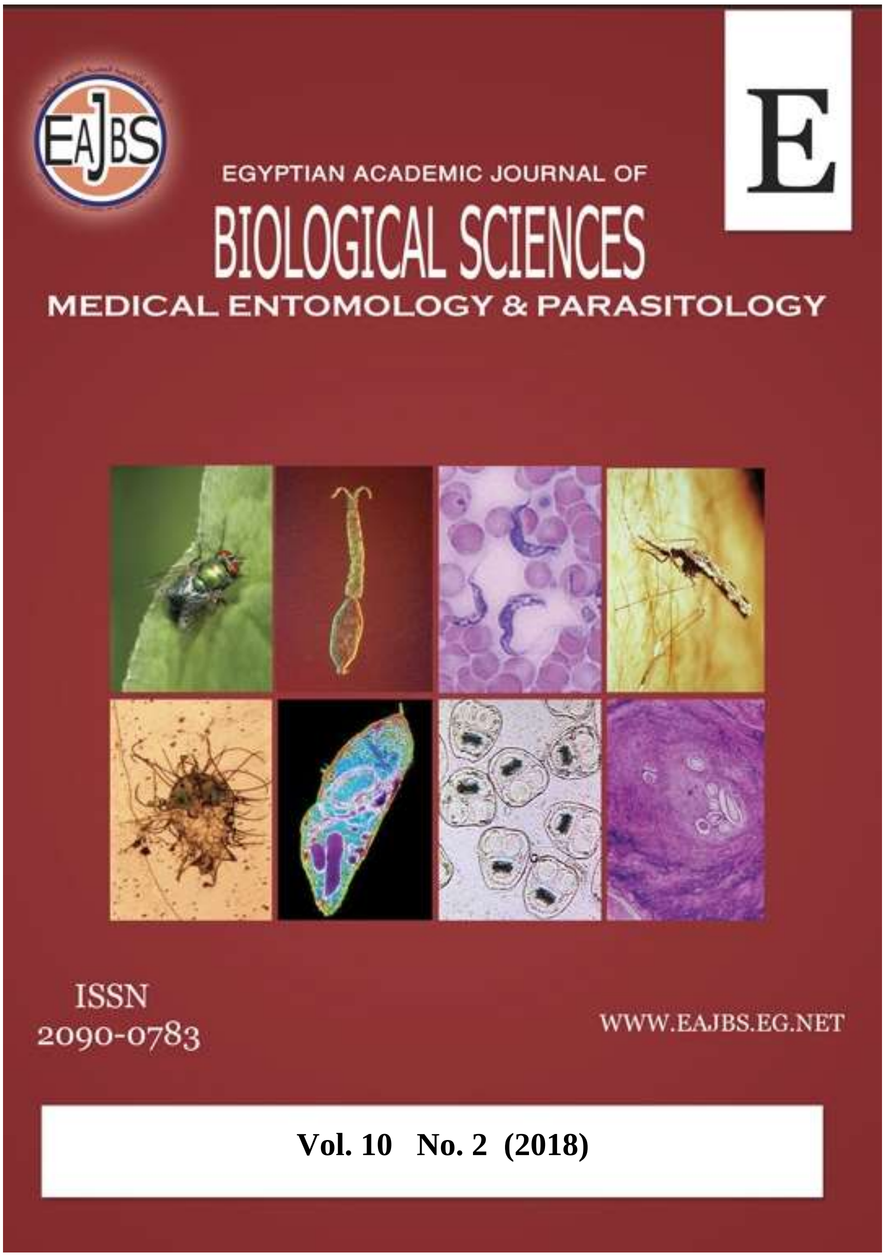

The Journal of Medical Entomology and Parasitology is one of the series issued quarterly by the Egyptian Academic Journal of Biological Sciences. It is an important specialist journal covering the latest advances in that subject.

It publishes original research and review papers on all aspects of basic and applied medical entomology, parasitology and host-parasite relationships, including the latest discoveries in parasite biochemistry, molecular biology, genetics, ecology and epidemiology in the content of the biological, medical entomology and veterinary sciences.

In addition to that, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc.

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.10(2) pp 71-77 (2018)*

Egypt. Acad. J. Biolog. Sci., 10 (2): 71 – 77 (2018)

Egyptian Academic Journal of Biological Sciences
E. Medical Entom. & Parasitology

ISSN: 2090 – 0783
www.eajbs.eg.net

Bioassay of Flavonoids *Crataegus oxyacantha* with Plant Bionanosensor

Faramarz Moradi*, Hossein Arouiee and Seyyed Hossein Neamati
Department of Horticulture Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
E-mail: moradifff@Gmail.com

ARTICLE INFO
Article History
Received:15/8/2018
Accepted:29/10/2018

Key words:
plant bionanosensor, bioassay, *Crataegus oxyacantha* extract.

ABSTRACT

Micro-organisms, tissues, cells, organelles, enzymes, peptides and DNA are used to identify the target substance called as a specific or non-specific biosensor. A bioassay is a biological testing procedure for estimating the concentration of a substance in a formulated product or bulk material. Due to the importance of flavonoids as antioxidants and expensive and time-consuming methods, bioassay of flavonoids via biosensor is growing. Plant bionanosensor is a new approach and a certain type of biosensor that converts the concentration of flavonoids into visible rotation and flavonoids are detected by different rotations of concentration. The rotations of C*crataegus oxyacantha* (Hawthorn) extract (flavonoids) with plant bionanosensor were analyzed in a completely randomized design with three replications by sas9.1 software. The different levels of flavonoid rotation are significant with a probability of 99%. So far, no report was made on plant biosensor.

INTRODUCTION

Hawthorn extract has a significant amount of flavonoid and is of high importance due to the antioxidant property. Flavonoids have two groups of anthocyanins and antoxantine. Anthocyanin has pigments and antoxantine is colorless (Kin and Young, 1999). Faramarz Moradi began his research of plant biosensors with the patent No. 26299 in Iran with the discovery of rotation organ (Moradi, 1999). Then, in 2000, he was chosen in Qwarizmi's Festival thanks to the invention named intelligent organ and could gain the third place (discovery of smart organ, 2000). He recorded the plant sensor No. 332/421 in Iran's scientific research and industrial organization in 2004. Foundation of the memory material was reported in Iran in 2005 (Moradi, 2005) and a type of bioreactor sensor was built (Moradi et al., 2003). From the intelligent organ, a plant engine generating bioenergy was created under Biotechnology Master's thesis at Tehran University with the participation of Sharif University and was patented in Iran (Moradi, 2005). Moreover, plant anchor cells as storage energy (Moradi, 2005) moisture signal sensor (Moradi, 2005) damp-proof Nano sensor of drugs and materials (Moradi, 2006) Mobile safety sensor (Recipient, grant and License, 2010) industry and materials micro-sensor (Moradi, 2005) were patented in Iran and one file named nanomotor was recorded in the US (Moradi, 2006). Foundation of plant sensors using discovering intelligent organ was accepted at the University of Arkansas of US (Moradi, 2009) and foundation of cellulosic rotating Nano composites, including NanoBiotech were accepted at Switzerland (Moradi, 2009) international nanotechnology conferences. Mobile safety sensor obtained the bronze medal in the Olympics (Recipient Bronze Medal from novel Technology, 2011) and was verified by the three levels of Iran's National Elite Foundation (Recipient, grant and License, 2011).

Biosensors are based on identifying the target material with high specific detection (Mello and Kubota, 2002) as follows:

1. Bio-recognition elements: in which enzymes, antibodies, a part of DNA, peptides, and even a tissue of microorganism are used (Gooding, 2006).
2. Amperometric biosensors that act based on oxidation and electrode reduction coated with enzymes (Vastarella, 2001). Absorption biosensors bind to a molecule that is to be determined. The electrode with the coated enzyme is one of them.
3. Carbon paste electrodes are widely used initially by Adams (Mailley et al., 2004). Carbon paste electrodes are simple and cheap (Ghobadi et al., 1996; Bolado et al., 2007).
4. Electrochemical analysis of polyphenolic compounds: quality and quantity of polyphenols are done by spectrometry or HPLC that are expensive and time-consuming. Graphite powder compound with non-electrolytic paste is inexpensive and easy to use. For more polyphenolic compounds, antioxidants containing phenolic groups are commonly used, which are based on electrochemical biosensors activity. They are a. polyphenol in the oil extraction (Capannesi et al., 2000), b. red polyphenol compounds (Gomes et al., 2004), c. polyphenols of various teas (Campanella et al., 2003), d. white and red polyphenols (Campanella et al., 2004a; Campanella et al., 2005) and a type of biosensor with Laccase enzyme for measuring the red polyphenol compounds. Laccase biosensor directly causes the oxidation of phenolic compounds (Freire et al., 2001; Freire et al., 2003a). Figure. 1 shows oxidation of flavonoids with Laccase (Pourcel *et al*., 2007).

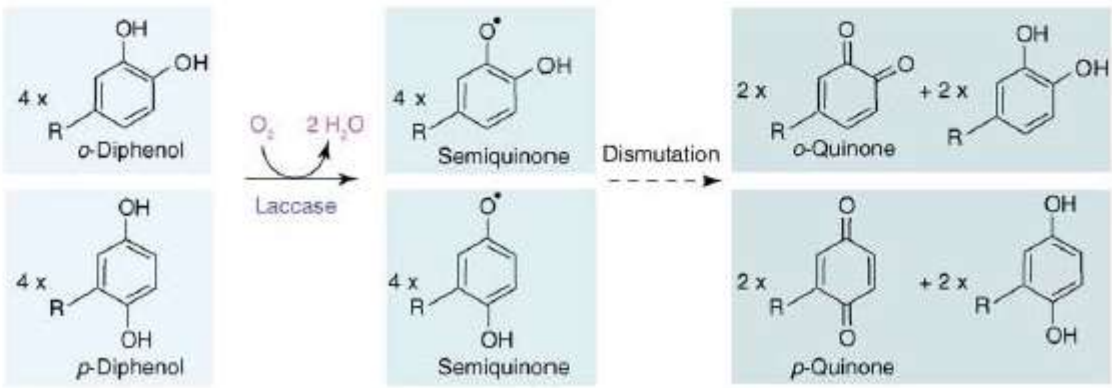

Figure. 1. Oxidation of flavonoids with Laccase

There are various methods for measuring antioxidant biological compounds. Qualitative and quantitative techniques of polyphenols measurement include chromatography such as HPLC and GC-MS. These techniques are expensive and time-consuming so the development of biosensors can overcome these limitations. In order to prevent damage by free radicals, the body has a defense system of antioxidants (Antolovich et al., 2002). Some plants have antioxidant properties, especially biological flavonoids are considered as neutralizing free radicals due to the antioxidant, natural origin and the effective ability of activity (Katalinic et al., 2006). The flavonoids (Urquiaga and Leighton. 2000), which can be seen in Fig. 2 are used to eliminate free radicals (Simone, 1992) which can be seen in Fig. 3. Plant bionanosensor is a certain type and new approach of biosensors that has been used by the Faramarz Moradi as inventor of this paper. The study was used to determine two types of concentrations of flavonoids in hawthorn extract through the rotation. So far, no report was made about such a plant biosensor at international level from other researchers.

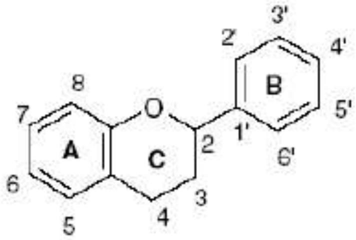

Fig. 2. Flavonoid structure: Ourkoyaga & Layton, 2000)

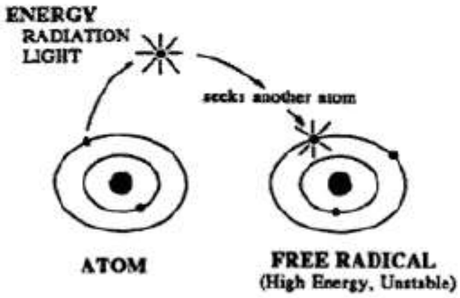

Figure. 3. Simon's free radicals (1992)

MATERIALS AND METHODS

Plant Nano-structure used in plant bionanosensor is the rotating Nano-structures as storage (i.e., it can turn to its previous memory such as memory metal) that has been fabricated by the Faramarz Moradi.

According to the following figure, plant bionanosensor has a graded plane divided by 310 sections, in which the amount of rotation could be read by a hand connected to a plant nano-structure (Figure 4and Figure 6). In the bottom of the bionanosensor, test site of the extract flavonoids can be seen (Figure. 5).

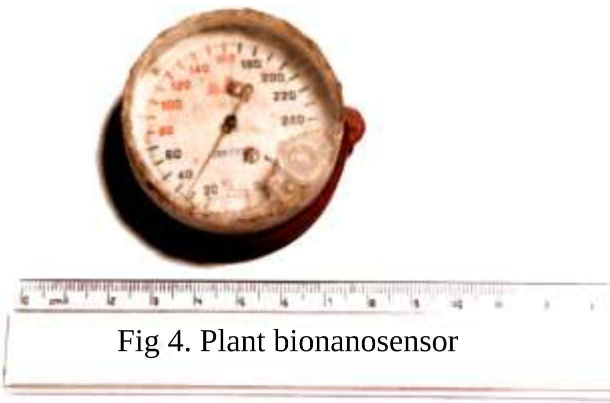

Fig 4. Plant bionanosensor

Fig 5. The site of flavonoids test

Using insulin syringe, a drop of treatment inserted at the test site and the test site was placed on the paper to read the degree of each treatment. 2-2.5 mg, 1-1.25 mg, 0.625-0.5 mg and 0 mg of flavonoid on hyperosoide/1 ml of hawthorn (*Crataegus oxyacantha*) in ethanol 70% was purchased by a pharmaceutical company of Iran Daru.

With regard to injection of 1.100 cc, the treatments were divided into 100 and the effect of treatments was analyzed by 02-0.025 mg, 0.0125- 0.01 mg, 0.0625-0.005 mg and 0 mg flavonoid in 1.100 cc as a completely randomized experiment with three iterations by software sas9.1. Comparison of average rotation was performed with LSD test at 1% level.

RESULTS AND DISCUSSION

Table 1 shows the results of the rotation bioassay.

Table 1. The Degree of plant bio nanosensor rotation

0 mg flavonoids in 70% ethanol as control	0.005-0.00625 mg of flavonoids as hyperozoid in 0.01 ml *crataegus oxyacantha* extract	0.01-0.125 mg of flavonoids as hyperozoid in 0.01 ml *crataegus oxyacantha* extract	0.02-0.025 mg of flavonoids as hyperosoide in 0.01 ml *crataegus oxyacantha* extract
261	230	150	80
262	200	140	70
255	215	135	75

Table 2. Analysis of variance of the treatments rotation of flavonoids extract

F**	Mean Square	Sum of squares	df	Source of changes
245.40	19802.97	59408.91	3	Treatment
	80.66	645.33	8	Error

$R2=0.97$ c.v= 5.19 **sig=1%

According to Table 2, at least two of the four treatments are statistically different from each other at 1% in order to decide which one of the treatments have a significant difference with other treatments. LSD method was used to compare means

.

Table 3. Comparison of the mean treatments of Flavonoid concentrations in ethanol (70% ethanol and 30% water)

Rank	The average degree of rotational memory	Flavonoid mg as hyperosoide in 0.01 ml of *crataegus oxyacantha* extract
A	295.33	0 (control)
B	215.00	0.005-0.00625
C	141.66	0.01-0.125
D	75.00	0.02-0.٠25

Table 3 shows that the A treatment has the greatest effect on rotational memory that is related to control and showed the greatest rotation by the greatest uptake by bio-nanosensor and the D treatment has the lowest effect on the rotational memory because it has the highest concentration. Therefore, it has low absorption and low rotation.

According to above tables, plant bionano sensor is capable to detect hawthorn extract with a probability of 99% (70% ethanol as a control), 0. 025-0.02 mg, 0.0125-0.01 mg, 0.00625-0.005 mg and 0 mg flavonoid on hyperosoide. The higher the flavonoid concentration, the lower the degree of rotation sensors will be. To examine the cause of rotation through an electron microscope manufactured by Zeiss German Factory, model 60A at Tehran University by SEM of plant sensor used in plant biosensor, a scan was run. The following picture is one of the images obtained.

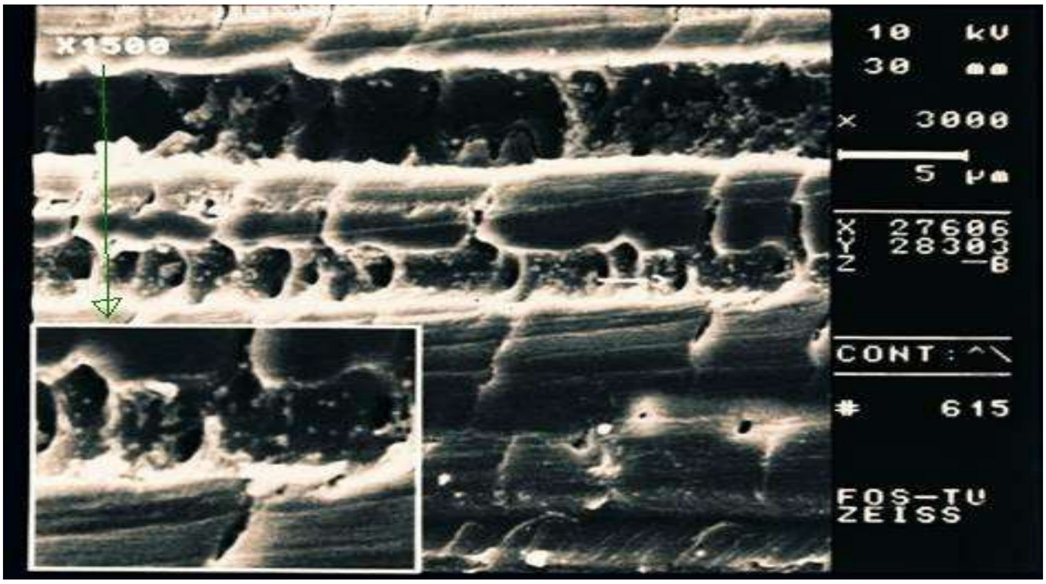

Fig. 6. Longitudinal section- electron microscopic image of plant nanostructure by SEM

The picture shows that the lower part of the picture magnified 1,500 times, in which nanoparticles less than the mean diameter of a nanometer or nano-holes can be seen that contribute to its rotation. Plant sensor longitudinal section shows that nano-holes and nanoparticles with absorption treatments convert the absorption treatment energy into the rotation with the help of specific structural features.

Plant bio-nanosensor advantage includes:

1. It is inexpensive compared to chromatography, spectroscopy techniques and other biosensors. 2. It has been the result of years of research since 1999. 3. It is simple and easy transportation makes its use in the desert. 4. It does not use any electrical energy source unlike other methods use it as flavonoid extract acts as an energy source of the biosensor. 5. If it is recorded at the international level, it can be commercialized at the international level. 6. If it is optimized, plant secondary metabolites can be directly studied by intact leaves and other physiological properties. 7. It is inexpensive and easy to use. 8. Any material is used in its performance. 9. The use of other methods requires purification of flavonoids.

REFERENCES

.F. Moradi (2005) Establishment of memorial plant material, 8th Iranian and International Congress of Agronomy and plant breeding, Aug 25-27, Guilan University, Rasht, Iran.

Antolovich, M., P.D. Prenzler, E. Patsalides, S. Mcdonald, K. Robards. 2002. Methods for testing antioxidant activity. The Analyst 127: 183-198.

Biol. Res. 33 (2): 89-96.

Bolado, P.F., D.H. Santos, P.J.L. Ardinasa, A.M. Pernia, A.C. Garcia. 2007. Electrochemical characterization of screen-printed and conventional carbon paste electrodes. Electrochimica Acta 53: 3635-3642.

Campanella, L., A. Bonanni, M. Tomassetti. 2003. Determination of the antioxidant capacity of samples of different types of tea, or of beverages based on tea or other herbal products, using a superoxide dismutase biosensor. Journal of Pharmaceutical and Biomedical Analysis 32: 725-736.

Campanella, L., A. Bonanni, E. Finotti, M. Tomassetti. 2004a. Biosensors for determination of total and natural antioxidant capacity of red and white wines: comparison with other spectrophotometric and fluorimetric methods. Biosensors and Bioelectronics 19: 641-651.

Campanella, L., E. Martini, M. Tomassetti. 2005. Antioxidant capacity of the algae using a biosensor method. Talanta 66: 902-911.

Capannesi, C., I. Palchetti, M. Mascini, A. Parenti. 2000. Electrochemical sensor and biosensor for poly phenols detection in olive oil. Food Chemistry 71: 553-562.

F. Moradi (1999) discovery of plant organ, that rotates by attracting and detracting Moisture, Iran patent.No.26299 .

F. Moradi (2005) design and manufacturing of plant nano-motor, Iran Patent No,3 1 39, supported by Tehran University .

F. Moradi (2005) design and produce of H2o signaling, Iran patent.No.3 1041

F. Moradi (2005) industrial and material Micro-sensors, Iran Patent.No.3 1037

F. Moradi (2005) Langar cells as a source of having Bio-energy, Iran Patent No.3 1040, supported by Tehran University .

F. Moradi (2006) design and manufacturing of plant nano-motor US patent file. No. U3 1P1US by Dr.Andrea Hrovat patent Attorney,[E-mail: hrovat@ksnatent.de] supported by Tehran University .

F. Moradi (2006) nanosensor of drugs and material (protection drugs and materials against humidity) Iran patent No, 38310233.

F.Moradi (2009) Establishment of rotary cellulose nano composites and plant biosensors using discovery smart organ , the best European conference on miniaturisation for the life sciences, Nano Bio Tech Montreux,Nov.16-18, Montreux, Switzerland.

F.Moradi (2009) Establishment of plant biosensors using discovery smart organ,4th BioNanoTox and Applicatin Research Conference,University of Arkansas, Oct. 21-22 ,Little Rock, Arkansas,USA.

F.Moradi, Naghavi M., Omidi M (2003) design and manufacturing of Sensors sensitive to saturated Steam pressure in Bioreactor: Application of intelligent organ biosensor in Biotechnology the third National Biotechnology Congress of Iran, Sep.9- 11, Mashhad, Iran

Moradi, F; Project title: discovery of smart organ ،Recipient, Qwarizmi Festival award, Tehran, Iran, 2000.

Moradi, F; Recipient Bronze Medal from novel Technology,The second national Olympiad of inventors, originators and innovators Islamic Azad University ,Isfahan Branch, Iran , oct 13-15 , 2011.

Moradi, F; Recipient, grant and License, Cell phone bio-safety sensor (against Cell phone microbes) Iran's National Elites, Iran, 2010, No.15.2270.

Moradi, F; Recipient, grant and License, nanosensor of drugs and materials (protection drugs and materials against humidity) Iran's National Elites, Iran, 2011, No.18.405.

Freire, R.S., M.M.C. Ferreira, N. Duran, L.T. Kubota. 2003a. Dual amperometric biosensor device for analysis of binary mixtures of phenols by multivariate calibration using partial least squares. Analytica Chimica Acta 485: 263-269.

Freire, R.S., N. Duran, L.T. Kubota. 2001. Effects of fungal laccase immobilization procedures for the development of a biosensor for phenol compounds. Talanta 54 : 681-686.

Ghobadi, S., E. Csöregi, G.M. Vaga, L. Gonton. 1996. Bienzyme carbon paste electrodes for L-glutamate determination. Current Separations 14: 94-102.

Gomes, S.A.S.S., J.M.F. Nogueiraa, M.J.F. Rebelo. 2004. An amperometric biosensor for poly phenolic compounds in red wine. Biosensors and Bioelectronics 20:1211-1216.

Gooding, J.J. 2006. Biosensor technology for detecting biological warfare agents:recent progress and future trends. Analytica Chimica Acta 559: 137-151.

Katalinic, V., M. Milos, T. Kulisic, M. Jukic. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry 94 : 550-557.

King, A. and G. Young. 1999. Characteristics and occurrence of phenolic phytochemicals. Journal of American Dieteteic Association 99: 213-218.

Mailley, P., E.A. Cummings, S. Mailley, S. Cosnier, B.R. Eggins, E. McAdams. 2004. Amperometric detection of phenolic compounds by polypyrrole-based composite carbon paste electrodes. Bioelectrochemistry 63: 291-296.

Mello, L.D. and L.T. Kubota. 2002. Review of the use of biosensors as analytical tools in the food and drink industries. Food Chemistry 77: 237-256.

Pourcel L., J. Routaboul, V. Cheynier, L. Lepiniec, I. Debeaujon. 2007. Flavonoidoxidation in plants: from biochemical properties to physiological functions.Trends in Plant Science 12: 29-36.

Project title: discovery of smart organ ؛ Recipient, Qwarizmi Festival award, Tehran, Iran, 2000

Recipient Bronze Medal from novel Technology, The second national Olympiad of inventors, originators and innovators Islamic Azad University, Isfahan Branch, Iran, oct 13-15, 2011

Recipient, grant and License, Cell phone bio-safety sensor (against Cell phone microbes) Iran's National Elites, Iran, 2010, No.15.2270.

Recipient, grant and License, nanosensor of drugs and materials (protection drugs and materials against humidity) Iran's National Elites, Iran, 2011, No.18.405.

Simone, Charles B. 1992. Free Radicals in Cancer and Nutrition, Simone Health Series New York: Elsevier Science Publishing Company Inc.

Thesis title: Design and Manufacturing of Bio-energy generator, using plant sensors, under Supervision of Dr.Bahman Yazdi Samadi from Tehran University, Dr. Sayed Khatiboleslam Sadrnezhad from Sharif University and Advisor Dr .Mohamad reza naghavi from Thehran University.

Urquiaga, I.. and F. Leighton. 2000. Plant poly phenol antioxidants and oxidative stress.

Vastarella, W. 2001. Enzyme Modified Electrodes in Amperometric Biosensors. University of Degli Studi di Bari, Thesis of PhD.