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Prospects of using carbohydrates as supplemented-diets and protein rich mixture as alternative-diet to improve the quality of venom produced by *Apis cerana* L.

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Abstract
In this study, an attempt to assess the effect of carbohydrate supplants and protein rich mixture was studied. Honeybees were fed on glucose, fructose and maltose for two months to guarantee feeding of the new generation on the new introduced diet, besides, bees were artificially fed on sugar (sucrose in water 1:1) and protein rich mixture consisting of soybean flour, dried yeast and dry skim milk, with different portions. Control group of bees was naturally fed of pollens grain, for comparison purposes. The results showed that among the supplemented-carbohydrate diets to the beehives, maltose sugar was found to be the best quality of bee venom, which gave the highest concentration of melittin, phospholipase A_2 and apamin of 535.21±17.73, 374.49±18.94 and 130.36±12.05 µg/µl respectively. The comparison of the alternative diets revealed that the protein rich mixture is better than sucrose diet. While, no significant difference in comparison with the natural pollen grains diet for yielding venom with high quality, and the venom major component concentrations were 585.67±12.89, 439.48±63.64 and 120.61±9.01 µg/µl for melittin, phospholipase A_2 and apamin, respectively.

Keywords: honey bee, bee venom, melittin, phospholipase A2, apamin

1. Introduction
Carbohydrates are the main source of fuel for metabolism in living organisms. They play a major role as structural components that assist in immune system, fertilization, preventing pathogenesis, blood clotting and development of brood (Brodschneider & Crailsheim, 2010) [2]. Honeybees attained carbohydrates from nectar, which consists of many sugars; sucrose, glucose and fructose, while pollen grains provide honey bees with proteins, vitamins, minerals and fats. The protein content of pollen is varying from 7 to 30% (by weight) with an average of about 22%. (Crailsheim *et al.,* 1992) [4]. Strong and energetic colonies of honeybees are highly required for successful beekeeping. Large populations of bees is easily to be achieved through fertility of the queen and ability of workers to store sufficient amounts of pollen grains and honey (Brodschneider & Crailsheim, 2010) [2]. For developmental purposes, worker larvae of bees are provided with royal jelly by nurses from egg hatching up to the age of 3.5 days, then they receive a mixture of royal jelly, honey and pollen with less protein and high amount of carbohydrates (Crailsheim, 1990) [3]. Increasing in the brood area/inch^2 and in the quantity of honey (gms) was obtained by (Abusabbah, Mahmoud, Mahjoub, Omar, & Abdelfatah, 2012) [1] due to feeding bees with compressed date, maize + yeast, chickpea + yeast and pollen grain + yeast. (Herbert Jr, Shimanuki, & CARON, 1977) [6] Reported that, the optimum protein level for caged honey colonies is ranged between 23–30% and not exceeded 50%.
Bee venom is one of the most complicated bee products used widely for medication purposes since ancient times. There is a contradictory on factors that governed the quality and quantity of bee venom, only information on the role of supplementary or/and alternative diets on honey production and brood enlargement is available. The quality of bee venom can be achieved through good content of melittin, phospholipase A_2 and apamin. A comprehensive study in order to provide primary information on the effect of supplementary or/and alternative diets on the quality and quantity of bee venom are highly required.
These two experiments were conducted to evaluate the effect of carbohydrate-supplemented diets and protein rich mixture as alternated-diet on the quality of bee venom of *A. cerana*.

2. Materials and Methods

2.1 Effect of carbohydrates supplemented-diets on the quality of bee venom of *A. cerana*

This experiment was conducted at University Putra Malaysia (UPM) apiary in the field No. 10 during the period from (August - 2012 to November - 2012). To test the effect of addition of glucose fructose and maltose on the quality of Bee venom of *A. cerana.* The lay out of the experiment was randomized complete block design (RCBD), replicated four times. The experimental unit was consisted of a single honeybee hive of *A. cerana.* One of the treatments was left without addition of any type of saccharides and its bees were left to forage naturally for the purpose of comparison (as control). Glucose, fructose and maltose were added separately as a solution of (200 ml/hive) each test hives every two days for two consecutive months to guarantee feeding of the new generation on the new introduced diet.

2.2 Effect of Protein Rich Mixture on the quality of bee venom

To assess the effect of protein rich mixture on the quality of bee venom of *A. cerana, an* experiment was arranged in a complete block design consisted of 4 treatments replicated thrice. Treatments were Sugar (sucrose in water 1:1), Protein rich mixture consisting of soybean flour, dried yeast and dry skim milk in proportion of 3:1:1. The other two treatments were set as control (natural flower nectar feeding). For three treatments, each beehive was caged in a plastic wire mesh measured (2m length x 2m width x 2m height). These cages were made to prohibit bees from feeding form other sources. Two types of control were applied, the first its hives were placed inside cages and the doors were remained opened while the other was left without cages as natural. The hives were provided with their determined diets according to the experiment every 2 days for 2 consecutive months. The bee venom samples were collected from the 2nd generation of honeybees and were analyzed using HPLC. All compiled data were statistically analyzed using JMP® (Institute, 2001) [8] computer based statistical program and means were separated using Tukey test.

2.3 Venom collection and analysis

The venom collector device model (BV0508) was used to collect samples as described by (Kim *et al.,* 2007) [10]. The collected bees' venom was stored in dark chemical analysis of bees' venom glass vial of measured 5 x 5 cm at -20 °C until further analysis.

The bees' venom solution was prepared by diluting 1g/ml distilled water. Prior to analysis, the solution was filtered through a 4.5µm membrane filter (Szczêsna, 2006) [14] then the collected venom samples were analyzed by high performance liquid chromatography (HPLC). The bee venom major component standards were purchased from SIGMA® to determine the standard retention period. A volume of 30µl venom from each sample was injected into C18 column (silica 300 Å) 250 x 4.6 mm. Two solvent system were used i. e., (A) 0.02 (TFA) in water and (B) 0.02 (TFA) in 90:10 acetonitrile: water for 85 min at flow rate of 1.0 ml/min. The temperature was 25 °C for linear gradient elution: 2% B - 60% B for 60 min, 60% B - 95%B, for 15 min, 5% B - 2% B for 25 min. Venom major components were identified using photo diode array detector at 220 nm wave length. The retention time and linear line of the standard melittin, phospholipase 2 and apamin were determined using HPLC in order to compare them with the component of the tested bee venom collected from bees foraged on the test seven plants. The retention time of the standard apamin, phosplipase A_2 and melittin were 25.7, 45.0 and 58.3 minutes respectively (fig.1).

The linear equation of the standard apamin, phospholipase A_2 and melittin were (y=47144x-104405) with (R^2 = 0.992), (y=296820x-985241) with (R^2 = 0.981) and (y=530107x-60707) respectively with (R^2 = 0.999) for all equations (Fig. 2).

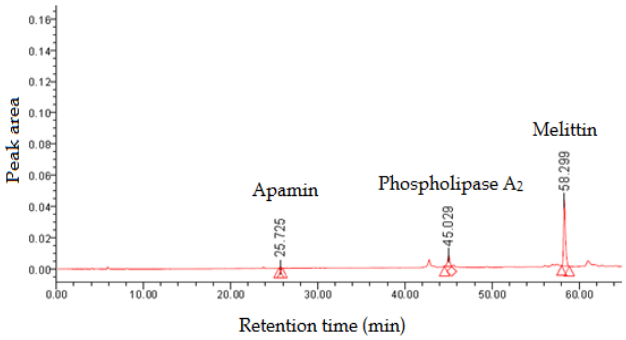

Fig 1: HPLC Chromatogram of the Standard of the Honeybee Venom Major Components.

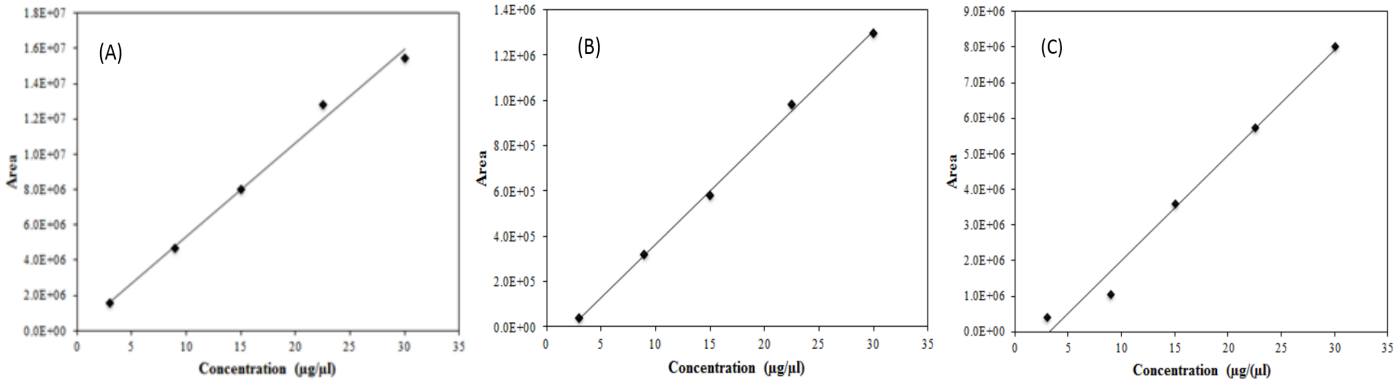

Fig. 2: The standard linear line of (A): Melittin, (B) Apamin; and (C) Phospholipase A_2.

2.4 Statistical analysis

All the obtained results were subject to analysis of variance (ANOVA). The statistical analysis applied by using JMP® (Institute & Publishing, 2009) [9] computer based statistical program and means were separated using Turkey test.

3. Results and Discussion

3.1 Effect of carbohydrates supplemented-diets on the quality of bee venom of *A. cerana*

Honeybee venom major component contents (melittin, phospholipase A_2 and apamin) of venom of *A. cerana* foraged naturally and on diets supplemented by glucose, fructose and

maltose were obtained. The melittin retention time was 55.1 minutes for venom of bees which their diets supplemented by fructose, glucose and maltose, respectively. Phospholipase A_2 took 42 minutes as retention time for bee venom extracted from bees their diets supplemented by fructose, maltose and glucose, respectively. Significant differences between venom components extracted from bees' supplemented-carbohydrates diets were reported.

Significantly, weight differences were recorded between melittin content of venom extracted from bees provided with different carbohydrates as supplemented diets. The Melittin of bees that their feed supplemented by Maltose gave (535.2 µg/µl) which found equivalent to that obtained by the Control (natural foraging) (546.6 µg/µl), while the bees that supplemented by glucose resulted in (415.8) and Fructose gave the lowest content (174 µg/µl) of melittin (Table. 1). Venom produced by bees that their diets were supplemented by three types of saccharides was found significantly different according to the type of added carbohydrates. The highest Phospholipase A_2 content was assessed from venom of bees that their diets supplemented by Maltose (374.2µg/µl) followed by bees foraged on natural diets (370.2µg/µl), glucose (310.8µg/µl) and fructose (211µg/µl). The highest apamin was resulted from venom of bees that were maltose-supplemented diet (130.4 µg/ml) followed by natural diet (control) (128.3 µg/ml) ml followed while glucose gave (111.8µg/ml) and fructose resulted in (95.3 µg/ml). All venom content of apamin in venom samples collected from honeybees supplemented by glucose, fructose and maltose were statistically similar to control content.

The important of suger in broods of honey bee bees studied by (Rortais, Arnold, Halm, & Touffet-Briens, 2005) [13] which they reported that each larva of honey bees consumed 59.4 mg while 18% of sugar content of brood food (fructose and sucrose) used for the first three days of larval development and 45% in the last two days. Carbohydrates, proteins, lipids, vitamins and minerals constitute the main pillar responsible for the reproduced progeny, health of bees' adults and for survival and productivity of different products of bees. (Brodschneider & Crailsheim, 2010) [2]. (Wykes, 1952) [15] proofed that, sugars in nectar were not equally attractive to bees and bees preferred solutions of single sugars of sucrose, glucose, maltose and fructose respectively. (Neupane & Thapa, 2005) [11] Assured that banana syrup can be used as supplementary carbohydrates for its potential on increase of brood frame and cheapness.

Neupane and Thapa (2005) [11] stated that bees hives fed routinely after harvesting honey or during droughts with sucrose solution, invert sugars, or various fruit syrups. Generally, maltose was found the best among the other tested saccharides; it increased the quality of bee venom by increasing the weight of melittin, phospholipase A_2 and apamin in venom.

3.2 Effect of Protein Rich Mixture as alternative diet on the quality of venom of *A. cerana*:

The three test alternative diets were found significantly affect the quality of venom components. Protein rich mixture was found to be the best of the two other alternatives and better than the control on increasing the properties of bee venom components. The result (Table 2) of the experiment showed that there are significant differences of major component of bee venom (melittin, phospholipase A_2 and apamin) among the treated beehives with different sources of diets. Venom produced from bees that artificially fed on protein rich mixture was found contain the same content of melittin as that foraged on natural flowers with both control and natural flowers were 585.7, 546.6 and 488.2 mg, respectively, while the melittin of bees that fed on sugar was the lowest with 364.3µg/µl.

In Table 2. High significant difference was obtained on the content of phospholipase A_2 that produced from bee venom of different treatments. Venom of bees that fed on protein rich mixture gave the highest phospholipase A_2 ca (439.5µg/µl) while the venom produced by bees left as control was (370.2µg/µl and 351.5µg/µl) were found statistically same. While, the lowest content of Phospholipase A_2 was attained from the venom of bees that fed on sugar (276.9µg/µl).

Using of alternative diets to feed bees gave significant differences on the content of apamin extracted from bee venom. Statistically, apamin produced from venom of bees that fed on protein mixture (120.6µg/µl) was same to that produced by bees left as control (128.3 µg/µl). While, the bees fed on sugar was found the least producer of apamin content (98.6). (Table. 2).

It is clear that, the protein mixture is the best among other alternatives to increase the bee venom content. Protein rich diets were found to promote ovarian and egg development in workers of the honeybee, *Apis mellifera* where (Human, Nicolson, Strauss, Pirk, & Dietemann, 2007) [7] stated that greater ovarian development was recorded to worker bees when fed on aloe pollen and sunflower pollen. Also highest activity of ovaries was recorded for bees fed royal jelly in a 1:3 Protein: Carbohydrates ratio (Pirk, Boodhoo, Human, & Nicolson, 2010) [12]. (Decourtye, Mader, & Desneux, 2010) [5] Reported that balanced nutrition supported by a diverse plantation consist of different pollens mixture is the optimal provider of proteins and vitamins for honeybees.

As stated above that, the food source affect the production of honeybees of honey, wax, and in affecting the brood changes on the quality and quantity is highly expected, however the obtained results of this study confirm this hypothesis.

Table 1: Effect of supplemented carbohydrates on the properties of melittin, Phospholipase A_2 and Apamin of venom of *A. cerana.*

Treatments	Mean weight µg/µl± SD		
	Melittin	Phospholipase A2	Apamin
Control	546.55 ± 29.98a	380.23 ± 21.77ab	128.29 ± 4.82a
Maltose	535.20 ± 17.73a	374.49± 18.49a	130.36 ± 6.95a
Glucose	415.77 ± 39.34b	310.78± 30.81b	111.83 ± 3.49ab
Fructose	197.73 ± 34.55c	211.01 ±20.12c	95.25 ± 6.47b
F values	90.8*	32.0*	9.7*

Table 2: Effect of alternative diets of *A. cerana* on Melittin, PhospholipaseA_2 and Apamin of bee venom

Treatments	Means µg/µl±SD		
	Melittin	Phospholipase A_2	Apamin
Protein mixture	585.7±12.8^a	440.8±32.1 ^{a}	122.0±7.9^a
Sugar	370.4±4309.1^b	239.5±41.9 ^{b}	98.5±10.8^b
Natural flower	546.5±29.9^a	380.9±19.2^a	128.3±4.8^a
F values	12.8*	8.0*	9.9*

4. Conclusion

In this study, the supplementary carbohydrate diet and the protein rich mixture alternative bees diet were found to have significant effects on the quality of the bee venom. The concentration of the melittin, phospholipase A2 and the apamin in the venom of the bees that fed with glucose and fructose, which was lower compared to the venom of the bees that were naturally fed. While, the quality of the venom of the bees fed on maltose, was found to be comparable with that produced by the bees foraged on natural flowers. The bee

venom extracted from the bees fed on a protein rich diet showed high significant differences in the quality of the venom. The melittin, phospholipase A2 and apamin concentrations were found to be higher that the concentration in the venom of bees that foraged on natural flowers, and fed by sucrose alternative diet. In addition, the relocation of bee hives and conversion of A. cerana foraged diets showed high significant differences in the content of phospholipase A2 of the bee venom extracted from pink powder and star fruit, whereas, no significant difference was observed between the melittin and apamin contents in the venom of the bees collected from the same plantation areas.

5. References

1. Abusabbah M, Mahmoud M, Mahjoub M, Omar D, Abdel fatah M. Promising alternative diets for honey bees to increase hive activities and sustain honey production during dry seasons in Saudi Arabia. International Journal of Agri Science. 2012; 2(4):361-364.
2. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. Apidologie 2010; 41(3):278-294.
3. Crailsheim K. The protein balance of the honey bee worker. Apidologie 1990; 21(5):417-429.
4. Crailsheim K, Schneider L, Hrassnigg N, Bühlmann G, Brosch U, Gmeinbauer R *et al*. Pollen consumption and utilization in worker honeybees (Apis mellifera carnica): Dependence on individual age and function. Journal of Insect Physiology. 1992; 38(6):409-419.
5. Decourtye A, Mader E, Desneux N. Landscape enhancement of floral resources for honey bees in agro-ecosystems. Apidologie, 2010; 41(3):264-277.
6. Herbert Jr EW, Shimanuki H, CARON D. Optimum protein levels required by honey bees (Hymenoptera, Apidae) to initiate and maintain brood rearing. Apidologie 1977; 8(2):141-146.
7. Human H, Nicolson S, Strauss K, Pirk C, Dietemann V. Influence of pollen quality on ovarian development in honeybee workers (Apis mellifera scutellata). Journal of Insect Physiology. 2007; 53(7):649-655.
8. Institute S. Jmp: The Statistical Discovery Software: Sas Inst., 2001.
9. Institute S, Publishing S. JMP 8 Statistics and Graphics Guide: SAS Institute, 2009.
10. Kim J, Lee HY, Kim MH, Han TS, Cho KR, Kim G *et al.* Antinociceptive efficacy of Korean bee venom in the abdominal pain of the mouse. Journal of Veterinary Clinics-Seoul. 2007; 24(3):320-324.
11. Neupane K, Thapa R. Alternative to off-season sugar supplement feeding of honeybees. Journal of the Institute of Agriculture and Animal Science. 2005; 26:77-81.
12. Pirk CW, Boodhoo C, Human H, Nicolson SW. The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). Apidologie 2010; 41(1):62-72.
13. Rortais A, Arnold G, Halm MP, Touffet-Briens F. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 2005; 36(1):71-83.
14. Szczêsna T. Protein content and amino acid composition of bee-collected pollen from selected botanical origins. J of Apicultural Science. 2006; 50(2):81-90.
15. Wykes G. The preferences of honeybees for solutions of various sugars which occur in nectar. Journal of Experimental Biology. 1952; 29(4):511-519.