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EFFECT OF GAMMA RADIATION ON THE ENZYME ACTIVITY OF HONEY BEE *APIS MELLIFERA* WORKERS

Sawires,S.G.[2]; Faiza A. Mariy[1]; Elbassiouny, A.M. [1] and Gharieb, O.H. [2]

[1]*Plant Protec., Dep., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.*

[2] *Nat. Prod., Dep., NC RR T, Atomic Energy Authority, Cairo, Egypt.*

ABSTRACT

The workers honey bee *Apis mellifera* which used in the present study was prepared from colonies headed with open mated local Carnica queens located at the apiary belonging to Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Samples of worker bees were transferred to National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt. for treating with of 20, 40, 50, 100, 150 and 200 rad of gamma radiation.

In the present study two isozyme systems esterase and malate dehydrogenase were estimated for identification and characterization effect of gamma radiation on honey bee (*Apis mellifera*). The zymogram reveals that esterase isozyme (EST) band no 1 (Est 0.4) is present in all bees where there is no difference in intensities. It was present till doses 20, 40, 50, 100, 150, 200 rad and control in a eminent activity in different doses. Band no 2 (Est 0.5) is present in moderate density different doses. Band no 3 (Est 0.6) appears in low activity in different doses by gamma radiation subjected to 20, 40, 50, 100, 150, 200 rad compared with the control. The zymogram of malic dehedrogenase (MDH) of different treatments by gamma radiation reveals different isozyme bands which were detected in some bees of different doses in maximum of four bands with different intensities. Band no 1 is the major band which nearly has the same degree of intensity in all treatment by gamma radiation. Isozyme band no 2 is present in all treatment of bees with different intensities. Band no 3 is absent in the all treatment bees and control expect dose 20 rad. Band no 4 appears in low activity in the bees, then it is nearly has the same intensity in all treatment.

Key word: honeybee -*Apis mellifera* - malate dehydrogenase (MDH) - esterase (EST)

INTRODUCTION

Insect uses fuels to power its flight, from which two major components: carbohydrates and the amino acid proline (Zebe and Gade, 1993). The consumption of these two substrates is well reflected by the presence of enzymes involved in the pathways of proline breakdown and glycolysis in the flight muscles, while the pathway of lipid oxidation is relatively poorly developed as judged by its associated enzymes (Auerswald and Gade, 1999).

The honeybee (*Apis mellifera* L.) is a heterothermic insect. During many duties inside the colony he become ectothermic. He changes to the endothermic state throughout the foraging cycle (Waddington 1990 and Stabentheiner 2001) and when need additional necessary heat to regulate the brood nest temperature (Koeniger, 1978 and Ritter, 1982) or to prevent chill coma and freezing in the swarm cluster (Heinrich, 1981) or winter cluster (Stabentheiner *et al*, 2003).During their life, individual honeybees can encounter a wide variety of ambient temperatures, from below freezing point to more than 40 ^{0}C (Heinrich, 1993). The energy turnover of the bees in both the ectothermic and endothermic state varies widely depending on the ambient temperature. With decreasing ambient temperature, it decreases in the resting (ectothermic) bees (Rothe and Nachtigall, 1989) but increases in the thermally active (endothermic) bees (Moffatt, 2001 and Blatt and Roces, 2001).

Multiple molecular forms of enzymes, or isozymes, are commonly investigated in genetic, ontogenetic and phylogenetic studies. Isozymes investigations in insects have been concerned primarily with genetic variations and developmental stages. Variation in flight metabolism has a clear genetic basis. Different genetic strains of honey bees often differ in flight metabolic rate, and these differences in flight physiology can be correlated with foraging effort, suggesting a possible pathway for selection effects on flight metabolism (Harrison and Fewell, 2002).

Investigated hemolymph esterase patterns during larval and pupal development of the two female colonies of the honeybee found pronounced differences with time between and within colonies

Subsequent studies revealed similar findings for a number of dehydrogenase isozymes (Tripathi and Dixon, 1968 & 1969).

Emphasis will be given to gamma radiation controlled enzyme patterns in the worker honey bees. The present experiment deals with malate dehydrogenase (MDH). This enzyme was chosen for several reasons. First, there are numerous reports regarding the low level or absence in different colonies of honey bees. Second, this enzyme has been found to exist in multiple-molecular forms (isozymes) in many species of higher organisms (Markert, 1962). Finally, malate dehydrogenase was chosen as a general indicator of aerobic metabolism. Since the synthesis of various enzymic forms is under genetic control. It was of interest to investigate the isozymic forms of this enzymes affected by different doses of gamma radiation.

MATERIALS AND METHODS

The workers honey bee *Apis mellifera* which used in the present study was prepared from colonies headed with open mated local Carnica queens located at the apiary belonging to Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Samples of worker bees were transferred to National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt, for treating with of 20, 40, 50, 100, 150 and 200 rad of gamma radiation.

Isozymes Electrophersis

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variations among studied worker bees using two isozyme systems according to Stegemann *et al*. (1985).

Samples of worker bees for each treated doses were used separately for isozymes extraction. The utilized isozymes are Peroxidase (Px), Polyphenyl Oxidase (PPO) and Alcohol dehydrogenase (Adh).

Gel preparation

The following stock solutions were prepared:

Acrylamide stock solution (30 %):

The solution was prepared by dissolving 30 g acrylamide and 0.8 g N, N- methylene bis–acrylamide in about 70 ml distilled water, then

the volume was completed to 100 ml by distilled water. The stock solution was kept at 4°C.

1.5 M Tris-HCl, pH 8.8:

The buffer was prepared by dissolving 18.15 g Tris in 50 ml distilled water and was shaked well with magnetic stirrer, and then pH was adjusted to 8.8 by HCl conc. solution. Then the volume was completed to 100 ml with distilled water and kept at °C.

Ammonium persulfate solution (APS 10 % W/V):

The solution was prepared by dissolving 1.0 g ammonium peresulfate in 10 ml distilled water. The solution is unstable, therefore it was immediately prepared before use.

Monomer gel preparation:

Acrylamide	8.3 ml
1.5 MTris	6.3 ml
D.W	9.9 ml
APS	250ul
TEMED	10 ul

Running buffer (5X)

This buffer was prepared by adding 15.0 g Tris and 72.0 g glycine to 1 liter distilled water and shaked well with magnetic stirrer. Then the volume was completed to 5 liter with distilled water and was kept at 4°C.

Extraction of isozymes:

Isozymes extraction was obtained from the different homogenizing 0.5 g fresh leaves samples in 1 ml extraction buffer (10% glycerol) using a mortar and pestle. The extract was then transferred into clean eppendorf tubes and centrifuged at 10000 rpm for 5 minutes. The supernatant was transferred to new clean eppendorf tubes and kept at –20 °C until use for electrophoretic analysis.

Application of samples:

A volume of 40 µl extract of each sample was mixed with 20 µl sucarose and 10 µ bromophenol blue, then a volume of 50 µl from this mixture was applied to each well.

Electrophoresis conditions:

The run was performed at 150 volt until the bromophenol blue dye has reached the separating gel end then the voltage was increased to 200 volt. Electrophoresis apparatus was placed inside a refrigerator during running duration.

Isozyme staining and detection:

After electrophoresis, the gels were stained according to their enzyme systems with the appropriate substrate and chemical solutions then incubated at room temperature in dark for complete staining. In most cases incubation for about 1 to 2 hours is enough.

Peroxidase (Px):

Benzidine HCl di	0.125 g
Glacial acetic acid	2 ml
D.W up to 50 ml	

Gel was placed into this solution and 5 drops of hydrogen peroxide were added. The gel was incubated at room temperature until bands were appeared.

Malate dehydrogenase (MDH):

0.1M Tris-pH (7.5)	100 ml
NAD	30 mg
MTT	20 mg
PMS	5 mg
Maleic acid	1.2g

Gel was placed into this solution and incubated at 30 ºC for 30 min until bands were appeared.

RESULTS AND DISCUSSION

Isozymes electrophoresis:

Isozyme analysis by electrophoresis offers a very well defined and effective tool to detect the differences, at gene levels, corresponding to gamma radiation.

In the present study two isozyme systems (esterase (EST) and malate dehydrogenase(MDH)) were estimated for identification and characterization gamma radiation effect on honey bee (*Apis mellifera*).

The isoenzyme patterns of EST and MDH found by studying irradiation bees *Apis mellifera* of seven different treatments by gamma radiation are depicted in Figs (1) and (2). The relative activity at each site is indicated by shading in the diagrams.

Esterase profiles:

The zymogram reveals that esterase isozyme band no 1 (Est1 0.4) is present in all bees where there is no difference in intensities. It was present till doses 20, 40, 50, 100, 150, 200 rad and control in a eminent activity in different doses, Fig. (1) and tables (1 &2). Also the same band shows an increase in its intensity in different doses. Band no 2 (Est 0.5) is present in moderate density in different doses. Band no 3 (Est 0.6) appears in low activity in different doses by gamma radiation subjected to 20, 40, 50, 100, 150, 200 rad compared with the control.

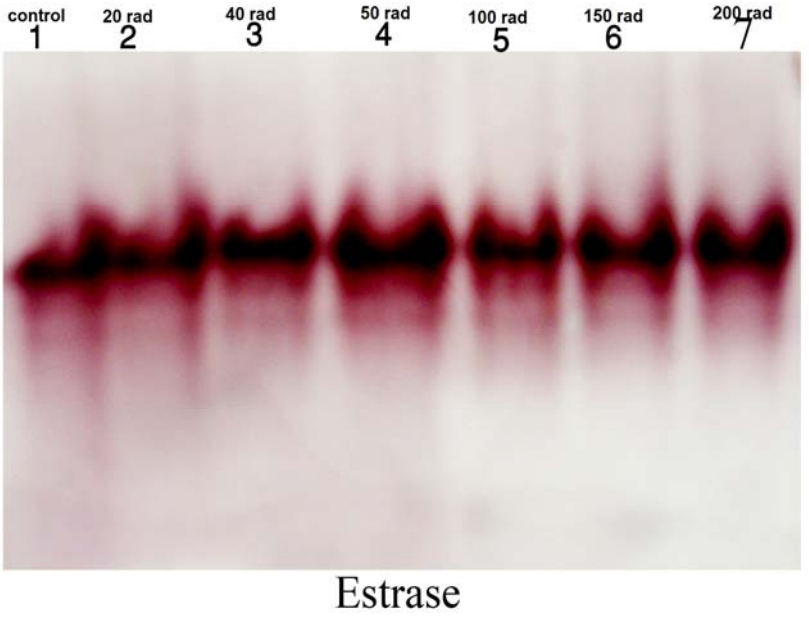

Fig (1). Electrophoretic patterns of esterase of seven different treatments by gamma radiation

Table (1): Edeogram analysis of Estrase isozyme bands patterns resulted from different treatments by gamma radiation compared with control (c)

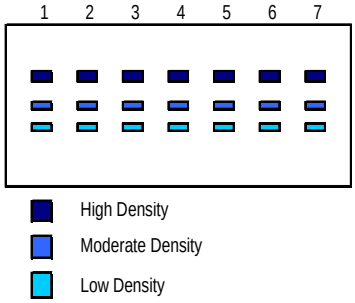

Table (2): Densitometric analysis for Estrase isozyme of the worker bee

Estrase Groups	Rf	1	2	3	4	5	6	7
Est1	0.4	++	++	++	++	++	++	++
Est2	0.5	+	+	+	+	+	+	+
Est3	0.6	-	-	-	-	-	-	-

Malic dehedrogenase :

Electrophoretic patterns of malic dehedrogenase of seven different treatments by gamma radiation are shown and illustrated in Fig (2). The zymogram, reveals different isozyme bands which were detected in some bees of different doses in maximum of four bands with different intensities.

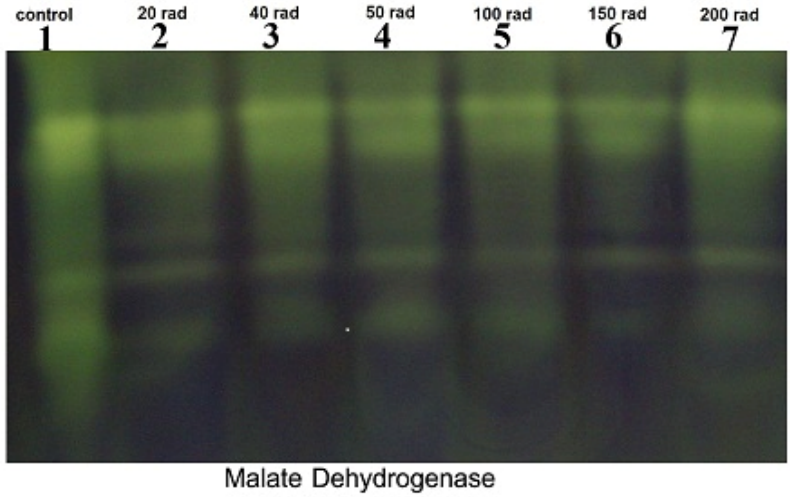

Fig (2). Electrophoretic patterns of malic dehedrogenase of seven different treatments by gamma radiation

Malic dehedrogenase isozyme band no 1 is the major band which nearly has the same ee of intensity in all treatment by gamma radiation. Isozyme band no 2 is present in all treatment of bees with different intensities. For instance, band no 2 appears in low activity in the dose 50 rad compared with the control. Also the same band is observed to be in low activity in the dose 200 rad compared with the control (table 3&4). Band no 3 is absent in the all treatment bees and control expect dose 20 rad. Band no 4 appears in low activity in the bees, then it is nearly has the same intensity in all treatments.

Table (3): Edeogram analysis of Malate dehydrogenase isozyme bands patter resulted from different treatments by gamma radiation compared with control (c)

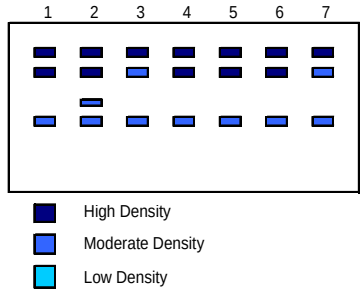

Table (4): Densitometric analysis for Malate Dehydrogenase isozyme of the worker bee

Malate dehydrogenase Group	Rf	1	2	3	4	5	6	7
Mdh1	**0.2**	++	++	++	++	++	++	++
Mdh2	**0.3**	++	++	+	++	++	++	+
Mdh3	**0.5**	0	+	0	0	0	0	0
Mdh4	**0.6**	+	+	+	+	+	+	+

The present study indicates that development of the workers of *Apis mellifera* is accompanied by changes in malate dehydrogenase and esterase activity. The number of MDH and EST isoenzymes increased as development proceeded, and there was an apparent increase in the quantity of some of the isoenzymes. It is quite possible that some isoenzymes that appeared suddenly during development are, in fact, present in concentrations too low to be detected at an earlier stage of development. Currently, we are investigating the MDH isoenzymes patterns in worker bees from around the world our results go in agreement with that reported by Sylvester's (1976) who stated that the MDH locus is controlled by three alleles (a, b, c) with heterozygous bees possessing three bands (i.e.: MDH is a dimeric molecule and homozygous bees have one band). Thus, the heterozygous bees used in this study were of the MDH $^{50/100}$ genotype. Earlier investigations showed that as the worker honey bee larva aged, the number of MDH and EST isoenzymes decreased and the minor bands became less intense and eventually disappeared (Nunamaker and Wilson, 1980).

Data from present study would seem to indicate that more isoenzymes (MDH3) are present dose 20 rad this indicate increased activity worker treatment a dose 20 rad compared other doses. Also, it shown that Malate dehydrogenase serves as a major source of energy during flight and is resynthesized during rest. Where Tripathi and Dixon (1969) found two and three malate dehydrogenase (MDH) isoenzymes in the hemolymph of queen and worker honeybees (Apis mellifera L.) ,respectively, throughout larval development; within each caste, the bands differed only in intensity, which decreased with age. In similar study, Tripathi and Dixon (1968) observed a marked difference in the hemolymph pattern of non-specific estreases (EST)

of queen and worker larvae: in 48-hr-old queen larvae there were two major and two minor bands that gradually decrteased in intensity until the larvae were 108 hr old, at which time one major and one minor band disappeared. In 48-hr-old worker larvae there were two major and four minor bands that also decreased in intensity until only one major and one minor band were present at 108 hr of age. In addition, Gilliam & Jackson (1972) found two MDH and two EST isoenzymes in the hemolymph of adult worker honey bees. However, Nunamaker & Wilson (1980) had also observed as many as seven MDH and seven EST isoenzymes in the hemolymph of newly emerged (<6hr old) adult workers. Kubicz and Galuszka (1971) found qualitative and quantitative differences in acid phosphatase isozymes of adult workers, queens and drones. They attributed these differences to altered physiological conditions related to the activities characteristic of each caste.Gilliam and Jackson (1972), in contrast, found that enzymes and other proteins do not change in mature worker honeybees, despite the fact that younger adult bees function as nurse bees and older ones as foragers.

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تأثيرات اشعة جاما علي النشاط الانزيمي لشغالات نحل العسل

سامح جرجس سويرس[2] – فائزة مرعي أحمد[1]- عادل محمد البسيوني[1] – اسامة حامد غريب[2]

[1] قسم وقاية النبات - كلية الزراعة جامعة عين شمس - القاهرة- مصر
[2] قسم المنتجات الطبيعية – المركز القومي لبحوث وتكنولوجيا الاشعاع-هيئة الطاقة الذرية - القاهرة – مصر

استخدمت شغالات نحل العسل من ملكات نحل كرينولي ملقحة طبيعية.تم تعريض الشغالات لاشعة جاما بجرعات 20و40و 50و100و150و200 راد في المركز القومي لبحوث وتكنولوجيا الاشعاع هيئة الطاقة الذرية لتقدير المشابهة الانزيمي لكلا من الاستريزومماليت ديهيدروجينيز لمعرفة تأثير اشعة جاما علي نحل العسل.

اظهرت النتائج الخاصة بالمشابهة الانزيمي الاستريز تواجد الحزمة رقم 1 (Est 0.4) في كل المعاملات بكثافة عالية ولم يحدث اي تغير بها مقارنة بالغير معامل بالاشعاع. الحزمة رقم 2 (Est 0.5) متواجدة بكثافة متوسطة في كل الجرعات وتواجدت الحزمة رقم 3 (Est 0.6) بنشاط منخفض في الجرعات المختلفة.

أظهرت النتائج الخاصة بالمشابهة الانزيمي Mdh المعاملات المختلفة بأشعة جاما وجود اربعة حزم بكثافات مختلفة حيث كانت الحزمة رقم 1 (Mdh1) هي الرئيسية في كل المعاملات والحزمة رقم 2 (Mdh2) وجدت بكثافة مختلفة في كل المعاملات والحزمة رقم3 (Mdh3) غابت في كل المعاملات ما عدا الجرعة 20 راد وجدت الحزمة رقم 4 (Mdh4) بنشاط منخفض في كل المعاملات.