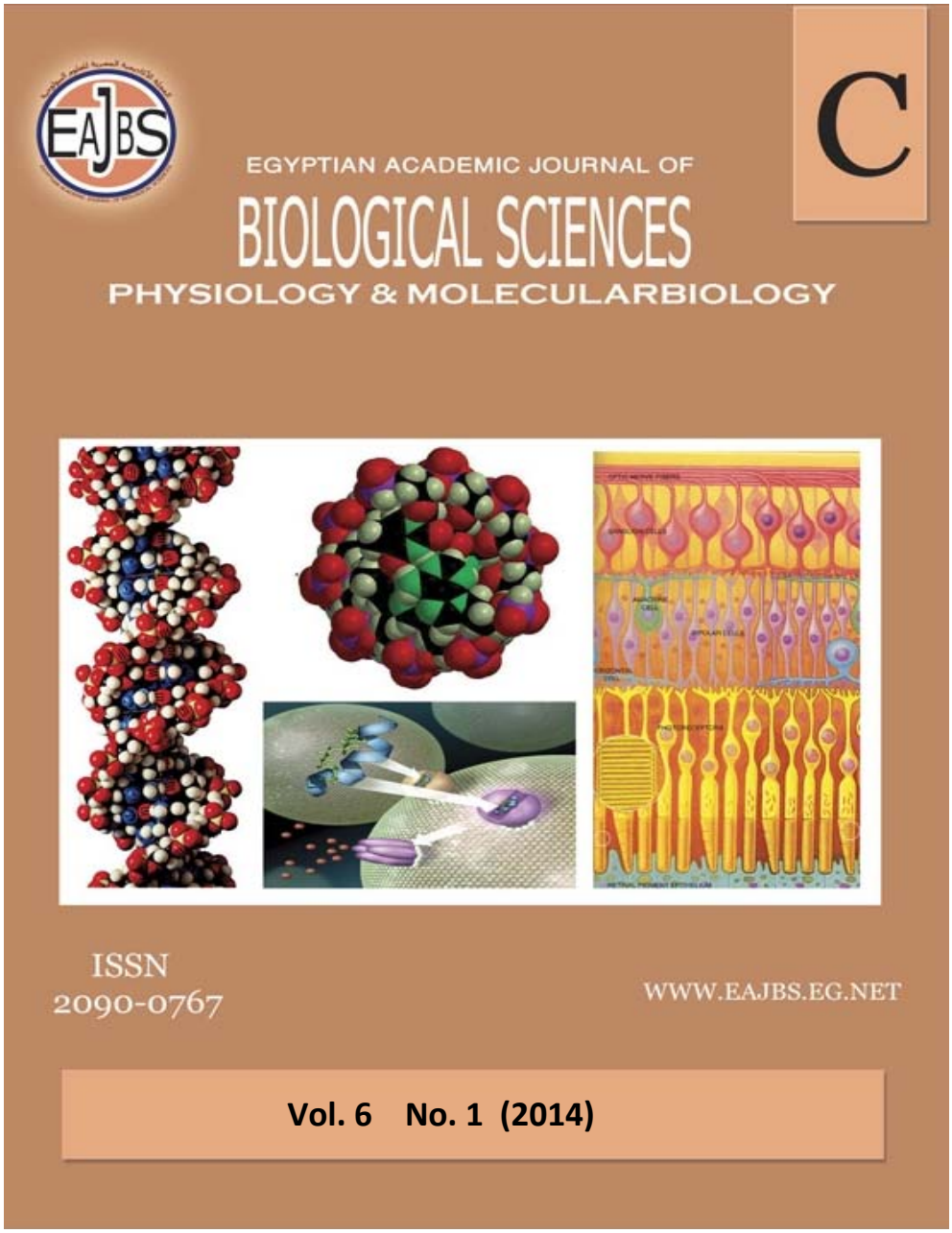

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Activity Level of Lactate dehydrogenase and β-glucosidase Enzymes in the honeybee colonies, (*Apis mellifera* L.) with different feeding

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ABSTRACT

The activity of Lactate dehydrogenase and β-glucosidase was determined in Carniolan workers honey bee, *Apis mellifera* L. after feeding on different diets antibiotic ,ripe milk and some natural plant extract (thyme, clove, cinnamon, black seeds and anise) . The results showed the highest increase in the β-glucosidase activity was occurred in the diet containing Ripe milk (106.17%) and the other diets scored decrease in the activity , as well as the Lactate dehydrogenase activity the results showed highly enzyme activity in diets antibiotic and clove 192.56%, & 215.70%. respectively as compared with the other diets used in this study.

INTRODUCTION

Carbohydrates and fatty acids play a predominant ro1e in the generation of energy for prolonged flights in insects (Beenakkers, 1969; Sacktor, 1970). Insects utilize various high-energy fuels for flight. They derive energy to meet the intense metabolic needs of flight from carbohydrates, fat or amino acids such as proline (Wheeler, 1989; Candy, 1989). During flight, honeybees increase their metabolic rate to relatively high values (Harrison and Fewell 2002; Feuerbacher *et al.,* 2003). In honey bee colonies requires proteins, carbohydrates, lipids, vitamins, minerals and water. These nutrients must be in the diet in a definite qualitative the food metabolism plays an important role in bee life. The physiological digestion different in bee body composition; percentage of glycogen, lipids and proteins (Hrassing and Crailsheim 2005). The source of feeding honey bees effects on induction haemolymph enzymes (Bounias and Morgan, 1990).

The enzyme glucosidase is ubiquitous and occurs in all the living kingdoms starting from bacteria to highly evolved mammals and performs varied functions in these organisms. In bacteria and fungi, β-glucosidases are mainly a part of the cellulase enzyme system and are responsible for the hydrolysis of short chain oligosaccharides and cellobiose (Bisaria and Mishra, 1989; Kubicek *et al.,* 1993). In insects and plants, β-glucosidase is involved in the release of cyanides from cyano-glucoside precursors. This is a part of a defense mechanism displayed in these systems (Esen, 1993).

Biochemical characteristics of β - glucosidase have been studied in the digestive system and salivary glands of many insect species belong to different orders, such as *Glyphodes pyloalis* Walker (Lep.: Pyralidae), *Apis mellifera* L. (Hymenoptera: Apidae), *Drosophila melanogaster* Meigen *(*Diptera: Drosophilidae), *Xanthogaleruca luteola* Mull. (Col.: Chrysomelidae) and *Rhynchophorus ferrugineus* Olivieri (Col.: Curculionide) (Ghadamyari *et al*. 2010; Riseh *et al*. 2012; Huber and Mathison 1976; Sharifi *et al*. 2011; Tanimura *et al*. 1976; Chipoulet and Chararas, 1985; Pratviel-Sosa *et al*. 1987; Santos and Terra, 1985; Terra and Ferreira, 1958). B-glucosidase can degrade cellobiose and cello-oligosaccharides to glucose in insects. Hemicelluloses and cellulose present in insect food are converted to di- and oligo-β-saccharides by some carbohydrases and digestive β-glucosidases that play an important role in their hydrolysis (Terra WR, Ferreira, 1994). Also, several investigations indicate the main role of β-glucosidases in insect-host plant interaction (Ferreira *et al.*1997; Ferreira *et al*.1998; Mattiacci *et al.*1995; Marana *et al.* 2000). β-glucosidases are enzymes that catalyze the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates. Several glucosidases are specific for the cleavage of glycosidic bonds depending on the number, position, or configuration of the hydroxyl groups in the sugar molecule. The activity of glucosidases is fundamental to several biochemical processes such as degradation of diet polysaccharides to furnish monosaccharide units, which are then able to be metabolically and used by the organism, lysosomal glycoconjugate catabolism and glycoprotein processing, and biosynthesis of oligosaccharide units in glycoproteins or glycolipids (Murray *et al*. 1994).

In insects, the fat body is the main organ responsible for energetic metabolism. This is also the organ of conversion and storage of fat, carbohydrates, and proteins (Arrese and Soulages 2010). The fat body is responsible for metabolism of carbohydrates and is especially enlarged in insect larvae. In honeybee larvae, it can constitute up to 65% of an individual's body mass (Bishop 1925).

Lactate dehydrogenase (LDH) is an important glycolytic enzyme present in virtually all animal tissues (Kaplan and Pesce, 1996). It is also involved in carbohydrate metabolism and has been used to indicate exposure to chemical stress (Diamantino *et al.,* 2001). LDH is involved in the production of energy, being particularly important when a considerable amount of additional energy is required immediately. A negative correlation between LDH activity and ambient oxygen levels for some aquatic organisms were suggesting a possible biochemical adjustment in response to the lowered oxygen levels. This probably occurs also in situations of chemical stress. Therefore, this enzyme may be a sensitive criterion in laboratory (Senthil Nathan *et al.,* 2005). LDH is, also, a parameter widely used in toxicology and in clinical chemistry to diagnose cell, tissue, and organ damage. However, the potential of this enzyme as an indicative criterion in the invertebrate toxicity tests has been scarcely explored (Senthil Nathan *et al.,* 2006).

The aim of this study is to evaluate the effect of different feeding period winter to help bee colonies to surmount on the malnutrition particularly at unsuitable conditions

MATERIALS AND METHODS

Feeding Honeybee Colonies

This work was carried out at the apiary of the Agriculture Research Station, Beni sweif Egypt. During the

period from 22nd Dec. 2012 to 10th of Feb. 2013. Twenty four healthy honeybee colonies from Carniolian Hybrid were conducted for this study.

Food supplement

A cake of 100gm food supplement consisted of one part medical dried yeast mixed with two parts powder sugar (w/w) according to zidan 2009.

Diet (1) content 100gm of food supplement and 2gm antibiotic (Sodium Sulphate demidin).

Diet (2) content 100gm of food supplement and 30ml of natural plant extract of Thyme.

Diet (3) content 100gm of food supplement and 30ml of natural plant extract of Clove.

Diet (4) content 100gm of food supplement and 30ml of natural plant extract of Cinnamon.

Diet (5) content 100gm of food supplement and 30ml of natural plant extract of black seeds.

Diet (6) content 100gm of food supplement and 30ml of natural plant extract of Anise.

Diet (7) content Ripe milk mixed with powder sugar (1: 3) (w: w).

Diet (8) Control (fed on food supplement without additives). 100gm of blend was placed on the top of the Combs from three colonies weekly to feed throughout the experimental periods. Samples of 100 adult workers were taken directly from the central comb of each colony at the experimental end. Bee Samples was transferred to the Laboratory and immediately frozen stored until analysis.

Preparation of extracts from bees

The samples were homogenized in a glass Potter homogenizer on an ice bath with 2.5 ml. cool 0.9% NaCl (1:10 w/v). Homogenates were centrifuged for 15 min at 1000 x g at 4 °C. In the supernatants.

Determination of enzymatic activity Determination of Lactate dehydrogenase activity:

The method described here is derived from the formulation recommended by the German Society for clinical chemistry (DGKC, 1972). Lactate dehydrogenase of catalyzes the conversion pyruvate to lactate, NADH is oxidized to NAD in the process. The rate of decrease in NADH is directly proportional to the LDH activity and determined photometrically.

Determination of β - glucosidase activity:

β - glucosidase activity was measured by assaying glucose liberated by enzymatic hydrolysis of salicin as described by Lindorth (1988).

RESULTS AND DISCUSSION

Data show in Table (1) indicated that the increase of percentage in the biological activity to area of sealed worker brood reared, the diets which content antibiotic and ripe milk recorded higher value percentage 429.96% and 384.95, respectively, the same diets recorded higher value of percentage for mean number of combs covered with bees (115.475% and 107.85, respectively) and recorded higher value of percentage pollen stored with all diets than control, but found the diets which content antibiotic and ripe milk recorded higher value percentage 38.80% and &33.78%, respectively.

Table 1: Effect of the different feeding on some biological activities of the honey bee Colonies.

treatments	Mean area of sealed worker brood (inch^2)			Mean No. of combs covered with bee /colony			Pollen grain	
	treatments		Increasing %	treatments		Increasing %	Mean area (inch^2)	Increasing %
	before	After		before	After			
Diet(1)	115.47	9.33	4.33	429.96	1190.67	224.67	276.67	38.80
Diet(2)	100.23	8.67	4.33	328.85	1060.67	247.33	262.67	31.78
Diet(3)	78.37	8.33	4.67	204.66	960.67	315.33	249.33	25.08
Diet(4)	92.38	8.33	4.33	204.24	910.67	299.33	255.33	28.09
Diet(5)	78.37	8.33	4.67	279.70	1035.33	272.67	245.33	23.10
Diet(6)	85.65	8.67	4.67	193.53	966.67	329.33	260.67	30.77
Diet(7)	107.85	9.00	4.33	384.95	1290.67	266.67	266.67	33.78
Diet(8)	64.24	7.67	4.67	138.41	740.67	310.67	199.33	-

Zidan (2009) fed honey bee colonies with food supplement mixed with 6 ml of each of the previously prepared mixture; eucalyptus, peppermint and thymol oils by different concentration and the control one was fed only with cake. He found that the increase of the biological activities of treated bee colony (sealed worker brood, covered combs with bee, pollen grain stored, royal jelly and honey),advice the beekeeper to using essential oils with sugar solution added to pollen supplement for their directly that help the bee workers to the reactivation after the season end.

The data resulted for the biochemical assay summarized in Table 2, the changes in β-glucosidase activity in the homogenate of honeybee, *Apis mellifera* (Fig.1) show the low activity in all types of different feeding, Antibiotic (Sodium Sulphate demidin), diet contain extract of Thyme, diet contain extract of Clove, diet contain extract of Cinnamon, diet contain extract of black seeds and diet contain extract of Anise (23.95 %, 31.71%, 34.77%, 24.95%, 43.01% and 56.58%, respectively). On the contrary, the diet containing Ripe milk show high activity (106.17%). The enzyme activity decreases as the glucose chain length increases (Bisaria and Mishra, 1989; Kubicek *et al*., 1993). That means the increase in activity of β-glucosidases due to degradation of diet polysaccharides to furnish monosaccharide units, which are then able to be metabolically and convert monosaccharide to energy (Murray *et al*. 1994). The activity of the enzyme depends on several factors including; age of bee, stage of the colony, nectar flow, environment conditions and the beekeeping practices β-glycosidase has been purified the ventricles and honey sac of *Apis mellifera* L.(De Moraes and Bowen 2000).

Table 2: Enzyme activity for β - glucosidase and Lactate dehydrogenase at homogenate honeybee

Treatment	Lactate dehydrogenase U x 10^3 /ml			β – glucosidase (ug glucose./min/mg)		
	Mean ± SE	A*	%	Mean ± SE	A*	%
Diet (1)	77.667 ± 0.752	1.93	192.56	2.860 ± 0.235	0.24	23.95
Diet (2)	32.000 ± 0.965	0.79	79.34	3.787 ± 0.133	0.32	31.71
Diet (3)	87.000 ± 0.769	2.16	215.70	4.153 ± 0.359	0.35	34.77
Diet (4)	28.667 ± 0.999	0.71	71.08	2.980 ± 0.141	0.25	24.95
Diet (5)	38.000 ± 0.661	0.94	94.22	5.137 ± 0.337	0.43	43.01
Diet (6)	29.333 ± 0.366	0.73	72.73	6.757 ± 0.344	0.57	56.58
Diet (7)	18.333 ± 0.717	0.45	45.45	12.680 ± 0.788	1.06	106.17
Control	40.333 ± 1.001	1.00	100.00	11.943 ± 0.618	1.00	100.00

Diet (1): Antibiotic (Sodium Sulphate demidin) Diet (2): extract of Thyme Diet (3): extract of Clove
Diet (4): extract of Cinnamon Diet (5): extract of black seeds Diet (6): extract of Anise
Diet (7): Ripe milk Diet (8): Control
A*= Enzyme activity for diet no. ( ) / Enzyme activity for control * % = percentage relative to control

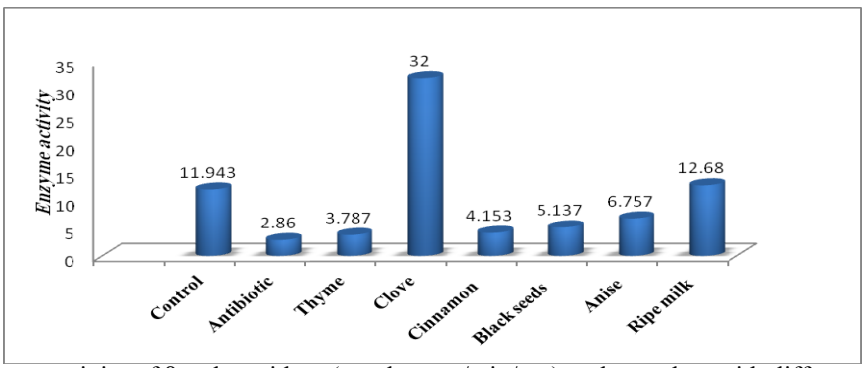

Fig. 1: Enzyme activity of β - glucosidase (ug glucose./min/mg) on honey bee with different feeding.

Data in the Table (2) and Fig. (2) show that, the lactate dehydrogenase activity showed highly enzyme activity in diet contain extract of Clove and Antibiotic (Sodium Sulphate demidin) 215.70% and 192.56%, respectively. Lactate dehydrogenase is an important glycolytic enzyme present in virtually all animal tissues (Kaplan and Pesce, 1996). It is also involved in carbohydrate metabolism and has been used to indicate exposure to chemical stress (Wu and Lam, 1997; Diamantino *et al.,* 2001). Higher LDH activity in insects is most probably due to consumption as well as utilization of large quantities of food and involved in the production of energy, being particularly important when a considerable amount of additional energy is required immediately. A negative correlation between LDH activity and ambient oxygen levels. (Wu and Lam, 1997; Diamantino *et al.,* 2001; Senthil Nathan *et al.,* 2005). The result in other diet used in this study show low activity of LDH in the diet contain extract of Thyme, diet contain extract of Cinnamon, diet contain extract of black seeds , diet contain extract of Anise and diet containing Ripe milk (79.34%, 71.08%, 94.22%, 72.73% and 45.45%%, respectively). From result found inverse relationship between β-glucosidase activity and Lactate dehydrogenase activity in diet contain Ripe milk that may be come back to increase of protein percentage and the diets which content antibiotic and ripe milk recorded higher value percentage to area of sealed worker brood reared, mean number of combs covered with bees and pollen stored so that recommended to use that diet for feeding honey bee.

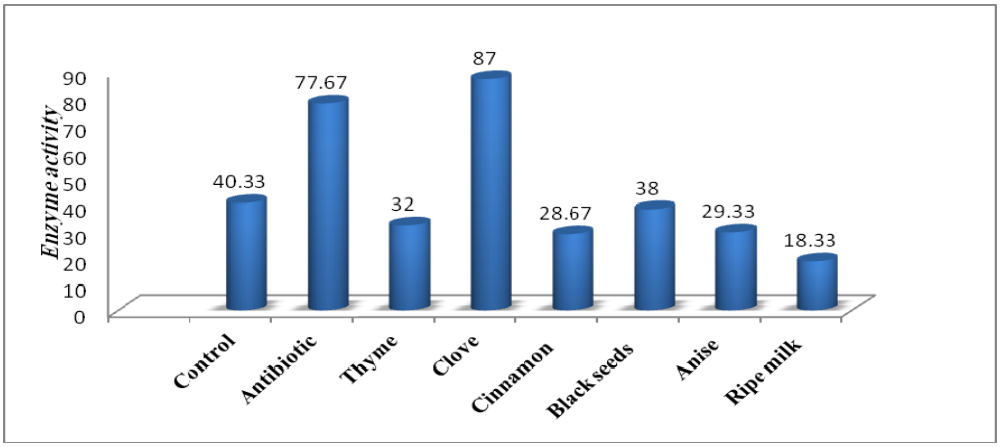

Fig. 2: Enzyme activity of lactate dehydrogenase (U x 10^3 /ml) on honey bee with different feeding.

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ARABIC SUMMARY

مستوى نشاط إنزيمي لاكتك ديهدروجينيز وبيتا جليكوسيديز في طوائف نحل العسل مع التغذيات المختلفة

سامح مصطفى عبد النبى ، ايهاب وفيق زيدان
معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى – الجيزة

نشاط إنزيم لاكتك ديهدروجينيز وبيتا جليكوسيديز تم تقديرها فى شغالات نحل العسل بعد التغذية على بيئات مختلفة (مضاد حيوى ، لبن رايب، مستخلصات نباتية طبيعية) (زعتر ، قرنفل، القرفة، الحبة السوداء، الينسون) وقد اظهرت النتائج ارتفاع نسبيى فى نشاط انزيم بيتاجليكوسيديزفي البيئة الغذائية التي تحتوي علي اللبن الرايب 106.17% وانخفاض فى جميع المعاملات الأخرى، بينما وجد ارتفاع فى نشاط إنزيم لاكتك ديهيدروجينيز في حالة البيئات الغذائية التي تحتوي علي المضاد الحيوي والقرنفل 192,56%و215,70 علي التوالي مقارنة مع البيئات الغذائية الاخري المستخدمة في هذه الدراسة