

PHYSIOLOGICAL CHARACTERISTICS OF CARNIOLAN AND ITALIAN HONEY BEE STRAINS WITH SPECIAL REFERENCE TO CITRUS HONEY COMPONENTS IN EGYPT

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ABSTRACT: Studies some of physiological properties on honey bee workers in both Carniolan and Italian pure strains in Order to reach a physiological method to differentiate between both strains and citrus honey reproduce from them with showed of DNA analysis of both strains by (PCR) polymerase chain reaction which indicated that: Deduce of Ca^{++} , Mg^{+++} , Na^+ , K^+ elements in citrus honey. Results were increase of Carniolan in the percentage of Ca^{++} and Mg^{+++} , mean reached 23.44, 3.10 (mg%) and decreases in the Italian bee to reached 16.81, 2.01 (mg%), respectively. And were reached of Na^+ and K^+ 8.86, 16.94 - 8,00 15.53 (m Eq%) of Italian and Carniolan bee, respectively. Increase of Carniolan bee of enzymes activities α and β esterase, Invertase, Trehalase and Amylase in reproduced citrus honey more than Italian bee. Increase of sugars (Glucose and Fructose) in field bee haemolymph in both strains bee. Increase of enzyme activities Invertase, Trehalase and Amylase in field bees haemolymph in both Carniolan and Italian strains. And not different between both strains in DNA analysis.

Key words: Strains, Biochemical analysis, Enzymes activity-DNA analysis - (PCR) polymerase chain reaction, haemolymph, physiological characteristics, components.

INTRODUCTION

Many studies on honey were carried out by Scientists with special Reference to it's composition (white *et al.*, 1962), (White and winters, 1989, white, (1979), AOAC, (1980), Siddique, (1970), Donner, (1977), Farghally, (1980) and El-Sherbiny and Rizk, 1979). Honey as it is found in the hive is a truly remarkable material prepared 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 inverting the sugar in the nectar, the bee mereases the attainable density of 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 (Hassan, 1985). Honey, is also known to contain a large numbers of polyphenols, flavonoides and antipoxidants (Blasa *et al.*, 2006). And honey is also known to contain sugar, pollen

grains, pigments, Minerals, Enzymes and water (Abd-El-Naby and Zidan (2014).

From these points of view this research was conducted to know and determined physiological characters of citrus honey and Carniolan, Italian bees for biochemical analysis component and DNA analysis of Carniolan and Italian bees strains (Besnard *et al.* (2001), Abdelrazik *et al.* (2007).

MATERIALS AND METHODS

Citrus honey and bee workers of Carniolan and Italian strains. All samples (3 replicates/ sample) at Menoufia Governorate were collected from apiary in Sadat Town during spring season from period March - April 2015/2016. All samples were analyzed at the Chemical microanalysis Unit, physiology Res. Dep., plant protection Res. Institute Giza, Egypt. For the following properties:

Apparatus:

Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST - 2 Mechanic-Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at -20°C till use for biochemical assays. Double beam ultraviolet / visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

Preparation of insects for analysis:

The insects were homogenized in distilled water (50 mg / 1 ml). 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.

Honey preparation:

Weight accurately a representative quantity of about 5 g of the homogenous honey sample, dissolve in distilled water and dilute to 100 ml in a calibration flask (diluted honey solution).

Calcium (Ca^{++}) determination:

Calcium ion was determined using Bioanalytics kit (email: bioanlab@bellsouth.net, Palm city, USA). Calcium reacts with cresophthalein in an alkaline medium to form a colored complex. The color developed has a maximum absorbance at 570 nm and is proportional to the calcium concentration in the sample. Measurement was against reagent blank and compared to calcium standard (10 mg / dl).

Magnesium (Mg^{+++}) determination:

Determination of Mg by the xylidyl blue method was followed using Quimica Clinica Aplicada S.A. kit (Spain). In an alkaline medium, the magnesium ions of the sample will produce a colored complex with xylidyl blue. Color intensity is directly proportional to the magnesium ions concentration. Ten

microliters of the sample were added to 1 ml reagent (0.1 mM xylidyl blue; 0.3 mM Tris buffer pH 11; 50 μM Glycoetherdiamine -N, N, N N -tetraacetic acid as a chelating agent) for 10 min at room temperature (20-25 °C). The Color produced was read at 520 nm against standard (4 mg / dl).

Na^+ and K^+ determination:

Ions measurements were made on a radiometer FLM3 flame photometer as described by Amin and El-Halafawy (2002). The standard solution contained sodium chloride (14 ± 1.4 mmol/L) and potassium chloride (5 ± 0.5 mmol/L) stored at room temperature (25 °C). Zero adjustment was against blank prepared by adding 5 ml of concentrated lithium chloride (300 ± 5 mmol/L) to 500 ml of distilled water.

Determination of phosphates activity:

Acid and alkaline phosphatases were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown color is produced.

Determination of Alpha and Beta esterases activity:

Alpha esterases (α -esterases) and beta esterases (β -esterases) were determined according to Van Asperen (1962) 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 hydrolysis of the substrate, respectively.

Determination of Invertase, Trehalase and Amylase activities:

Digestive enzymes were determined according to the method described by Ishaaya and Swirski (1976) using trehalose, sucrose, and soluble starch as substrates

for trehalase, invertase and α -amylase, respectively.

Generally, 20 μ l of diluted enzyme solution was incubated for 10 min at 30 °C with 250 μ l 4% sucrose solution and 230 μ l phosphate buffer (pH, 5.4, 0.1 M). The reaction was stopped by adding 250 μ l DNS reagent to each tube in boiling water for 5 min. Samples were cooled, diluted with 2.5 ml H₂O, and read at 550 nm.

Glucose determination:

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 assayed using Stanbio kit (Stanbio Laboratory, Inc. 2930 East Houston street, San Antonio, Texas 78202). Glucose oxidase catalyses the oxidation of α -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 oxidises it to a red chromophore product that read at 500 nm by spectrophotometer, and the optical density compared by standard (cone. 100 mg %) to obtain the results, Bogdanov and Baumann (1988).

Fructose determination:

Determination of fructose was done using Biodiagnostic kit (info@bio-diagnosvtic.com). Fructose forms a pink color when heated with resorcinol 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 (1 M). Mix well. Let stand for 10 min. Centrifuge at 3000 rpm for 10 min. Add 50 μ l of the supernatant to 100 μ l of resorcinol (9 mM) and 1 ml of HCL (9 M). 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), Bogdanov and Baumann (1988).

Analysis of DNA from haemolymph of bee workers both strains of Carniolan and Italian using bead-beating method (Abdel-Razik *et al.*, 2007).

PCR reactions (25 μ l) with primers were contained 25 to 50 mg of DNA (1 μ l of diluted DNA), 105 μ l of 10x reaction buffer, 200 μ M dNTPs (sigmas chemical Co. St. Louis), 0.8 μ M primer. Primers were screened against DNA from haemolymph. Tested primers that produced strong, reproducible PCR products (bands) of both strains. The reproducibility of the RAPD markers was tested by performing PCR reactions with different concentrations (20-200 μ g/ μ l) of DNA template, with at least three independent DNA extractions from the same sample (Besnard *et al.*, 2001). Analysis of DNA of both Carniolan and Italian bees was conducted at the Insect biotechnology and Molecular biology unit, plant protection Res. Institute.

Statistical analysis:

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's, 1955 multiple range test).

RESULTS AND DISCUSSION

The determination of some minerals in citrus honey:

Data in Table (1) reveal the mean percentage of calcium reached 16.81 and 23.44 (mg%) at Italian and Carniolan bee, respectively.

While the mean of Magnesium (mg%), sodium and potassium (mEq%) reached 2.01, 3.10-8.86, 8.00 - 16.94, 15.53 (%) at Italian and Carniolan bee, respectively.

The increased the mean percentage of calcium and potassium in Carniolan and Italian bees but not determined of house and field bees from both strains for Ca⁺⁺ , Mg⁺⁺⁺ , Na⁺ and K⁺ .

Table (1): The mean biochemical analysis components (Minerals/honey) of Carniolan and Italian bees strains

Minerals	Carniolan bee $\pm$SD (Citrus honey)	Italian bee $\pm$SD (Citrus honey)
Calcium (mg%)	23.44 $\pm$ 2.37a	16.81 $\pm$ 1.16a
Magnesium (mg%)	3.10 $\pm$ 0.27a	2.01 $\pm$ 0.21b
Sodium (mEq%)	8.00 $\pm$ 0.26a	8.86 $\pm$ 0.3a
Potassium (mEq%)	15.53 $\pm$ 0.21a	16.94 $\pm$ 1.56a

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's multiple range test)

The determination of enzymes activities: Alkaline phosphatase enzyme:

Data in Table (2) show that the average of Alkaline phosphates activities reached 1315.7, 1372.0, 1539.3 and 1220.3 ($U \times 10^3$ /insect (worker)) in house bees and field bees Carniolan and Italian strain, respectively.

The highest activity of alkaline phosphatase enzyme in haemolymph of house bees of Italian strain more than field bees. And house, field bees of Carniolan strain. Alkaline phosphatase in honey of both strains was Nil because bee honey acidity (pH=3.9-4.1) Table (3).

Alpha esterase enzyme:

Data presented in Table (2) show that mean activity of Alpha esterase enzyme reached 97.67, 90.70, 139.00 and 154.00 (μ g α -naphthol/min/insect (worker)) in Carniolan and Italian (house, field bees), respectively.

And the mean reached 2388.00 and 2366.33 (μ g α -naphthol/min/ml) in Carniolan and Italian honey, respectively.

The highest activity of Alpha esterase enzyme in haemolymph of Italian bees and highest in honey of Carniolan bees. Table (3).

Beta esterase enzyme:

As shown in Table (2) mean activity of Beta esterase enzyme reached 86.33, 79.70, 112.70 and 171.70 (μ g - β -naphthol/min./insect (worker)) in Carniolan and Italian (house, field bees), respectively.

And the mean reached 2201.70 and 2011.30 (μ g - β -naphthol/min./ml) in Carniolan and Italian honey respectively.

The highest activity of Beta esterase enzyme in haemolymph of Italian bees and highest activity in honey of Carniolan bees. Table (3).

Invertase enzyme:

Data in Table (2) mean activity of invertase enzyme reached 499.33, 701.70, 539.33 and 823.00 (μ g glucose/min./insect) in Carniolan and Italian (house, field bees) respectively.

And the mean reached 2316.70 and 1410.00 (μ g glucose/min./ml) in Carniolan and Italian honey respectively. Table (3).

The highest activity of invertase enzyme in haemolymph of field bees Carniolan and Italian bees Table (2) and highest activity in honey of Carniolan bees more than of Italian honey (Table 3).

Physiological characteristics of carniolan and italian honey bee

Table (2): The mean biochemical analysis components (enzymes/bees) of Carniolan and Italian bees strains

Treatments	Carniolan bee $\pm$ SD		Italian bee $\pm$ SD	
	House bees	Field bees	House bees	Field bees
Enzymes:				
Alkaline Phosphatase	1315.70 $\pm$ 20.5b	1372.00 $\pm$ 30.1 ab	1539.30 $\pm$ 56.5a	1220.30 $\pm$ 118b
α -esterase	97.67 $\pm$ 3.51c	90.70 $\pm$ 4.0c	139.00 $\pm$ 4.35b	154.00 $\pm$ 7.9a
β -esterase	86.33 $\pm$ 3.21c	79.70 $\pm$ 3.2c	112.70 $\pm$ 7.37b	171.70 $\pm$ 7.63a
Invertase	499.33 $\pm$ 14c	701.70 $\pm$ 7.6b	539.33 $\pm$ 10.5c	823.00 $\pm$ 25.2a
Trehalase	154.33 $\pm$ 8.14b	155.33 $\pm$ 13.6b	165.33 $\pm$ 8.5b	440.00 $\pm$ 26.8a
Amylase	140.70 $\pm$ 4.0c	184.00 $\pm$ 15b	136.33 $\pm$ 4.16c	399.33 $\pm$ 11a

Alkaline Phosphatase=U $\times 10^3$ /worker)

α -esterase = μ g α -naphthol/min/worker

β -esterase = μ g β -naphthol/min/worker

Invertase + Trehalase + Amylase = μ g glucose/min/worker

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's multiple range test)

Table (3): The mean biochemical analysis components (enzymes/honey) of Carniolan and Italian bees strains

Treatments	Carniolan bee $\pm$ SD (Citrus honey)	Italian bee $\pm$ SD (Citrus honey)
Enzymes:		
Alkaline Phosphatase	Nil	Nil
α -esterase	2388.0 $\pm$ 164a μ g α -naphthol/min/ml.	2366.33 $\pm$ 138a μ g α -naphthol/min/ml.
β -esterase	2201.7 $\pm$ 93a μ g β -naphthol/min/ml.	2011.3 $\pm$ 127a μ g β -naphthol/min/ml.
Invertase	2316.7 $\pm$ 125a μ g glucose/min/ml.	1410.0 $\pm$ 147b μ g glucose/min/ml.
Trehalase	2101.7 $\pm$ 60a μ g glucose/min/ml.	2020.0 $\pm$ 72a μ g glucose/min/ml.
Amylase	1985.7 $\pm$ 106a μ g glucose/min/ml.	826.33 $\pm$ 66b μ g glucose/min/ml.

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's multiple range test)

Trehalase enzyme:

Data in Table (2) mean activity of Trehalase enzyme reached 154.33, 155.33, 165.33 and 440.00 (μg glucose/min/insect) in Carniolan and Italian honey (house, field bees), respectively. And the mean reached 2101.7 and 2020.0 (μg glucose/min/ml.) in Carniolan and Italian honey, respectively. The highest activity of Trehalase enzyme in haemolymph of field bees Italian and the highest activity of Trehalase in honey of Carniolan bees. Table (3)

Amylase enzyme:

As shown in Table (2) mean activity of Amylase enzyme reached 140.7, 184.00, 136.33 and 399.33 (μg glucose/min/insect) in Carniolan and Italian (house, field bees), respectively.

And the mean reached 1985.7 and 826.33 (μg glucose/min/ml) in Carniolan and Italian honey, respectively (Table 3). The highest activity of Amylase enzyme in haemolymph of field Italian bees. (table 2).

And highest enzyme activity in honey of Carniolan bees. More than Italian honey table (3).

The main digestive enzymes of carbohydrates present in the alimentary track of adult bees has been studied. The α -amylase that hydrolysis starch contained in pollen grains (Ohashi *et al.*, 1999).

The determination of Glucose Sugar:

As shown in table (4) mean reached of Glucose sugar were 323.33, 1084.66, 311.00 and 2305.33 (μg /insect) in Carniolan and Italian (house, field bees), respectively.

And the mean reached 27.13 and 32.43 (gm %) in Carniolan and Italian honey, respectively (Table 5). The highest concentration of glucose sugar in haemolymph of field bees in Carniolan and Italian bees (Table 4) and significant between of glucose sugar in honey of Carniolan and Italian bees. 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. (Panzenböck and Crailsheim, 1977).

Trehalose is the main sugar found in haemolymph of honeybee. Its concentration is very high and varies from 2 mg/ml. to 40 mg/ml. (Blatt and Roces 2001). Other sugars found in the haemolymph are glucose and fructose. Their concentrations are relatively low: 15 μg /l and 7 μg / ml for glucose and fructose, respectively (Leta *et al.* 1996).

The determination of fructose sugar:

Data in Table (4) mean reached of fructose sugar were 275.33, 611.66, 310.00 and 925.66 (μg /insect) in Carniolan and Italian (house, field bees), respectively. And the mean reached 38.17 and 28.88 (gm%) in Carniolan and Italian honey, respectively (Table 5). The highest concentration of fructose sugar in haemolymph of field bees in Italian bees more than Carniolan bees and the highest concentration of fructose sugar in honey of Carniolan bees more than Italian honey. Generally the mean of biochemical analysis components of Carniolan and Italian honey bee strains of minerals, enzymes and sugars in citrus honey and haemolymph for bees from both strains (Table 6).

DNA analysis on Carniolan and Italian bees strains

As shown in Fig (1) show results of sequence of DNA for strains using PCR (Polymers Chain Reaction) and bands for DNA genome. Upper MW (Molecular Weights) had upper bands and lower molecular weights had lower bands. This analysis was obtained by primer Light bands (results (DNA) on gel image. DNA was not different in the two samples of strains.

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Table (4): The mean biochemical analysis components (sugars/bees) of Carniolan and Italian bees strains

Treatments	Carniolan bee $\pm$ SD strain		Italian bee $\pm$ SD strain	
	House bees	Field bees	House bees	Field bees
Sugars in bees:				
Glucose (μ g/worker)	323.33 $\pm$ 10.4c	1084.66 $\pm$ 74b	311.00 $\pm$ 8.9c	2305.33 $\pm$ 94.5a
Fructose (μ g/worker)	275.33 $\pm$ 17.9c	611.66 $\pm$ 34.5b	310.00 $\pm$ 11.1c	925.66 $\pm$ 65.7a

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's multiple range test)

Table (5): The mean biochemical analysis components (sugars/honey) of Carniolan and Italian bees strains

Treatments	Carniolan bee $\pm$ SD (Citrus honey)	Italian bee $\pm$ SD (Citrus honey)
Sugars in honey:		
Glucose (gm%)	27.13 $\pm$ 0.96a	32.43 $\pm$ 0.81b
Fructose (gm%)	38.17 $\pm$ 1.3a	28.88 $\pm$ 0.96b

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's multiple range test)

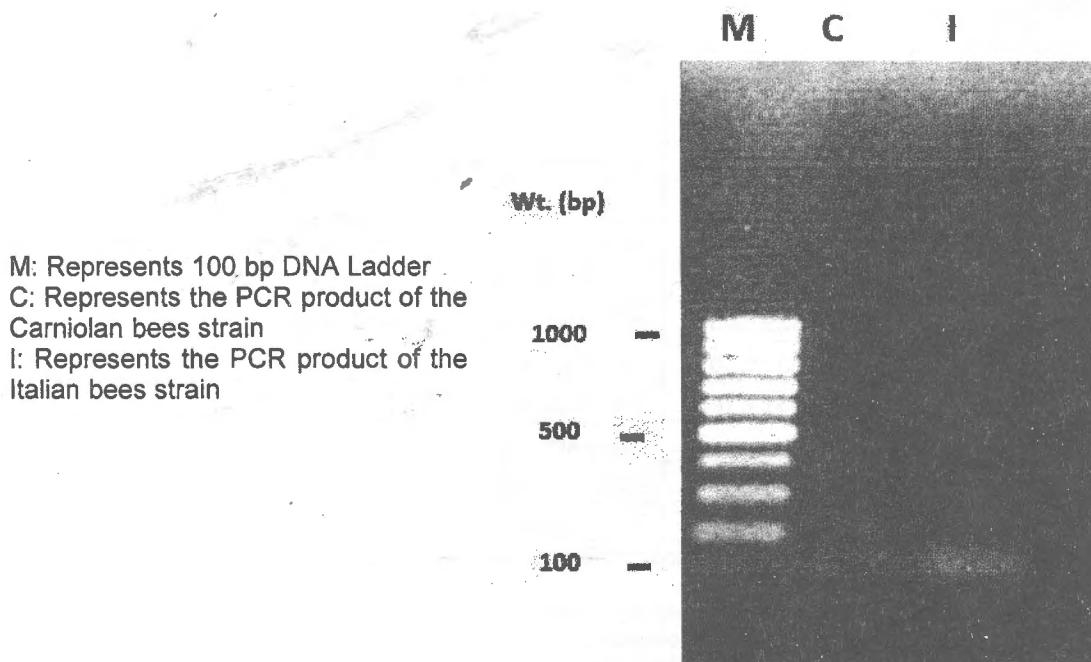


Figure (1): Agarose gel 0.8% of the amplified DNA samples with universal primers.

Table (6): The mean biochemical analysis components of Carniolan and Italian bee strains.

Treatments	House bees	Field bees	Citrus honey	Values
Carniolan bee strain ±SD				
Minerals				
Calcium (Ca ⁺⁺)	not determined	not determined	23.44±2.37a	mg%
Magnesium Mg ⁺⁺⁺			3.10±0.27a	mg%
Sodium (Na ⁺)			8.00±0.26a	mEq%
Potassium (K ⁺)			15.53±0.21a	mEq %
Enzymes				
Alkaline phosphatase	1315.70±20.5b	1372.00±30.1ab	Nil	Ux10 ³ /worker
α-esterase	97.67±3.51c	90.70±4.0c	2388.0± 164a	*
β-esterase	86.33±3.21c	79.70±3.2c	2201.7±93a	**
Invertase	499.33±14c	701.70±7.6b	2316.7±125a	***
Trehalase	154.33±8.14b	155.33±13.6b	2101.7±60a	***
Amylase	140.70±4.0c	184.00±15b	1985.7±106a	***
Sugars				
Glucose	323.33±10.4c	1084.66±74b	27.13±0.96a	****
Fructose	275.33±17.9c	611.66±34.5b	38.17±1.3a	****
Italian bee strain ±SD				
Treatments	House bees	Field bees	Citrus honey	Values
Minerals				
Calcium (Ca ⁺⁺)	not determined	not determined	16.81±1.16a	mg%
Magnesium Mg ⁺⁺⁺			2.01±0.21b	mg%
Sodium (Na ⁺)			8.86±0.3a	mEq%
Potassium (K ⁺)			16.94±1.56a	mEq%
Enzymes				
Alkaline phosphatase	1539.30±56.5a	1220.30±118b	Nil	Ux10 ³ /worker
α-esterase	139.00±4.35b	154.00±7.9a	2366.33±138a	*
β-esterase	112.70±7.37b	171.70±7.63a	2011.3±127a	**
Invertase	539.33±10.5c	823.00±25.2a	1410.0±147b	***
Trehalase	165.33±8.5b	440.00±26.8a	2020.0±72a	***
Amylase	136.33±4.16c	399.33±11a	826.33±66b	***
Sugars				
Glucose	311.00±8.9c	2305.33±94.5a	32.43±0.81b	****
Fructose	310.00±11.1c	925.66±65.7a	28.88±0.96b	****

Means bearing different subscripts (within column) are significantly different ($p < 0.01$, ANOVA, Duncan's multiple range test)

* α -esterase values in Bee = $\mu\text{g } \alpha\text{-naphthol/min/worker}$

* α -esterase values in Honey = $\mu\text{g } \alpha\text{-naphthol/min/mil}$

** β -esterase values in Bee = $\mu\text{g } \beta\text{-naphthol/min/worker}$

** β -esterase values in Honey = $\mu\text{g } \beta\text{-naphthol/min/mil}$

*** Invertase + Trehalase + Amylase in Bee = $\mu\text{g glucose/min/worker}$ *** Invertase + Trehalase + Amylase in Honey = $\mu\text{g glucose/min/mil}$. **** Glucose + Fructose.

in Bee = $\mu\text{g /worker}$ **** Glucose + Fructose. in Honey = gm%

Generally the statistical analysis of the obtained data show significant differences in the minerals, enzymes and sugars in citrus honey and haemolymph for bees between Carniolan and Italian honeybee colonies.

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الخصائص الفسيولوجية لسلاسل نحل العسل الكرنيولي والإيطالي و علاقة ذلك بمكونات عسل الموالح في مصر

أمانى سعد مصطفى محمد أبو ليلة

قسم بحوث النحل - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - دقي - جيزة - مصر

الملخص العربي

يهدف البحث إلى التوصل لطريقة فسيولوجية للتمييز بين سلاسل نحل العسل الكرنيولي والإيطالي النقية و عسل الموالح الناتج منهما بالإضافة إلى تحليل الـ DNA للشغالات عن طريق تفاعل البلمرة التسلسلي (PCR) في فصل الربيع (مارس وأبريل) عامي ٢٠١٥/٢٠١٦ بأحد مناحل مدينة السادات ، محافظة المنوفية وذلك لتقدير:

١- عناصر Ca^{++} , Mg^{+++} , Na^{+} , K^{+} في العسل الناتج من كلا السلالتين.

٢- أنشطة إنزيمات : الفوسفاتيز (في وسط قلوي) - الفاوييتا استيريز - الانفريتيز - التريهاليز - الأميليز في الشغالات الحاضنة والحقلية و العسل الناتج.

٣- كميات سكري الجلوكوز و الفركتوز في الشغالات الحاضنة والحقلية و العسل الناتج.

٤- تحليل الـ DNA للشغالات بإجراء تفاعل البلمرة التسلسلي.

وأوضحت النتائج وجود فروق معنوية واضحة بين السلالتين كما يلي :

أولاً: عسل الموالح:

زادت النسب المئوية للكالسيوم و المغنسيوم في سلالة النحل الكرنيولي وبلغت في المتوسط ٢٣.٤٤ ، ٣.١ مليجرام % على التوالي و انخفضت في السلالة الإيطالي حيث بلغت ١٦.٨١ ، ٢.٠١ مليجرام % على التوالي، بينما تقاربت النسبة للصوديوم و البوتاسيوم في سلاسل نحل الإيطالي والكرنيولي فبلغت في المتوسط ٨.٨٦ ، ١٦.٩٤ - ٨ ، ١٥.٥٣ mEq % على الترتيب ولم يتم تقدير (تحديد) هذه العناصر في الشغالات لكلا السلالتين .

ثانياً: الإنزيمات والسكريات:

- ١- تفوقت سلالة النحل الكرنيولي في زيادة أنشطة أنزيمات الألفا و البيتا استيريز و الانفرتيز والتريهاليز و الأميليز في العسل الناتج عن السلالة الإيطالية.
- ٢- زادت كميات سكري الجلوكوز والفركتوز في هيموليمف النحل الحقلّي عن النحل الحاضن لكلا السلالتين.
- ٣- زيادة نشاط أنزيمات الانفرتيز و التريهاليز و الأميليز في دم النحل الحقلّي عن النحل الحاضن لكلا السلالتين.
- ٤- تفوقت سلالة النحل الإيطالي في زيادة أنشطة الانزيمات محل الدراسة في هيموليف الشغالات عن السلالة الكرنيولي.

ثالثاً: تحليل الـ DNA :

- لم تظهر فروق واضحة عند تحليل الـ DNA لعينات هيموليمف الشغالات لسلالتي النحل الكرنيولي والإيطالي ولذلك تحتاج هذه النقطة لمزيد من الدراسة.
- وطبقاً للنتائج السابقة يوصي بما يلي:
- ١- ضرورة الاهتمام والاستمرار في تربية كلا السلالتين في المناطق المصرية حيث تتفوق السلالة الكرنيولي في إنتاج العسل أكثر من الحضنة ، و تتفوق السلالة الإيطالية في إنتاج الحضنة أكثر من العسل لأن لكل سلالة صفاتها الفسيولوجية التي تميزها.
 - ٢- التوصية باستيراد كلا السلالتين النقية بطريقة رسمية منعاً لحدوث التربية الداخلية Inbreeding (تربية الأقارب) والتي تسبب انخفاض صفات تلك السلالات و قلة الإنتاج.