FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (42), No.1, pp. 33-48, 2022

INFLUENCE OF SUGARY FEEDING WITH SOME VITAMINS ON PHENOLOXIDASE AND ANTIOXIDANT ACTIVITY IN HONEYBEE WORKERS, *Apis Mellifera L.*

Hosafy. M. Eshbah1, Ehab. W. Zidan2, Samah. F. Abu Al-layl1
1 Department of plant protection (Apiculture), Fac. Agric., Minia Univ. Egypt.
[2]Department, plant protection Res Institute. Inst. Agric. Res. Center, Dokki, Giza, Egypt

Received 20 Feb. 2022 Accepted 22 March 2022

ABSTRACT

There were few attempts about the requirements of honeybee to vitamins. Our results highlighted the effectiveness of Phenoloxidase (PO) and Antioxidant (AO) activity in honeybee workers and vitamin types added with sugar solution as early and vitamins important indicators. There was a clear improvement in the antioxidant system in bee colonies that were provided with sugary feeding with vitamins mixture compared to other vitamins individually (A, B, C and E) and control ones. This suggests that honeybee workers need artificial feeding with mixed vitamins during winter season possess mechanisms that reduce their oxidative stress. Results suggest that vitamins in winter feeding for 14 weeks (from 1 December to 14 March) has the potential to improve colony development and health during overwintering, a period of high colony losses. The results showed that vitamins diet increased bee activity and colony health escpicially in the dearth period, which reduce varroa infection and caused the absence of European foulbrood compared to the control.
Key words: Honeybee, Phenoloxidase, Antioxidant, Vitamins.

INTRODUCTION

Honeybee population fluctuated according to the component of nutrients. These nutrients affected also the honeybee immunity and the absence of diseases. **(Somerville,2005)**. When the vitamins content increased three times they caused an increase in honeybe larval weight. **(Weaver, 1974)**. Moreover, vitamin C is considered as enzyme cofactor and a source of antioxidant activity,so it's the

best outrient to honeybee. **(Navon *et al.* 1985)**. Colony population offected greatly when the temperature decreases in winter time. **(Brodschneider et al., 2010; Van Engelsdorp et al., 2009)**. **Di Pasquale et al. (2013)** showed that phenol-oxidase (PO) in hemolymph of insects is affected by the quality of the diet. PO plays an important role in the regulation of immunity system in the organisms. **(Brookman et al., 1989; Söderhäll and Cerenius, 1998; Wilson et al., 2001; Sugumaran, 2002)**. Within these defense mechanisms, and the existence of PO as a key enzyme in the haemolymphas pro-phenoloxidase result in the ability of melanin production **(Ashida and Brey 1997; Schmid-Hempel, 2005; Siva-Jothy et al.,2005; Leclerc et al.,2006; Mullen and Goldsworthy, 2006; Nakhleh et al.,2017).** Many recent researches threw light on using β-glucans, lipopolysaccharides and peptidoglycans as activators of PO and haemocytes **(Charles and Killian,2015; Mazzei et al., 2016; Nakhleh, 2017;). Lourenço *et al.*, (2005)** in Brazil pointed out that PO pathway is used as a basic component to the reactions occur in the hemolymph of invertebrate immune. PO enzyme is one of the first immune molecules that was identified in invertebrates. Among innate immune system factors, PO is critical in insects' defense **(Ajamhassani et al., 2012)**. Honeybee body contains glutathione S-transferase, superoxide dismutase, catalase, and peroxidase which considered the most important antioxidant **(Collins et al., 2004)**. Glutathione, ascorbic acid, vitamin E, uric acid and thioredoxin are non-enzymatic components in the antioxidant system of insects according to **Krishnan and Fergus. (2009).** Feeding honeybee colonies in winter with (Vitamin C) minimizes bee losses and increases health immunity by reducing infestation of *Varroa destructor*. **(Navajas et al., 2008; Farjan et al., 2012 and 2014; Andi and Ahmadi, 2014)**. Feeding honeybee colonies in winter with vitamin C removed the toxic metabolites from bee bodies and increased the anti-oxidative system efficiency **(Chan et al., 2009; Azzami et al., 2012 and Steinmann et al., 2015)**. Furthermore, the positive impact results of honeybee nutrition were revealed on certain aspects of bee immunity according to the effects of viruses and *Paenibacillus larvae* **(Alaux et al., 2010 and Wang et al., 2014)**. In addition the longevity of honeybee and the production of antimicrobial peptides affected mainly by pollen feeding. **(Alaux et al., 2011 and Li et al., 2014)**. Therefore, this study check the role of winter feeding with syrup solution supplemented with vitamins A, B, C, E and a mixture of them on the colonies of honeybee during winter season.

This work aimed to evaluate the effect of artificial feeding to honeybee colonies in winter and the activity of phenoloxidase and antioxidant systems of honeybee colonies. Also, determination of the time and periods of feeding honeybee colonies with artificial diet supplies is another goal of the study which is applied on colonies during the winter season in Minia Governorate.

MATERIALS AND METHODS

This study was conducted from 22th Dec. 2019 to 28th of Feb. 2020 in a private apiary in Samalout district, Minia, Egypt. The trial was extended till moving the honeybee colonies to the flowering citrus trees, and then the supplemental feeding

was no longer applied. Eighteen colonies of the 1st hybrid Carniolan bees (*Apis mellifera carnica*) were selected among the colonies of the apiary. The experimental colonies were nearly equalized in strength and headed by sister queens. The tested colonies were divided to 6 groups, group was included three colonies and offered one of the following feeding processes:

Group (1): 200ml sugar syrup + 0.1mg. vitamin A/ colony/ week intervals.

Group (2): 200ml sugar syrup (1:1) + 0.1mg. vitamin B/ colony/ week intervals.

Group (3): 200ml sugar syrup (1:1) + 1.5 mg. vitaminC/ colony/ week intervals.

Group (4): 200ml sugar syrup (1:1) +0.9 mg. vitaminE/ colony/ week intervals.

Group (5): 200ml sugar syrup (1:1) +vitamins mix (A 0.05 mg. +B0.05 mg +C0.5 mg +E 0.25 mg) / colony/ week intervals.

Group (6): 200ml sugar syrup (1:1)/ colony/ week intervals.

Collection of honeybee workers:

Thirty adult honey bee workers of each group were collected from colonies at the end of the trial. These samples were aseptically collected in sterile screwed cups and kept in -80 ℃ in the central laboratory of Faculty of Agriculture, Minia University.

Preparation of extracts from honeybee workers:

Adults worker bees were homogenized separately in an ice bath for 2 minutes with Buffer Phosphate Sodium (BPS) **(Contreras-Garduno et al., 2007)**, (PH6.5) , at a 1:5 (w/v) ratio. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was used in further analyses.

Phenoloxidase activity:

To measure Phenoloxidase (PO) activity, PO sample (20 µl) was mixed with 20 µl of 15 mM L-dihydroxphenyl alanine (L-DOPA)dissolved in 20 mMTris- Hcl buffer (PH 6.5). To stop reaction, after 40 min of incubation at 28 °C, 260 µl of ice-cold distilled water was added to each sample. Then the reaction of mixture was measured with auv- 2450 spectrophotometer at wavelength of 490 nm. The PO activity was estimated as the increment in the rate of absorbance. An increase of 0.001 per minute was taken to be 1 unit (U): activity = A490x 10 -3 /min. **(Ashida and Dohke, 1980).**

Antioxidant activity

The free radical scavenging effect of the three ethanolic extracts bees was assessed by the discoloration of a methanolic solution of 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical (violet color) according to **(Viturro et al., 1999; Astudillo et al., 2000; Feresin et al., 2002, and Lee et al., 2002).** A freshly prepared DPPH(Sigma) solution (20 mg/l) was used for the assay. Phosphate buffer extraction solution(100 ml) mixed with 3ml of DPPH solution. After 30 min in dark place, the absorbance was measured at 517 nm using Shimadzu UV-120-02 Spectrophotometer, vitamin C was used as a reference free radical scavenger (positive

controls) at the same concentration and the methanolic DPPH served as a control. All reactions were carried out in triplicates and results are given as mean ± s.d. The percentage of DPPH discoloration was calculated as follows:

DPPH discoloration was calculated as follows:

DPPH discoloration % (I) = 100 X (A1 - A2/ A1) Whereas:

Al: absorbance of the control. A2: absorbance in the presence of the tested extract.

Field test of antioxidant and Phenoloxidase

Each group was examined for the presence of European Foulbrood (EFB) and varroa diseases according to Zidan, (2014) and **Zidan et al., (2009).**

Statistical analysis

The obtained data was analyzed as one way ANOVA, using Proc. ANOVA in SAS **(Anonymous, 2003)** and means were compared by LSD (*P*= 0.05 level) in the same program .The reduction rate of varroa infestation was evaluated according to the equation of **Henderson and Tilton (1955).**

RESULTS AND DISCUSSION

Effects of vitamins A, B, C, E and Mix of them added to sugary feeding on phenoloxidase activity in honeybee workers were illustrated in Table (1), and Fig. (1). The highly significant of phenoloxidase activity recorded 49.83 U/gm. with vitamin mix followed by 42.07 U/gm. for vitamin C. The phenoloxidase activity had no significant differences with vitamins C, A, E, and B which showed 42.07, 37.50, 31.23 and 30.97 U/gm, respectively. Phenoloxidase activity were recorded the lowest value with control (23.33 U/gm), (F= 5.77, t-test: $p<0.05$) and LSD (0.05) were 12.02 among all tested treatments.

Also, the obtained data which determined in honeybee workers following the same feeding of sugary solution with vitamins (Table 1), gave high significant values comparing with control (F=5.42 , t-test: $p< 0.05$). The highest antioxidant activity was obtained using vitamins mixture 19.67U/gm while the lowest value was recorded for samples with vitamins B the lowest recoded which gave 11.30 U/gm .These results showed the importance of adding vitamins to the sugary syrup of honeybee colonies in winter to increase immunity of honeybee workers by the activity of phenoloxidase and the total antioxidant**.**

Data illustrated in Table, (2) and figures (3,4) showed that no infections by EFB were observed with feeding with all tested vitamins or with the vitamins mix but the infestation of EFB appeared in control at months Feb., Mar., Apr., May, Jun and Sept. the average were 3, 80, 197, 12, 2 and 1 cells/colony respectively. The vitamins feeding syrup at all months compared to control, recorded the highly reduction in the percentage infestation of varroa mites on workers adults. While the vitamins mix recorded reduction infestation reached to 100% at Mar. and Apr. as well as vitamin B at May and Jun. In general, the vitamins tested can be ranked in descending order according to the reduction in varroa infection as followed; vitamins mix> vitamin B > vitamin E > vitamin C >vitamin A .

This study defined standard work parameters to measure Phenoloxidase (PO) and Antioxidant (AO) activity in honeybee worker bodies . Based on vitamins that had been addedt to a sugary diet. The results showed significant differences in PO activity between the vitamin types, confirming PO modulation as a consequence of stress factors. The PO cascade can be activated by some molecules such as β-glucan, lipopolysaccharides, and peptidoglycans as observed also in recent studies focused on the effect of these molecules on both phenoloxidase activity and haemocytes populations **(Charles and Killian, 2015; Mazzei et al., 2016; Nakhleh, 2017 and Sheehan, 2018)**.Moreover, monoculture plantings reduced the diversity of flowering plant species with vitamins and indicated evidences that honeybees had a requirement for a multitude of vitamins. **Nation and Robinson (1968) and Haydak (1972)** showed that no brood was reared by honeybee colonies which fed sugar syrup without vitamins. Honey bees were experienced widespread declines in abundance and diversity and this proved the relationship between diet and stress resistance in honeybee **(Cameron et al., 2011 and Kosior et al., 2007)**.Nutrition was considered the first line of defense of honeybee colonies and was the vital in dealing with honeybee diseases. Honeybee larvae fed on a nutritionally poor diet were showed to be significantly more susceptible to various diseases **(Foley et al., 2012)**.Water soluble vitamins was common in pollen, but the content of vitamin C, for example, varies throughout the season according to various floral sources **(Ashida, 1990 and Huang, 2012). Herbert and Shimanuki, (1978)** stated that honeybee reared more brood by feeding them with vitamin A and vitamin K. The addition of more than 400 mg/kg ascorbic acid to an artificial diet appears to be synthesised by bees, brood rearing capacity has been increased **(Herbert et al., 1985)**. A mixture of vitamins A, D, E and K in the diet substantially improved the amount of brood produced **(Beck and Strand, 2007)**. **Herbert and Shimanuki (1978)** stated that honeybee reared more brood by feeding them with vitamin A and vitamin K. The addition of more than 400 mg/kg ascorbic acid to an artificial diet appears to be synthesized by bees; brood rearing capacity has been increased **(Herbert et al., 1985)**. stated that more brood was reared when the artificial diet supplemented with 2000 mg/kg ascorbic acid and the high brood rearing may be due to the high antioxidant capacity of ascorbic acid. The growth of larvae increased sharply when vitamin content of the diet was increased three times **(Weaver ,1974)**. **Hagedorn and Burger (1968)** suggested that pollen storage caused a decline in ascorbic acid content. To increase the immunity of honeybee, it's important to feed the bees with vitamin C during winter, as honeybees lived in different conditions, increased environmental the energy in the hemo lymph and generat.Reactive Oxygen Species (ROS) which cause oxidative stress **(Bounias, 1980)**. Promotes energy conservation by decreasing the activity of respiratory enzymes **(Garg and Mahajan, 1994)** and modulates humoral and cellular immune responses **(Kumaret al., 2003)**.

This result was particularly important for the management of honeybee colonies used for pollination of crops that bloom in the winter or the early spring.

Additionally, during winter season nutrition had an important role in physiological stress resistance by providing key nutrients such as vitamins, protein and carbohydrate. Further work was needed to understand more detail the relationship between nutrition content of vitamins and stressors in honey bee. To achieve well healthy colonies it was recommend balanced vitamins added for honeybee colonies, especially when they are placed in a unsuitable environment such as winter, hot summer or after honeybee harvested directly.

Table (1): Effect of some types of vitamins added to sugary feeding on Phenoloxidase (PO) U/gm and % Antioxidant (AO) activity in honeybee worker .

Treatment	Phenoloxidase (PO) U/gm	Antioxidant (AO)%
Vitamin A	37.50b	16.17 AB
Vitamin B	30.97bc	11.30 BC
Vitamin C	42.07ab	16.90 AB
Vitamin E	31.23bc	16.03 AB
Vitamin Mix	49.83a	19.67 A
Control	23.33c	5.80 C
F Value	5.77**	5.42**
P value	0.01**	0.01**
$L.S.D_{(0.05)}$	12.02	6.57

Table (2): Effect of some types of vitamins added to sugary feeding on the infection with EFB and varroa mite reduction percentage in honey bee colonies.

Treatment / Month	Vit. A		Vit. B		Vit. C		Vit. E		Vit. Mix		Control	
	EFB Infec.	% Red. of varroa	EFB Infec.	% Red. of varroa	EFB Infec.	% Red. of varroa	EFB Infec.	% Red. of varroa	EFB Infec.	% Red. of varroa	EFB Infec.	% Red. of varroa
Pre. Treatment	0.0	15.0	0.0	85.0	0.0	79.0	0.0	82.0	0.0	73.0	0.0	8.0
Jan.	0.0	44.0	0.0	79.0	0.0	59.0	0.0	92.0	0.0	56.0	0.0	5.0
Feb.	0.0	72.0	0.0	97.1	0.0	57.0	0.0	98.0	0.0	97.0	3.0	6.0
Mar.	0.0	22.3	0.0	89.9	0.0	98.0	0.0	46.0	0.0	100	80.0	5.3
Apr.	0.0	94.0	0.0	50.0	0.0	41.0	0.0	76.0	0.0	100	197.0	3.6
May	0.0	88.7	0.0	100	0.0	29.7	0.0	44.7	0.0	90.0	12.0	5.3
Jun	0.0	84.7	0.0	100	0.0	41.0	0.0	70.4	0.0	78.4	2.0	6.0
Jul.	0.0	42.0	0.0	37.0	0.0	27.0	0.0	26.4	0.0	72.2	0.0	4.6
Aug.	0.0	34.7	0.0	41.0	0.0	32.0	0.0	50.0	0.0	97.1	0.0	7.0
Sep.	0.0	37.4	0.0	66.0	0.0	75.0	0.0	41.0	0.0	62.0	1.0	5.0
Oct.	0.0	58.0	0.0	89.0	0.0	46.2	0.0	29.4	0.0	82.0	0.0	6.4
Nov.	0.0	45.0	0.0	61.0	0.0	50.0	0.0	34.7	0.0	88.7	0.0	9.3
Dec.	0.0	61.0	0.0	99.0	0.0	59.8	0.0	66.2	0.0	72.8	0.0	9.0

Vit.=vitamin %Red.=percent of reduction Infec.= infection

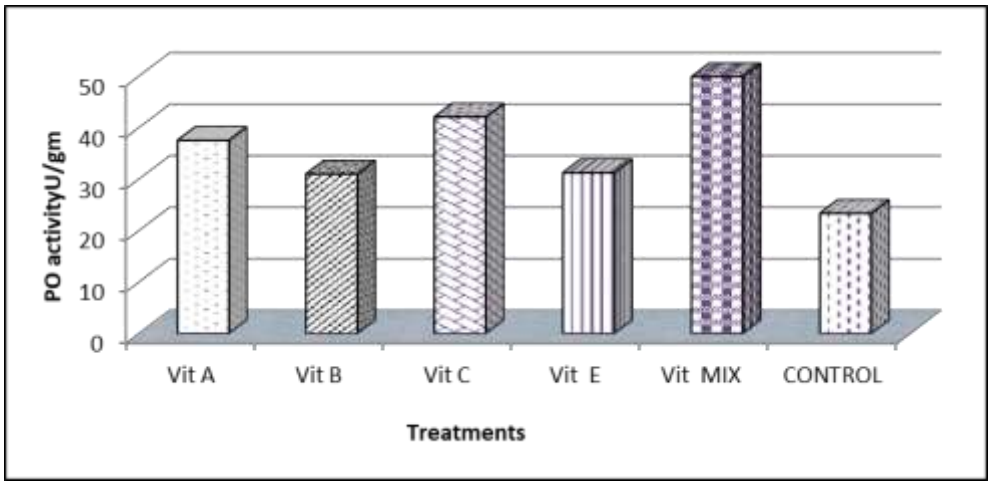

Figure (1) : Phenoloxidase activity in worker bodies of honeybees fed artificial sugar with vitamins.

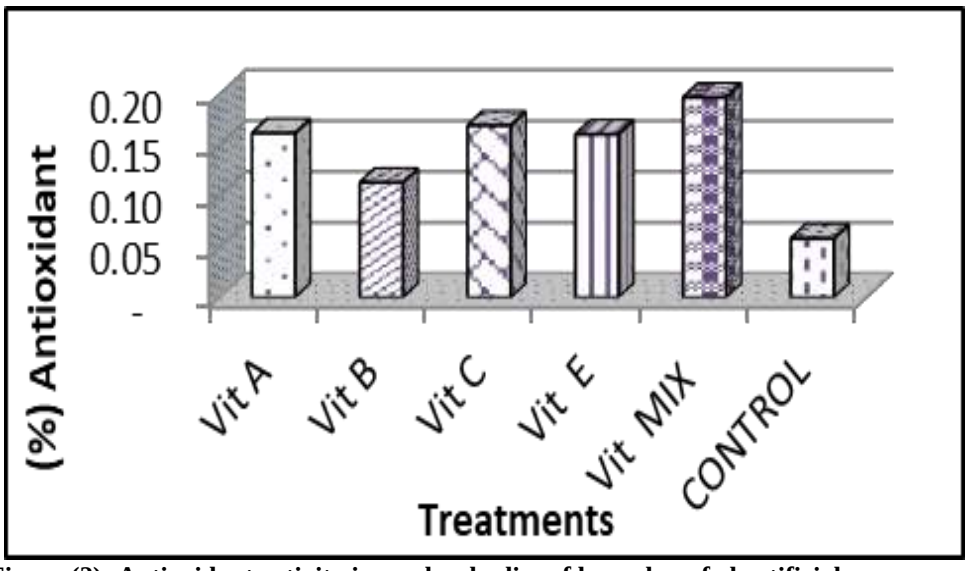

Figure (2): Antioxidant activity in worker bodies of honeybees fed artificial sugar with vitamins.

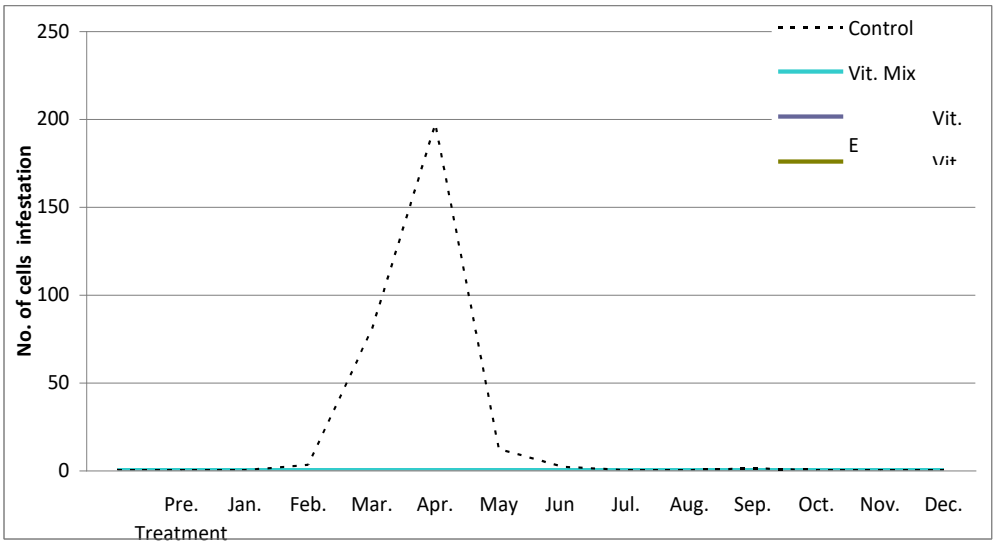

Figure (3): Effect of some types of vitamins added to sugary feeding on the infection with EFB in honey bee colonies.

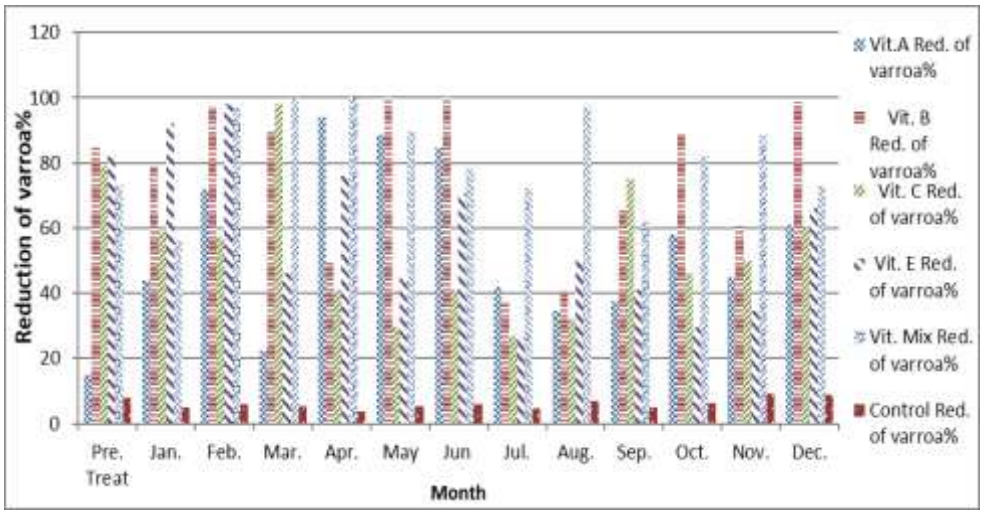

Figure (4): Effect of some types of vitamins added to sugary feeding on the varroa mite reduction percentage in honeybee colonies.

REFERENCES

Ajamhassani, M.; Sendi, J. J.; Farsi, M. J.; and Zibaee, A. (2012). Purification and characterization of phenoloxidase from the hemolymph of *Hyphantria cunea* (Lepidoptera: Arctiidae). Invert. Surv. J., 9(1): 64-71.

Alaux, C.; Dantec, C.; Parrinello, H.; and Le Conte, Y. (2011). Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. BMC genomics,12(1): 1-14.

Alaux, C.; Ducloz, F.; Crauser, D.; and Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. Biol. lett., *6*(4): 562-565.

Anderson, L. M. and Dietz, A. (1976). Pyridoxine requirement of the honey bee (*Apis mellifera*) for brood rearing. Apidol., 7(1): 67-84.

Andi, M. A. and Ahmadi, A. (2014). Influence of vitamin C in sugar syrup on brood area, colony population, body weight and protein in honey bees. Int. J. Biol., 4(6): 32-36.

Anonymous. (2003). SAS Statistics and graphics guide, release 9.1. SAS Institute, Cary, North Carolina 27513, USA.

Ashida, M. (1990). The prophenoloxidase cascade in insect immunity. Res. Immunol.,141(8):908-910.

Ashida, M. and Dohke, K. (1980). Activation of pro-phenoloxidase by the activating enzyme of the silkworm, *Bombyx mori*. Insect Biochem.,10(1): 37-47.

Ashida,M. and Brey,P. (1997). Recentadvancesontheresearchoftheinsect prophenoloxidasecascade, in Molecular Mechanism sofImmune Responsesin Insects, edsP.Brey and D.Hultmark(London:Chapman and Hall),135–172.

Astudillo, L.; Schmeda-Hischmann, G.; Herrea, J. P. and Cortes, M. (2000). Proximate composition and biological activity of *Chilean prosopis* species. J. Sci.food and Agric., 80(5): 567-573.

Azzami, K.; Ritter, W.; Tautz, J.; and Beier, H. (2012). Infection of honey bees with acute bee paralysis virus does not trigger humoral or cellular immune responses. Archives of virology, *157*(4): 689-702.

Beck, M. H. and Strand, M. R. (2007). A novel polydnavirus protein inhibits the insect prophenoloxidase activation pathway. Proc. Nati. Acad. Sci., 104(49):19267-19272.

Bounias, M. (1980). Effects of ascorbic and dehydroascorbic acids per os on the larval glycemia and amino-acidemia of artificially fed *Laspereysia pomonella* (Lepidoptera). Insect Biochem., *10*(5): 521-527.

Brodschneider, R.;Moosbeckhofer,R. and Crailsheim, K. (2010). Surveys as a tool to record winter losses of honey bee colonies: a two year case study in Austria and South Tyrol. J. Apic.Res., 49(1): 23-30.

Brookman, J. L.; Ratcliffe, N. A. and Rowley, A. F. (1989). Studies on the activation of the prophenoloxidase system of insects by bacterial cell wall components. Insect Biochem.,19(1): 47-57.

Cameron, S. A.; Lozier, J. D.; Strange, J. P.; Koch, J. B.; Cordes, N., Solter, L. F.; and Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. Proc. Nation. Acad. Sci., 108(2): 662-667.

Candy, D. J.; Becker, A. and Wegener, G. (1997). Coordination and

integration of metabolism in insect flight. *Comparative* Biochemistry and Physiology Part B: Bioche.Molec. Biol., 117(4): 497-512.

Chance, B. and Maehly, A. C. (1955). [136] Assay of catalases and peroxidases.

Charles, H. M.; and Killian, K. A. (2015). Response of the insect immune system to three different immune challenges. J.Insect physio., 81, (97)-108.

Chan, Q. W.; Melathopoulos, A. P.; Pernal, S. F.; and Foster, L. J. (2009). The innate immune and systemic response in honey bees to a bacterial pathogen, *Paenibacillus larvae*. BMC genomics,*10*(1): 1-9.

Collins, A. M.;Williams, V. and Evans, J. D. (2004). Sperm storage and antioxidative enzyme expression in the honey bee, *Apis mellifera*. Insect Mole.Bio., *13*(2): 141-146.

Contreras-Garduño, J.; Lanz-Mendoza, H. and Córdoba-Aguilar, A. (2007). The expression of a sexually selected trait correlates with different immune defense components and survival in males of the American rubyspot. J.Insect physiol., 53(6): 612-621.

Di Pasquale, G.; Salignon, M.; Le Conte, Y.; Belzunces, L. P.; Decourtye, A.; Kretzschmar, A. and Alaux, C. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter?. PloS One, 8(8): e72016.

Farjan, M.; Dmitryjuk, M.; Lipiński, Z.; Biernat-Łopieńska, E. and sŻółtowska, K. (2012). Supplementation of the honey bee diet with vitamin C: The effect on the antioxidative system of *Apis mellifera* carnica brood at different stages. J.Apic.Res., 51(3): 263-270.

Farjan, M.; Łopieńska-Biernat, E.; Lipiński, Z.; Dmitryjuk, M.; and Żółtowska, K. (2014). Supplementing with vitamin C the diet of honeybees *Apis mellifera carnica* parasitized with *Varroa destructor*: effects on antioxidative status. Parasitol., *141*(6): 770-776.

Feresin, G. E.; Tapia, A.; Angel, G. R.; Delporte, C.; Erazo, N. B.; and Schmeda-Hirschmann, G. (2002). Free radical scavengers, anti-inflammatory and analgesic activity of Acaena magellanica. J.pharmacy and pharmacol., 54(6): 835-844.

Foley, K.; Fazio, G.; Jensen, A. B. and Hughes, W. O. (2012). Nutritional limitation and resistance to opportunistic *Aspergillus* parasites in honey bee larvae. J.Invert. Pathol. , 111(1): 68-73.

Garg, S. K. and Mahajan, S. (1994). Effect of ascorbic acid on longevity and biochemical alterations in *Callosobruchus maculatus* F.(Coleoptera : Bruchidae). Archi. Geronto. Geria., 18(2): 149-157.

Henderson, C. F. and TILTON, E. W. (1955). Tests with acaricides against the brown wheat mite. *Journal of economic entomology*, *48*(2), 157-161.

Hagedorn, H. H. and Burger, M. (1968). Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. II. Effect of vitamin content of pollens. J.Apic. Res., 7(2): 97-101.

Hagedorn, H. H. and Moeller, F. E. (1968). Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands, and brood rearing. J.Apic. Res., 7(2): 89-95.

Haydak, M.H. and Dietz, A. (1972). Cholesterol, pantothenic acid, pyridoxine and thiamine requirements of honeybees for brood rearing. J.Apic.Res., 1(2): 105–109.

Herbert Jr.; E. W. and Shimanuki, H. (1978). Effect of fat soluble vitamins on the brood rearing capabilities of honey bees fed a synthetic diet. Ann.Ento.Soci.Am.,71(5): 689-691.

Herbert Jr, E. W.;Vanderslice, J. T. and Higgs, D. J. (1985). Effect of dietary vitamin C levels on the rate of brood production of free-flying and confined colonies of honey bees. Apidol., 16(4):385-394.

Huang, Z. (2012). Pollen nutrition affects honey bee stress resistance. Ter.Arth.Rev., 5(2): 175-189.

Kosior, A.; Celary, W.; Olejniczak, P.; Fijał, J.; Król, W.; Solarz, W. and Płonka, P. (2007). The decline of the bumble bees and Cuckoo bees (Hymenoptera:Apidae:Bombini) of Western and Central Europe. Oryx., 41(1): 79-88.

Krishnan, D. and Fergus, R. (2009). Fast image deconvolution using hyper-Laplacian priors. Advances in neural information processing systems. 22, 1033-1041.

Kumar, S.; Christophides, G. K.; Cantera, R.; Charles, B.; Han, Y. S.; Meister, S. and Barillas-Mury, C. (2003). The role of reactive oxygen species on Plasmodium melanotic encapsulation in *Anopheles gambiae*.Proce.Natio.Acad.Sci., 100(24): 14139-14144.

Laughton, A. M.; Boots, M. and Siva-Jothy, M. T. (2011). The ontogeny of immunity in the honey bee, *Apis mellifera* L. following an immune challenge. J.Insect Physiol., 57(7): 1023-1032.

Lee, J.C; Kim, H.R.; Kim, J., and Jang, Y.S. (2002). Antioxidant property of an Ethanol extract of the stem of Opuntia ficus˘indica var. Saboten. J. Agric. And Food Chemi.,50(22):6490-6496.

Leclerc, V.; Pelte, N.; Chamy, L. E.; Martinelli, C.; Ligoxygakis, P.; Hoffmann, J. A. and Reichhart, J. M. (2006). Prophenoloxidase activation is not required for survival to microbial infections in Drosophila. Embo.Rep., 7(2): 231-235.

Li, C.; Xu, B., Wang, Y.; Yang, Z.; and Yang, W. (2014). Protein content in larval diet affects adult longevity and antioxidant gene expression in honey bee workers. Entomologia Experimentalis et Applicata,1*51*(1): 19-26.

Lourenço, A. P.; Zufelato, M. S.; Bitondi, M. M. G. and Simões, Z. L. P. (2005). Molecular characterization of a cDNA encoding prophenoloxidase and its expression in *Apis mellifera*. Insect Biochem. Mol. Biol., 35(6): 541-552.

Mazzei, M.; Fronte, B.; Sagona, S.; Carrozza, M. L.; Forzan, M.; Pizzurro, F. and Felicioli, A. (2016). Effect of 1, 3-1, 6 β-glucan on natural and experimental deformed wing virus infection in newly emerged honeybees (*Apis mellifera* ligustica). PLoS One, 11(11): e0166297.

Mullen, L. M. and Goldsworthy, G. J. (2006). Immune responses of locusts to challenge with the pathogenic fungus Metarhizium or high doses of laminarin. J.Insect Physiol., 52(4): 389-398.

Nakhleh, J.; El Moussawi, L. and Osta, M. A. (2017). The melanization response in insect immunity. Advances in Insect Physio., 52, 83-109.

Nation, J. L. and Robinson, F. A. (1968). Brood rearing by caged honey bees in response to inositol and certain pollen fractions in their diet. Ann.Ent.Soc.Am.,61(2): 514-517.

Navajas, M.; Migeon, A.; Alaux, C.; Martin-Magniette, M. L.; Robinson, G. E.; Evans, J. D.; and Le Conte, Y. (2008). Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection. BMC genomics, *9*(1): 1-11.

Navon, A.; Nesbitt, J.; Henzel, W. and Lipke, H. (1985). Effects of ascorbic acid deficiency on growth and cuticle composition of *Manduca sexta* and *Spodoptera littoralis*. Insect Biol., 15(2): 285-291.

Schmid-Hempel, P. (2005). Evolutionary ecology of insect immune defenses. Ann.Rev.Entomol., 50, 529-551.

Siva-Jothy, M. T.; Moret, Y.; and Rolff, J. (2005). Insect immunity: an evolutionary ecology perspective. Advances in insect physio., 32(1):-48.

Söderhäll, K. and Cerenius, L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. Curr.Opin. Immunol., 10(1): 23-28.

Somerville, D. (2005). Fat bees skinny bees. A manual on honey bee nutrition for beekeepers. Australian Govern. Rural Indust Res.Develop.Corpor., Goulburn. 1-142.

Steinmann, N.; Corona, M.; Neumann, P. and Dainat, B. (2015). Overwintering is associated with reduced expression of immune genes and higher susceptibility to virus infection in honey bees. PloS one, 10(6): e0129956.

Sugumaran, M. (2002). Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects Pig.Cell. Res., 15(1): 2-9.

Van Engelsdorp, D.; Evans, J. D.; Saegerman, C.; Mullin, C.; Haubruge, E.; Nguyen, B. K. and Pettis, J. S. (2009). Colony collapse disorder: a descriptive study. PloS One, 4(8): e6481.

Viturro, C.; Molina, A. and Schmeda-Hirchmann, G. (1999). Free radical scavengers from *Mutisi friesiana* (Asteraceae) and *Sanicula gravelens* (Apiaceae). Phyto.Res., 13(5): 422-424.

Wang, H.; Zhang, S. W.; Zeng, Z. J.; and Yan, W. Y. (2014). Nutrition affects longevity and gene expression in honey bee (*Apis mellifera*) workers. Apidol., *45*(5): 618-625.

Weaver, N. (1974). Control of dimorphism in the female honeybee 2. Methods of rearing larvae in the laboratory and of preserving royal jelly. J.Apic.Res., 13(1): 3-14.

Wilson, K.; Cotter, S. C.; Reeson, A. F. and Pell, J. K. (2001). Melanism and disease resistance in insects Ecol.lett., 4 (6): 637-649.

Zidan, E.W. (2014). Eval uation efficacy of certain antibiotics and essential oils for contorlling the american foulbrood dise in honeybee colonies *Apis mellifera* L. Fayoum J. Agric. Res. Develop., 28(1): 1-10.

Zidan, E.W.; Khattab, M.M.; Omar, R.E. and Khattaby, A.M.A(2009). Evaluation of the efficiency of some essential oils and chemical acaricides for varroa control. Ann.Agric. Sci., Moshtohor, 47(3): 85-91.

الملخص العربي

تأثير التغذية السكرية وبعض الفيتامينات على نشاط الفينول أوكسيديز ومضادات الاكسدة في شغالات نحل العسل

حصافي محمد عشبه1 – أيهاب وفيق زيدان2 – سماح فولي أبوالليل1

1 قسم وقاية النبات– كلية الزراعة – جامعة المنيا– مصر.

2 معهد بحوث وقاية النباتات– مركز البحوث الزراعية–الدقي– الجيزة.

أجري هذا البحث لدراسة نشاط أنزيم الفينول أوكسيديز ومضادات الأكسدة في أجسام شغالات نحل العسل نتيجة تغذيتها علي محلول سكري مضاف إليه فيتامينات أ , ب ,ج , هـ وخليط منهم.

وأظهرت النتائج :

فعالية نشاط أنزيم الفينول أوكسيديز ومضادات الأكسدة في أجسام شغالات نحل العسل حيث أعطت الفيتامينات المضافة إلي المحلول السكري مؤشرات مهمة في وقت مبكر نتيجة زيادة تركيز الأنزيم ومضادات الأكسدة في معاملة خليط الفيتامينات يليه معاملة فيتامين ج يليه معاملة فيتامين أ مقارنة بالكنترول وهذا يشير إلى أن طوائف نحل العسل تحتاج إلى التغذية بالفيتامينات خلال فصل الشتاء حتي يملكون آليات تقلل من إجهاد الأكسدة لديهم . أدت التغذية الشتوية بالمحلول السكري والفيتامينات لطوائف نحل العسل المختبرة لمدة 14 أسبوع خلال شتاء 2021/2020إلي تحسين نشاط الطائفة وزيادة مناعة الشغالات حيث لم تسجل إصابات مرضية بالفاروا أو مرض تعفن الحضنة الأوروبي مقارنة بالكنترول التي ظهرت به نسب مختلفة من الإصابة بكلا المرضين .

كلمات مفتاحية: نحل العسل – الفينول أوكسيديز – مضادات الأكسدة – الفيتامينات .