 g/100g)، تم دراسة تأثير خمس تركيزات من المستخلص المائي للبروبوليس المصري كرش ورقي على أوراق نبات الطماطم وكانت التركيزات كالتالي (1, 2, 10, 20, 100mg propolis ml^{-1} Distilled Water)، حيث أظهرت نباتات الطماطم المعالجة بالبروبوليس بتركيز (100mg/ml) تأثيراً معنوياً على محتواها من مضادات الأكسدة وغيرها من المركبات النشطة حيويا مقارنة بالنباتات الأخرى غير المعاملة.

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