J. Plant Production, Mansoura Univ., Vol. 5 (2):295 - 303, 2014

EFFECT OF AMINO ACIDS AND ALDEHYDES ON TYROSINASE ACTIVITY FROM MARROW

El-Shora, H. M. and Rania M. Hegazy
Botany Department, Faculty of Science, Mansoura University

ABSTRACT

Tyrosinase (monophenol, *O*-diphenol: oxygen oxidoreductase, EC 1.14.18.1) is a copper-containing protein widely distributed in animals, plants and microorganisms. The enzyme was extracted from *Cucurbitapepo*var. cylindrica, (marrow, family:Cucurbitaceae). Proline, tryptophane, aspartic acid, cysteine, histidine, glycine, *β*-alanine and valinewere assayed for their effect on tyrosinase activity in different concentrations (2.5, 5, 7.5, 10 and 12.5 mM)*in vitro*. Histidine, aspartic acid, glycine and *β*-alanine induced tyrosinase activity gradually from 2.5 to 10 mM after which there was a decline in the enzyme activity. Tryptophane, valine and cysteine induced the activity up to 5 mM, while proline induced the activity from 2.5 to 7.5mM. Also, tyrosinase activity was assayed in presence of benzaldehyde, anisaldehyde (*P*-methoxybenzaldehyde) and acetaldehyde (0.1, 0.2, 0.3, 0.4 and 0.5 mM).It was found that the enzyme activity was inhibited by the three tested aldehydes.
Keywords:Tyrosinase, Tyrosine, Amino Acids, Benzaldehyde, Anisaldehyde, Acetaldehyde.

INTRODUCTION

Tyrosinases (EC 1.14.18.1) are widely distributed in nature; these enzymes are known as type 3 copper proteins having a diamagnetic spin-coupled copper pair in the active center (Lerch, 1983). It is known to be the key enzyme inmelanogenesis as well as the browning phenomenon in fruits.The enzyme provides the major driving force towards melanin formation *O*-hydroxylation of its phenolic substrate and successive oxidation of the produced *O*-dihydroxy compound to the corresponding *O*-quinone in the presence of molecular oxygen (Fenoll*et al*., 2002). The consecutive catalytic functions of tyrosinase are named cresolase and catecholase; respectively (Van Gelder*etal*., 1997).

Most of the reported tyrosinases are intracellular enzymes. The characterized plant tyrosinases have been intracellular enzymes, possibly bound to organelles (Duarte *et al.,* 2012).

Since tyrosinase is a key enzyme for melanin biosynthesis in plants and animals, the inhibition of tyrosinase activity may be useful for the treatment of disorders associated with melanin hyperpigmentation (Masamoto*et al.,* 2003).Tyrosinase inhibitors have become increasingly important in cosmetic and medical products in relation to hyperpigmentation (Pérez-Bernal *et al*., 2000; Kim *et al*., 2002).

The enzyme is also important in the food industry because during the processing of fruits and vegetables any wounding may cause cell disruption and lead to quinine formation. The enzymatic browning implies a considerable economic loss in the commercial production of fruits and vegetables. The appearance of food and beverages may be affected, as may

the taste and its nutritional value, often decreasing the quality of the final product (Martinez and Whitaker, 1995; Whitaker, 1995).

Davies (1982) reported that amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins, which are formed by a process in which ribosomes catalyze the polymerization of amino acids.

Tyrosinase from various fruits and vegetables has been studied (Paranjpe*et al.,* 2003; Neves*et al.,* 2009), but the enzyme from *Cucurbitapepo*has been rarely reported. This study was undertaken to investigate the effect of various amino acids and aldehydes on tyrosinase activity from *Cucurbitapepo*.

MATERIALS AND METHODS

Plant material

The experimental plant used in this investigation was*Cucurbitapepo*var. cylindrica, (marrow, family:Cucurbitaceae). Pure strain of seeds was obtained from Egyptian Ministry of Agriculture.

Enzyme preparation

Tyrosinase was extracted from marrow according to the method ofEl-Shora(2001). Five gmcotyledonary leaveswere macerated in 25 ml of pre-chilled 100 mM potassium phosphate buffer (pH 8.0). The resultant homogenate was centrifuged at 3000 rpm, 4^{o}C for 10 min. The obtained supernatant constitutes the crude extract which was used for enzyme assay.

Enzyme assay

The reaction was carried out in 100mM potassium phosphatebuffer (pH 8.0, 1.7 ml) containing 0.3 ml 20 mM L-tyrosine as a substrate and 1ml crude enzyme extract in a total volume of 3 ml. The reaction mixture was incubated at 40^{o}C for 40 min. The reaction was stopped by adding 0.5 ml NaN_3 then the optical density (O.D) of the developed color was measured at 520 nm using NV203 spectrophotometer. One unit of the enzyme activity was defined as the amount of the enzyme increasing 0.01 absorbance at 520 nm under experimental conditions.

Treatment with amino acids

Proline, tryptophane, aspartic acid, cysteine, histidine, glycine, *β*-alanine and valine were tested for their effects on tyrosinase activity. Different concentrations of each amino acid (2.5, 5, 7.5, 10 and 12.5 mM) were added to the reaction mixture followed by incubation and then assaying the enzyme activity.

Treatment with aldehydes

Tyrosinase activity was assayed in presence of different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mM) of benzaldehyde, anisaldehyde and acetaldehyde in the assay mixtureindividually under the same experimental conditions.

RESULTS AND DISCUSSION

Effect of amino acids

This experiment aimed to investigate the effect of different amino acids on tyrosinase activity *in vitro*. The results shown in Figs (1-8) indicate that *β*-alanine, aspartic acid, glycine and histidine stimulate tyrosinase activity up to 10mM after which the enzyme activity was inhibited. However, cysteine, valine and tryptophane increased the activity up to 5mM. Also, it was observed that 7.5 mM concentration was the best proline concentration for stimulating the enzyme activity.

Amino acids are considered as osmolytes which keeps the enzyme from disruption. In addition, amino acids stabilize the enzyme proteins. Proline as amino acid was found to protect enzyme-proteins from ion inhibitory effect (Solomon *et al.,* 1993).

Effect of aldehydes

Various concentrations of benzaldehyde, anisaldehyde and acetaldehyde (0.1, 0.2, 0.3, 0.4 and 0.5 mM) were testedfor their effect on tyrosinase activity. The enzyme activity was measured at each concentration. The obtained results are shown graphically in Figs (9-11).

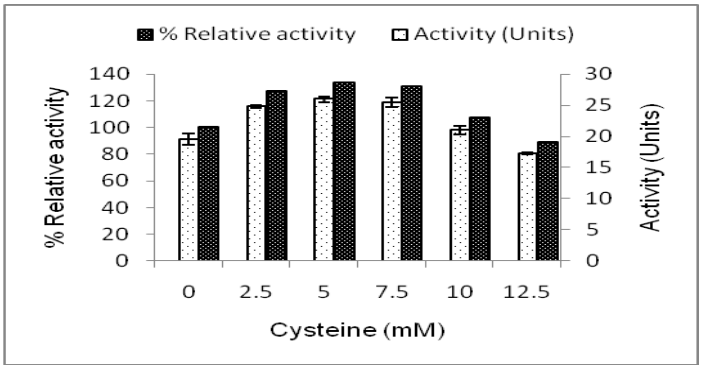

Fig. 1: Effect of cysteine on tyrosinase activity.

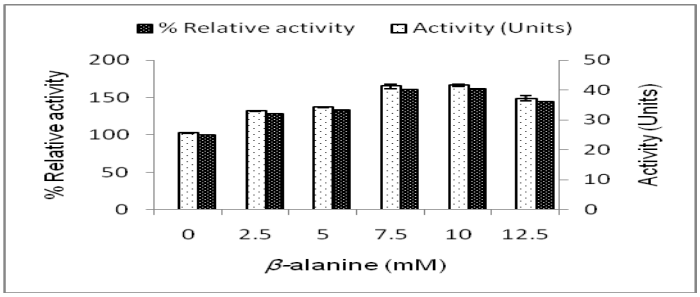

Fig. 2: Effect of *β*-alanine on tyrosinase activity.

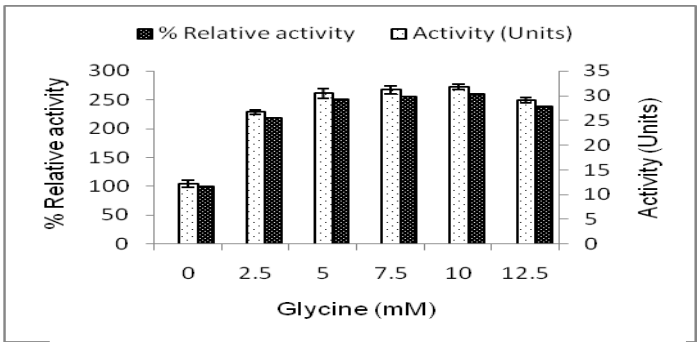

Fig. 3: Effect of glycine on tyrosinase activity.

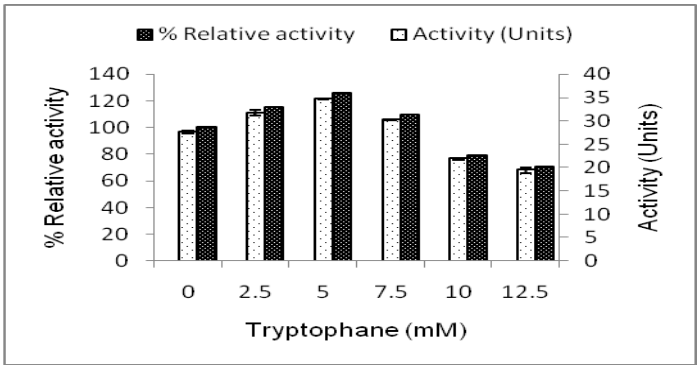

Fig. 4: Effect of tryptophane on tyrosinase activity.

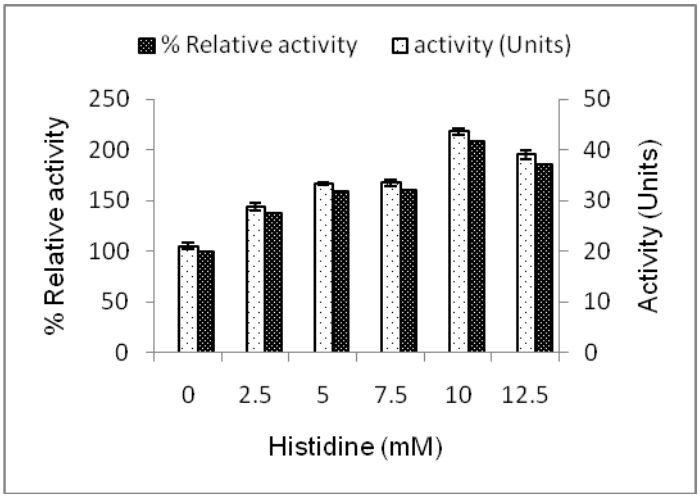

Fig. 5: Effect of histidine on tyrosinase activity.

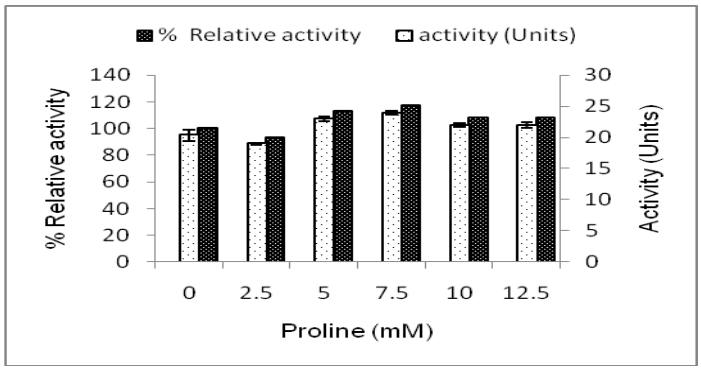

Fig. 6: Effect of proline on tyrosinase activity.

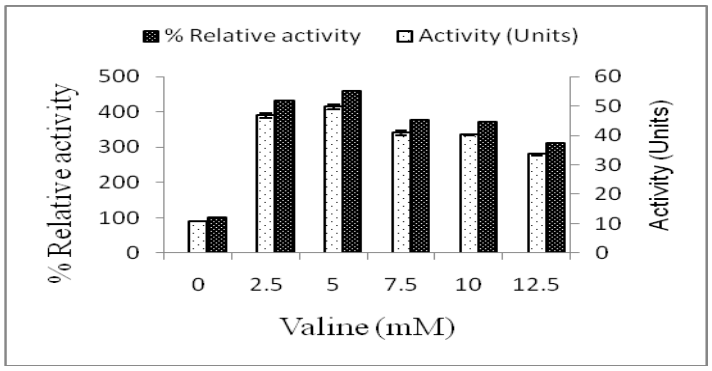

Fig. 7: Effect of valine on tyrosinase activity.

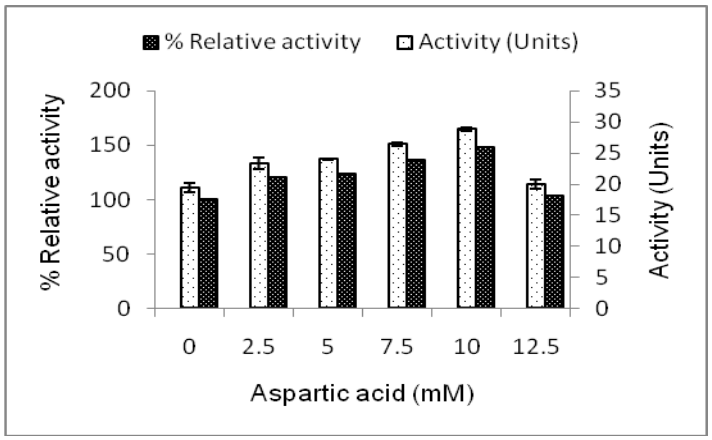

Fig. 8: Effect of aspartic acid on tyrosinase activit

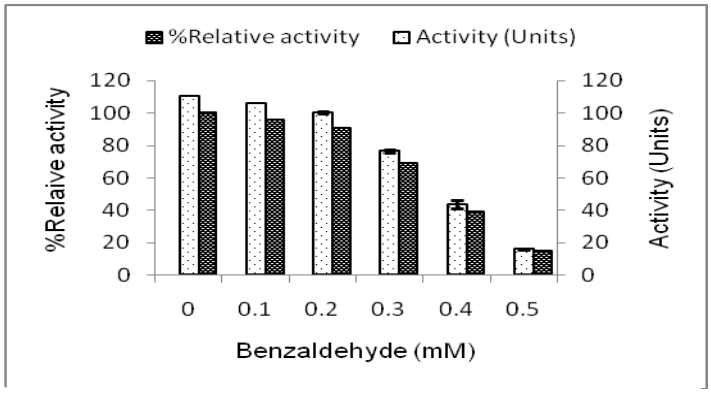

Fig. 9: Effect of benzaldehyde on tyrosinase activity.

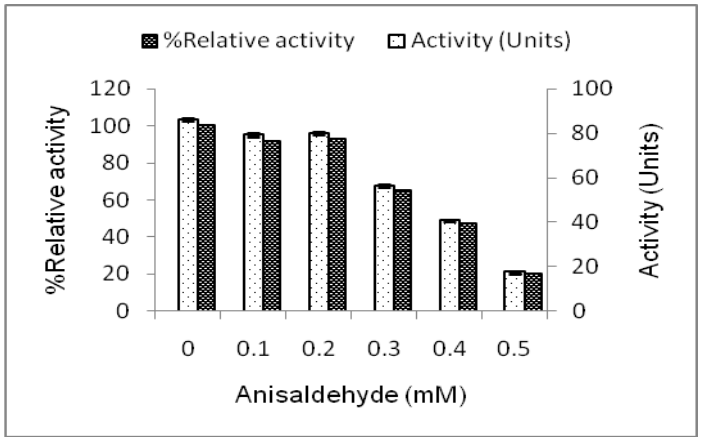

Fig. 10: Effect of anisaldehyde on tyrosinase activity.

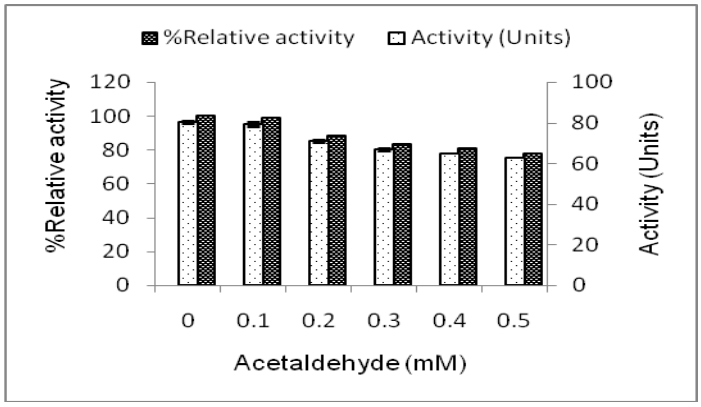

Fig. 11: Effect of acetaldehyde on tyrosinase activity.

These results indicate that there was a continuous inhibition of tyrosinase activity with increasing the aldehyde concentration. It was observed that the percentage of relative activity at 0.5 mM was 14.63%, 20.28% and 77.99% in presence of benzaldehyde, anisaldehyde and acetaldehyde, respectively.

It is possible thatthe aldehyde is attached to a site different from the active site and hindered binding of the substrate to the enzyme through steric hindrance or by changing the protein conformation (Walker and Wilson, 1975).

The aldehyde group is known to react with biologically important nucleophilic groups such as sulfhydryl, amino, and hydroxyl groups. The tyrosinase inhibitory mechanism of aldehyde-type inhibitors comes from their ability to form a Schiff base with a primary amino group in the enzyme(Kubo and Kinst-Hori, 1999).

Anisaldehyde is a classical noncompetitive inhibitor to tyrosinase (Ha *et al.,* 2005). It can combine with both free enzyme and the enzyme-substrate complex, and there are same binding intensity between anisaldehyde and both of the enzyme forms (Kubo *et al.,* 2003).

In conclusion, the results obtained from this study showed that amino acids stimulate the activity of tyrosinase isolated from marrow and this stimulation is concentration dependent. These amino acids can be used to activate the enzyme in its industrial applications. On the other hand, aldehydes inhibited the activity of the enzyme from the same source so they can be used in cosmetics and to alleviate the undesirable effect of tyrosinase during its use in food applications.

FEFERENCES

Davies, D. D. (1982).Physiological aspects of protein turnover.*Encycl. Plant Physiol. New Series*, 14 A (Nucleic Acids and Proteins: Structure, Biochemistry and Physiology of Proteins, Eds., Boulter, D. and B. Partier, Springer Verlag, Berlin, Heidelberg & New York, pp: 190-228.

Duarte,L. T., Tiba, J. B., Santiago, M. F., Garcia, T. A. and Bara, M. T. F. (2012). Production and characterization of tyrosinase activity in *Pycnoporussanguineus* CCT-4518 crude extract. *Braz*. *J. Microbiol.,* 43(1): 21-29.

El-Shora, H. M. (2001).Effect of growth regulators and group modifiers on NADH-glutamate synthase of marrow cotyledons.*J. Biol. Sci.,* 1: 579-602.

Fenoll, L. G., Rodriguez-Lopez, J. N., Garcia-Molina, F., Garcia-Canovas, F. and Tudela, J. (2002).Michaelis constants of mushroom tyrosinase with respect to oxygen in the presence of monophenols and diphenols.*Int. J. Biochem. Cell Biol.*, 34: 332-336.

Ha, T. J., Tamura, S. and Kubo, I. (2005).Effects of mushroom tyrosinase on anisaldehyde.J. Agric. Food Chem., 53(18): 7024-7028.

Kim, Y. M., Yun, J., Lee, C. K., Lee, H. H., Min, K. R. and Kim, Y. (2002).Oxyresveratrol and hydroxystilbene compounds.Inhibitory effect on tyrosinase and mechanism of action.*J. Biol. Chem.*, 277: 16340-16344.

Kubo, I. and Kinst-Hori, I. (1999). Tyrosinase inhibitory activity of the olive oil flavor compounds. *J. Agric. Food Chem*., 47: 4574-4578.

Kubo, I., Chen, Q., Nihei, K., Calderon, J. S. and Céspedes, C. L. (2003).Tyrosinase inhibition kinetics of anisic acid.*Z.Naturforsch*, 58: 713-718.

Lerch, K. (1983).Neurosporatyrosinase: structural, spectroscopic and catalytic properties. *Mol. Cellular Biochem*., 52(2): 125-138.

Martínez, M. V. and Whitaker, J. R. (1995).The biochemistry and control of enzymatic browning.*Trends Food Sci. Technol*., 6: 195-200.

Masamoto, Y., Ando, H., Murata, Y., Shimoishi, Y., Tada, M. and Takahata, K. (2003). Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of *Euphorbia lathyris*. L. *Biosci.Biotech*.*Biochem*., 67: 631-634.

Neves, V. A., Picchi, D. G. and de Siva, M. A. (2009).Some biochemical properties of polyphenoloxidase from spearmint (*Menthaarvensis*).*Braz. J. Biol. Technol.,* 52(4): 1001-1010.

Paranjpe, P. S., Karve, M. S. and Padhye, S. B. (2003).Characterization of tyrosinase and accompanying laccase from *Amorphophalluscampanulatus*.*Ind. J. Biochem.Biophys.,* 40: 40-45.

Pérez-Bernal, A., Muñoz-Pérez, M. A. and Camacho, F. (2000).Management of facial hyperpigmentation.*Am*. *J*. *Clin. Derm.*,1: 261-268.

Solomon, A., Beer, S., Waisel Y., Jones, G. P. and Paleg, L.G. (1993). Effect of NaCl on the carboxylating activity of Rubisco from *Tamarixjordanis* in the presence and absence of proline-related compatible solutes. *Physiol. Plantarum,* 90:198-204.

Van Gelder, C. W. G., Flurkey, W. H. and Wichers, H. J. (1997).Sequence and structural features of plant and fungal tyrosinases.*Phytochem*., 45: 1309.

Walker, J. R. L. and Wilson, E. L. (1975).Studies on the enzymatic browning of apples.Inhibition of apples *O*-diphenol oxidase by phenolics acid.*J*. *Sci.Food Agric*., 26: 1825-1831.

Whitaker, J. R. (1995). Polyphenol oxidase, In: Wong, D. W. S. (ed.). Food Enzymes, Structure and Mechanism, Chapman & Hall, New York, pp: 271-307.

تأثير الأحماض الأمينيه والألدهيدات علي نشاط إنزيم التيروزينيز من نبات القرع

حامد محمد الشوري و رانيا مجدي حجازي
قسم النبات – كلية العلوم – جامعة المنصوره

تيروزينيز(EC: 1.14.18.1) هو بروتين يحتوي علي النحاس في مركز النشاط الإنزيمي وهو إنزيم واسع الإنتشار حيث أنه يوجد في النباتات، الحيوانات بالإضافة إلي الكائنات الدقيقة. تم إستخلاص الإنزيم من فلقات نبات القرع (التابع للعائلة القرعية) المنزرع لمدةأسبوع ثم دراسة تأثير ثمانية أحماض أمينية (البرولين- التريبتوفان - حمض الأسبارتيك - السيستين- الهيستيدين- الجلايسين- الفالين والبيتا ألانين) علي نشاط الإنزيم المستخلص. كانت التركيزات المستخدمة من هذه الأحماض الأمينية هي (٢.٥، ٥، ٧.٥، ١٠، ١٢.٥ ميللي مول).ولقد أثبتت النتائج التي تم الحصول عليها أن الأحماض الأمينية (الهيستيدين- الجلايسين- حمض الأسبارتيك والبيتا ألانين) قد حفزت النشاط الإنزيمي من تركيز ٢.٥ وحتي ١٠ ميللي مول ثم انخفض النشاط الإنزيمي عند التركيزات الأعلي. أما بالنسبة للتريبتوفان- الفالين والسيستين فلقد حفزت هذه الأحماض الأمينية النشاط الإنزيمي من تركيز ٢.٥ ميللي مول وحتي تركيز ٥ ميللي مول، بينما حفز البرولين النشاط الإنزيمي من ٢.٥ حتي ٧.٥ ميللي مول. ولقد تم أيضا قياس نشاط التيروزينيز المستخلص من نبات القرع في وجود بعض الألدهيدات مثل البنزالدهيد، الأنيزالدهيد والأسيتالدهيد كلا علي حده بتركيزات مختلفة (٠.١، ٠.٢، ٠.٣، ٠.٤ و٠.٥ ميللي مول). ولقد وجد أن هذه المركبات قد ثبطت النشاط الإنزيمي عند التركيزات المختلفة.

قام بتحكيم البحث

ا/ د محمود محمد درويش	**كلية الزراعة – جامعة المنصورة**
ا/د عصام ابو القاسم	**كلية العلوم – جامعة طنطا**