Journal of Plant Protection and Pathology

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Available online at: www.jppp.journals.ekb.eg

Role of the Date Palm Pollen Grains (*Phoenix dactylifera* L.) in Induced Antimicrobial Peptides Synthesis and Improved the Royal Jelly Protein Properties in Honey Bees

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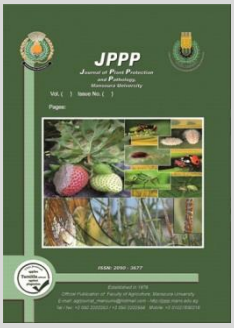

ABSTRACT

Date Palm Pollen Grains (*Phoenix dactylifera* L.) (DPPG) were used by feeding honey bee colonies to study their role in raising immunity peptides and improving the Royal Jelly (RJ) protein properties. The Haemolymph and RJ of adult nurse honey bee workers following the feeding process at two concentrations (10 & 30%) were collected. Total protein and Electrophoretic protein analysis (SDS–PAGE) were done. From the results obtained, it could be concluded that treated honey bee colonies by the DPPG led to increased total Haemolymph protein numbers, particularly of the lower molecular weights with both tested concentrations. 2-The RJ protein molecular weights were reduced from (240 kDa) (maximum molecular weights in the control) to (71 kDa). 3-The RJ at (10%) conc. of the feeding process showed four proteins ≤ 14 kDa, among them known as Hymenoptaecin (12 kDa), Defencin (4.8 kDa), and Apidaecin (2 kDa). While with the feeding conc. (30%), it recoded one of the AMPs only, Apidaecin (2 kDa). 4–The RJ in its normal state (as a control) contains no proteins with molecular weights less than 14 kDa. So, it could be recommended that beekeepers feed their bee colonies with DPPG, particularly at the concentration (10%) that allows them to increase the antimicrobial peptides in the RJ, which is healthy nutrition for both the bees and people consumption.

Keywords: Pollen Grains, Date Palm, Royal Jelly, Honeybee, Haemolymph, Immunity.

INTRODUCTION

Date Palm Pollen Grains (*Phoenix dactylifera* L.) are a natural product that is well-known in traditional medicine. It was analyzed for its physical and chemical properties. It could be used in the pharmacological field. Date Palm Pollen (DPP) is a dioeciously wind-pollinated species. Pollen is a natural source of biochemical and nutritional substances such as protein, carbohydrates, amino acids, minerals, sterols, hormones, and many different kinds of enzymes (Hassan, 2011). This values proved that (DPP) can be considered as a rich source of natural bioactive compounds that can be used to prevent the oxidation of ma ny food products. That has aroused great interest for consumers, since phenolic substances act as antioxidants and present multiple biological effects, including the reduction of the risk of heart disease, cancer, and cataracts. These compounds also prevent the oxidation of LDL lipoprotein, platelet aggregation and dam-age to red blood cells. Additionally, DPP had a very low content of flavonoids. The total phenolic and flavonoids contents could be influenced by geographical origin, storage and drying procedure (Haifa *et al.*, 2019). The Date Palm fruit extracts have also been shown to have antifungal (Shraideh *et al*., 1998), antibacterial (Saddiq and Bawazir, 2010), antiparasitic (Metwaly *et al*., 2012), antiviral, hepatic protective (Saafi *et al.*, 2011), anti-coccidial, anti-inflammatory, and anti-apoptotic properties (Metwaly *et al*., 2014). Overall, DPP could be considered a promising source of new natural antioxidant and antimicrobial agents for use in various food and pharmaceutical products and formulations (Daoud *et al*., 2015). Due to the high content of protein present in Royal Jelly (RJ), honey bees need a diet rich in sugars, vitamins, fatty acids, minerals, and all essential amino acids, which are mainly obtained from pollen consumption with the possible addition of a small amount to honey (Lercker *et al.*, 1993). RJ has inhibitory activity against *Escherichia coli*, *Salmonella spp*, Proteus, *Bacillus subtilis*, and *Staphylococcus aureus*. RJ has a high content of polar aromatic organic acid compounds and unsaturated compounds, especially 10-hydroxy-2-decenoic acid (10-HDA). The insoluble fraction in ether contains two peptides, called Royalisin and Jellein, with antimicrobial activity for gram-positive, gram-negative bacteria, fungi, and yeasts (Fontana *et al.* 2004). Honey bees defense against many pathogens by producing antibiotic substances such as Propolis and RJ. The innate immune system is the first line of defense against pathogens in plants and invertebrates. It is also critical for vertebrate immunity before the acquired immune system generates a specific response. Various antimicrobial peptides are the key elements of the insect immune system. After the honey bees are infected by pathogens, four antimicrobial peptide families are synthesized, representing a broad spectrum of antimicrobial activity in the haemolymph. All of these are Cationic peptides identified as: Apidaecin, Apaecin, Hymenoptaecin and Defencin (Xu *et al.*, 2009). So this paper is aimed at studying the role of the DPPG as a supplementary feeding process in inducing antimicrobial peptides synthesis and improving the Royal Jelly protein properties in honey bees.

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DOI: 10.21608/jppp.2021.220025

MATERIAL AND METHODS

The current research was conducted in the Department of Bee Research, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt, in late winter 2020.

For this study, twelve honey bee colonies were studied and divided into three groups.

Date Palm Pollen Grains (*Phoenix dactylifera* L.) were fed as a powder in a sugar solution with concentrations of 10% and 30% for two months, twice a week.

The last one was done just as a control by feeding sugar solution.

Haemolymph samples

The haemolymph of adult nursing bee workers following the end of the feeding process was collected using the method of Gilliam and Shimanuki (1971).

Royal Jelly collection

The Royal Jelly (RJ) from nursing bee workers was collected naturally after ending the treatments by reserving the queens partially in the same colony (partial orphan) according to the method of Fu-Liang *et al.*, (2017).

Electrophoretic analysis

The Haemolymph and the Royal Jelly were analyzed electrophoretically using (12%) SDS–PAGE (Weber and Osborn 1969 & Laemmli, 1970). Coomassie Brilliant Blue R-250 was utilized in staining the proteins.

The Haemolymph and Royal Jelly protein determination

The dye binding assay approach was used to assess the protein content of both the Haemolymph and Royal Jelly of honey bee workers treated and non-treated by Date Palm Pollen Grains. Albumin from bovine serum (BSA) was used as a standard (Bradford, 1976).

RESULTS AND DISSCUSION

I-Total protein concentration

As shown in Table 1, the haemolymph protein concentration in the worker honey bees after the feeding treatments showed higher concentrations (10.4 & 9.7 mg/ml), respectively with both tested feeding conc. (10 & 30%) than those with control one (9.1 mg/ml). In comparison to their control (10.5 mg/ml), the RJ protein concentration collected from nursing honey bee workers fed on the DPPG with the feeding concentration (10 & 30%) showed the lowest values (9.5 & 8.7 mg/ml).

Table 1. Total Protein concentration in the Haemolymph and Royal Jelly of honey bee workers as the feeding process by DPPG.

Samples		T. Protein (mg/ ml)
Haemolymph	Control	9.10
	10%	10.40
	30%	9.70
Royal Jelly	Control	10.50
	10%	9.50
	30%	8.70

II-The Haemolymph and the Antimicrobial peptides (AMPs)

As shown in Table (2) and Figs. (1,2), feeding honey bee colonies on DPPG reflects 20 protein bands in the haemolymph of honey bee workers fed on (30% conc.), while with the feeding concentration of 10% they recorded 16 bands. Whereas with the control treatment it recorded 14 bands only. It could be concluded that the haemolymph of worker honey bees not treated with any treatments as control contains 3 proteins of low molecular weights ≤ (14 kDa), of which two are known as anti-microbial peptides (AMPs); Hymenoptaecin (14 kDa) and Defencin (4.8 kDa) (high similarity ratio exists between the proteins of 5 kDa). While with the feeding process at concentrations (30%), there are four proteins ≤ (14 kDa) (14, 10, 8 & 5 kDa). From them, we know Hymenoptaecin (14 kDa) and Defencin (4.8 kDa).

Table 2. The Haemolymph protein Molecular weights collected from nursing bee workers using feeding by the (*(Phoenix dactylifera*).

The Haemolymph Protein Molecular Weights Of Honey Bee Workers (kDa.)			
St	Control	(10%)	(30%)
240	75	161	153
140	63	119	113
115	55	72	72
80	51	57	59
62	47	48	52
50	41	46	48
43	37	45	47
27	27	35	46
15	18	29	45
7	17	23	34
	15	18	28
	12	17	24
	9	15	22
		11	19
		8	17
	5	5	15
			14
			10
			5
	14	16	20

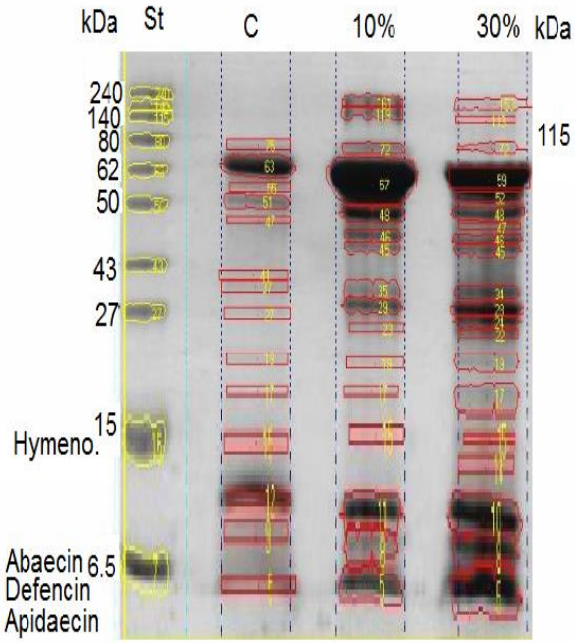

Fig.1. Stimulate synthesis the antimicrobial peptides in the Haemolymph of worker bee as treated by Date Palm Pollen Grains *(Phoenix dactylifera* L.).

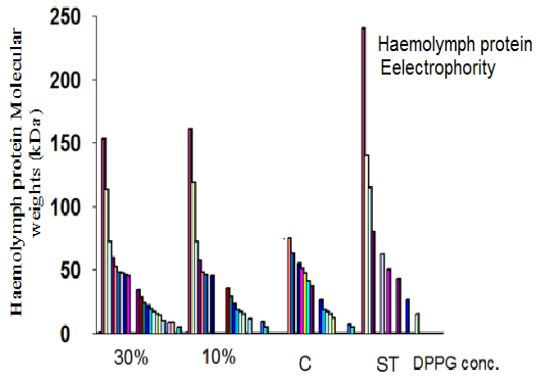

Fig.2. Reflect of feeding honey bee colonies by DPPG on the Haemolymph protein Molecular weights of nurse bee workers.

III-The Royal Jelly and Antimicrobial Peptides (AMPs)

From the results obtained from Table (3) and Figs. (3,4), it could be established that the RJ total protein recorded 13 protein bands when bee colonies fed on the DPPG at a level of 10%, while with the high feeding concentration of 30%, it recorded only 8 proteins, whereas with control one, 12 proteins were recorded.

From Table (3) and Figs. (3,4), the RJ collected from nursing honey bee workers treated by DPPG at a concentration of 10% showed four proteins of lower molecular weights ≤ 14 kDa. (12, 8, 5 & 2 kDa), respectively. From them, we know Defencin (4.8 kDa.) and Apidaecin (2 kDa). While with the feeding process, 30% recoded one AMP, which is Apidaecin (2 kDa). whereas RJ collected from non-treated honey bee colonies as a control showed none of the AMPs.

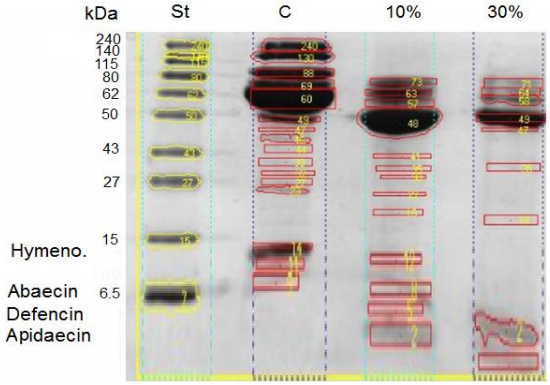

Fig.3. Stimulate the antimicrobial peptides synthesis in the Royal Jelly of worker bees in state of the treated by DPPG.

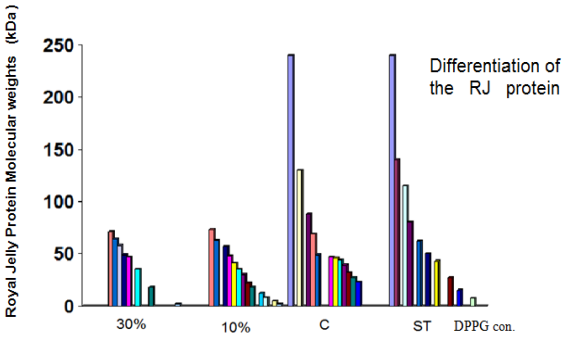

Fig.4. Effect of feeding honey bee colonies on DPPG on the Royal Jelly protein Molecular weights of nurse bee workers.

Table 3. Royal Jelly protein Molecular weights collected of nursing bee workers according to the feeding by the DPPG.

Royal jelly Protein Molecular Weights (kDa)			
St	**Control**	**(10%)**	**(30%)**
240	240	73	71
140	130	63	64
	88	57	58
115	69	48	49
80	49	41	47
62	47	35	35
50	46	30	18
43	44	22	2
27	39	18	
15	32	12	
7	27	8	
	23	5	
		2	
10	12	13	8

From the results obtained, it could be concluded that treated honey bee colonies by the Date Palm Pollen Grains (DPPG) led to: 1-Increased Haemolymph protein numbers with both tested concentrations reached 20 protein bands at level (30%) and 16 protein bands at level (10%), while with control one they recoded 14 proteins only. It could be noted that increasing the molecular weights with both two concentrations reached 161 and 153 kDa, respectively, in comparison to 75 kDa in the control. 2-Increased the Haemolymph protein numbers of the lower molecular weights ≤ 14 kDa reached to 4 proteins (12, 8, 5 & 2 kDa) with the conc. (10%) in comparison to one protein only (2 kDa) with the feeding conc. (30%) and nothing with the control. 4-Clinically clear reduction in the RJ protein molecular weights from 240 kDa (maximum molecular weights in the control) to (73 & 71 kDa) with both tested concentrations (10 & 30%). 5-The Royal Jelly in its normal state does not contain any proteins of lower molecular weights ≤ (14 kDa).

It could be established that the RJ collected from nursing honey bee workers treated by DPPG with the preferred concentration (10%) is categorized by the presence of higher AMPs and considered very healthy against the bacteria of both negative and positive gram. We can advise the beekeepers to use DPPG (10%) to feed their bee colonies to produce a Royal Jelly with higher antimicrobial peptides that is healthy for feeding honey bee members and for human consumption.

Discovery of the Apidaecin, isolated from lymph fluid of the honey bee (*Apis mellifera*). Apidaecin represents a new family of inducible peptide antibiotics that are active against a wide range of plant-associated bacteria and some human pathogens (Casteels *et al*, 1989**).** In studies on some plant extracts on the stimulation of the honey bee immune system in addition to their effect on the survival time of worker bees through five plant extracts of Artemisia herba- albam, *Thymus serpyllum, Citrus aurantium, and aqueous extract of plant leaves of Laurus nobilis and Aloysia citriodora* Barhoum *et al.* (2018) found that all five tested plant extracts had a significant effect on increasing both the mean and median survival time of honey bees as compared to the control. The plant extracts can induce the synthesis and assembly of antimicrobial peptides depending on the con

centration used and the period of application. Rodrigue s *et al*., (2010**)** decided that the natural immune system of insects has the ability to activate long-term immune responses to the pathogen, and this is contrary to previous beliefs that insects lack immune memory. In honey bees, four peptide families were detected within the fluid that supplied them, and four different peptides were found in Royal Jelly produced by workers (Romanelli *et al.*, 2011).

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دور طلع النخيل في الببتيدات المضاده للميكروبات وتحسين خصائص بروتين الغذاء الملكي في نحل العسل

محمود عزت زكريا ، هبه الله سيد السايح ، محمد علي إبراهيم عبد العظيم و ساره حسن الدريني

قسم بحوث النحل – معهد وقاية النباتات – مركز البحوث الزراعية – وزارة الزراعة.

في هذا البحث تم استخدام محلول سكري مضاف له طلع النخيل بتركيزين (١٠% - ٣٠%) وتم اختيار ٩ خلايا متساويه في القوه علي ان يكون لكل تركيز ٣ خلايا والكنترول ٣خلايا .وكان الهدف من هذاهو التحفيز علي زيادة انتاج ببيتيدات المناعه وخصائص بروتين غذاء ملكات النحل ورفع مناعة نحل الطوائف المستخدمه ومقارنتها بالكنترول .وذلك بسحب عينات هيموليمف من اليرقات بعد تغذية الطوائف ثم عمل لها فصل بروتينات . اظهرت النتائج:- خفض الاوزان الجزئية للبروتين من (٢٤٠ كيلودالتون) الي (٧١ كيلودالتون) .اظهرت التغذية بروتينات ذات اوزان جزيئية اقل من ١٤ كيلودالتون عند التغذية بـ ١٠%. لذالك يوصي بتغذية طوائف النحل بمحلول سكري يحتوي علي ١٠% طلع نخيل.