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Haemolymph enzymes and sugars levels in foraging hybrid of honey bees (*Apis mellifera* L.) at giza governorate, Egypt

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Abstract
Studies at some physiological properties in foraging honey bee workers in order to reach a physiological method. Results were to reached 1296.15, 762.35, 297.66, 291.65, 125.70 and 122.40 (ux 10^3 / workers, µ g glucose / min./ worker of (Amylase, Trehalase and Invertase), µg β- naphthol/ min./worker and µ g α - naphthol/ min./worker of enzymes / worker, respectively. And were to reach 1694.99 and 768.66 µg/ woker of Glucose and Fructose sugars, respectively. Before study in foraging honey bee for enzymes and sugar levels in haemolymph.
This research deduces the importance of Energy Enzymes and sugars with their relationship to field bees activity in increase of both foraging and production.

Keywords: enzymes activity, sugars, foraging, honey bee, haemolymph, levels

Introduction
Physiological Studies on honey bee (*Apis mellifera* L.) is a social insect that lives in a community in which the behavior of all individuals is highly controlled and sugars. Many studies on honey were carried out by scientists with special reference to its composition [1-7]. Honey as it is found in the hive is a truly remarkable material preferred by the bees from the natural sugar solutions we know as nectar, it is changed from an easily spoiled, then, sweet liquid to a stable high density, high energy, food. By investing the final product and thus raises efficiency of the process in terms of caloric density. The chemical composition of honey is complex and its contents of individual constituents are very considerably [8]. Honey is also known to contain a large numbers of polyphenols, flavonoids and antioxidants [9]. And honey is also known to contain sugar, pollen grains, pigments, Enzymes and water [10].
From these points of view this research was conducted to know and determined physiological characters of enzymes and sugars (glucose and fructose) in levels in foraging hybrid of honey bees for biochemical analysis component [11].

Materials and Methods
Bee workers of hybrid. All samples (3 replicates / sample) at Giza Governorate were collected from apiary in Beekeeping Research Department, Dokki during March 2022. All Samples were analyzed at the chemical microanalysis unit, physiology Res., plant protection Res., institute Giza, Egypt.

For the following properties
preparation of insects for analysis
The insect were homogenized in distilled water (50 gm/ 1ml.). Homogenates were centrifuged at 8000 rpm for 15 min at 5 ˚c in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use.

1. Determination of Amaylase, and Invertase activity
Digestive enzymes were determined according the method described by [12] using trehalose, sucrose, and soluble starch as substrates for trehalase, Invertase and α- amylase, respectively. Generally, 20 ml of diluted enzyme solution was incubated for 10 min. at 30 ˚c with 250 ml. 4 % sucrose solution and 230 ml. phosphate buffer (PH, 5.4, 0.1 M).
The reaction was stopped by adding 250 ml. DNS reagent to each tube in boiling water for 5 min. samples were cooled, diluted with 2.5 ml. H_2O and read at 550 nm.

2. Determination of Beta and Alpha esterases activity
Beta esterases (β- esterases) and Alpha esterases (α – esterases) were determined according to [13] using α-naphthyl acetate or β - - naphthyl acetate as substrates, respectively**.** The developed color was read at 600 or 555 nm for α and β – naphthol produced from hydrolosis of the substrate, respectivel.

3. Determination of phosphates activity
Acid and alkaline phosphatases were determined according to [14]. In this method, the phenol released by enzymatic hydrolysis of disodium phenyl phosphate reacts with 4-amino antipyrine, and by the addition of potassium ferricanide, the characteristic brown color is produced.

4. Determination of Glucose
Glucose is widely distributed simple sugar with an active aldehyde group. Estimation of glucose by glucose oxidase gives the true glucose concentration eliminating the interference by other reducing sugars. Glucose was assyed using stanbio kit (Stanbio lab., Inc. 2930 East Houston st., san Antonio, Texsas 78202). Glucose oxidase catalyses the oxidation of alpha-D-glucose to D-glucono-1,5 lacyone (gluconic acid) with the formation of hydrogen peroxide. The oxygen liberated from hydrogen peroxide by peroxidase reacts with the o-dianisidine and oxidisis it to a red

chromophore product that read at 500 nm by spectrophotometer, and the optical density compared by standard (con. 100mg %) to obtain the results, [15].

5. Determination of Fructose

Was done using biodiagnostic Kit (info@biodiagnostic.com) Fractose forms a pink color when heated with resorcimol in the presence of hydrochloric acid, which can be directly measured photometrically at 495 nm. Fifty microliters of sample were added to 0.5 ml. of trichloroacetic acid (1M). mix well. let stand for 10 mn. Centrifuge at 3000 rpm for 10 mn. Add 50 ml. of the supernatant to 100 ml. of resorcinol (mM) and 1 ml. of H cl (9M) Mix well, place into a boiling water bath for exactly 5 min. Allow to cool in cold water. Then measure the absorbance against reagent blank and compared to that of standard fructose (300 mg/ dl), [15].

Statistical Analysis

Means bearing different subscript (within column) are significantly different ($P < 0.01$, ANOVA, [16] multiple rang test).

Results and Discussion

Data in Tables (1, 2, 3): show that the average of Alkaline phosphatase activities reached 1296.15 (UX 10^3 / worker) in field bees hybrid. The highest activity of alkaline phosphatase enzyme in heamolymph of field bees. A-esterase enzyme in the same tables show that mean activity reached 122.40 mg α – naphthol / min. / worker, β esterase reached 125.70 mg β- naphthol / min. / worker, Invertase, Trehalase and amylase were reached 762.35, 297.66 and 291.65 mg glucose / min/ worker respectively. The highest activity of Invertase enzyme more than Trehalase and Amaylase enzymes. The main digestive enzyme of carbohydrates present in the alimentary tract of adult bees has been studied.

The α – amylase that hydrolysis starch contained in pollen grains [17]. Sugars are degraded into glucose and fructose by digestive enzymes in the alimentary tract. The carbohydrate economy of adult bees is well understood, especially the distribution and sugar concentration in tissues [18]. Trehalose is the main sugar found in haemolymph of honey bee. Its concentration is very high and varies from 2mg. / ml. to 40 mg / ml [19]. Other sugar found in the haemolymph is glucose and fructose. Their concentrations are relatively low, 15 mg / ml. and 7 mg / ml. for glucose and fructose, respectively [20]. Table (3) show that the average of glucose and fructose activities reached 1694.99 and 768.66 mg / worker, respectively.

Table 1: Biochemical analysis component (Enzymes and sugars / workers) in haemolymph in foraging of honey bee

Treatments (Enzymes)	Rep.	Level/ enzyme/ workers	Treatments Sugars	Rep.	Level/ Sugars/ workers
Amylase	1	97.30	Glucose	1	564.99
	2	98.14		2	564.00
	3	96.21		3	566.00
Mean		291.65±13.00	Mean		1694.99± 84.20
Trehalase	1	99.22	Fructose	1	257.0
	2	99.0		2	255.44
	3	99.44		3	256.22
Mean		297.66±19.90	Mean		768.66 ± 49.60
Invertase	1	254.11			
	2	253.24			
	3	255.00			
Mean		762.35± 16.40			
β-esterase	1	41.90			
	2	42.00			
	3	41.80			
Mean		125.70 ±5.40			
α-esterase	1	42.00			
	2	39.40			
	3	41.00			
Mean		122.40±5.95			
Alkaline Phosphatase	1	432.05			
	2	431.00			
	3	433.00			
Mean		1296.15±30.15			

Table 2: The main Biochemical analysis component (Enzymes/ bees) of field honey bee workers

Treatments (Enzymes)	Values
Amylase	291.65± 13.00=mg glucose /min./worker
Trehalase	297.66± 19.90 = mg glucose /min./worker
Invertase	762.35± 16.40 = mg glucose /min./worker
β-esterase	125.70± 5.40 = mg β-naphthal / min./ worker
α-esterase	122.40 ± 5.95 = mg α- naphthal / min./ worker
Alkaline Phosphatase	1296.15± 30.15= U x 10^3 / worker

Table 3: The main Biochemical analysis component (sugars/ bees) of field honey bee workers

Treatments (sugars)	Values
Glucose	1694.99±84.20 mg./ worker
Fructose	768.66± 49.60 mg./ worker

Tables (1, 2, 3) the means bearing different subscripts (Within column) are significantly different ($P< 0.01$, ANOVA, Duncans multiple rand test)

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