ISSN: 03875547
Volume 45, Issue 02, April, 2022

The role of iron oxide nanoparticles in the growth and propagation of the broccoli plant Brassica Oleracea

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Keywords:
iron oxide, nanoparticles, Brassica Oleracea

ABSTRACT

This study was conducted in the Plant Cell and Tissue Culture Laboratory of the Department of Life Sciences at the College of Education for Pure Sciences at the University of Diyala during the period from September 2020 to July 2021. This study was carried out with the aim of obtaining typical suspended cell cultures. Of the broccoli plant Brassica Oleracea of medicinal and pharmaceutical value, which is derived from the callus of the subcotyledonous stems of broccoli grown on MS medium supplemented with different concentrations of 2,4-(2,4-D) Dichlorophenoxy acetic acid (interlaced with Benzyl adenine (BA) and then follow-up The results of its cultivation, the results indicate the following. The stems grown on MS medium supplemented with growth regulators have superiority and ability to induce callus compared to their peers in the control treatment. It was found that the MS medium supplemented with concentrations of 1.5,2.00 mg.l-1,4-D + 0.5 mg.l-1 BA stimulated callus induction and achieved the highest value of 100% compared to the control treatment whose induction was 0%, and the percentage of Induction to 50% in the treatment at a concentration of 0.5,1.00 mg.l ¬¬-1,4-D + 0.5 mg.l-1 BA. The results also indicate that the highest average fresh weight of the callus was recorded when treatment of 1.5.2.00 mg.l ¬¬-1 of growth regulators, which reached 1.166,2.343 g.piece -1 in a row, outperforming the treatment with a concentration of 0.5,1.00 mg. L-1 2,4-D + 0.5 mg.L-1 BA, whose value started decreasing to 0.313,0.860 g.pc-1, respectively. The results showed that the concentration exceeded 2.00 mg. 1 liter of 2,4-D + 0.5 of BA added to it nano iron oxide was significantly higher than the rest of the concentrations, as it recorded the highest mean of 4.453 gm of fresh weight compared to the control treatment of 2.343 gm. for the stem and amounted to 4.333 gm compared to the control treatment which amounted to 2.650 gm for the leaf the.

1. INTRODUCTION

The cruciferous family Brassicaceae includes many important vegetables, one of which is broccoli, whose

scientific name is Brassica oleraceae var. Italica, broccoli is grown for its flowering inflorescences, which are eaten while they are in the process of flowering buds with their thick, smooth stalks [4]. The pink tablets are often eaten cooked or boiled, and sometimes they are eaten in their natural state, or they may be used in pickles. Broccoli is an annual herbaceous plant native to the Mediterranean region [11]. Where it grew there for the first time during the Roman rule in Italy, and was first planted in England in the year 1720 AD, and then its cultivation moved to the United States of America in the year 1806 AD. In terms of commercial production, the year 1923 AD witnessed the first cultivation of broccoli for the purpose of commercial marketing in USA [10].

The broccoli plant is characterized by high nutritional value, as it contains many nutrients, vitamins, proteins, fats and carbohydrates, except for the high content of glucosides, which have anti-cancer properties [6], and every 100 gm of the eaten part contains the following nutritional components: 89.1 g moisture, 22 Calories 3.6g Protein 0.3g Fat 5.9g Carbs 1.5g Fiber 103mg Calcium 78mg Phosphorous 1.1mg Iron 15mg Sodium 38mg Potassium 2500IU Vitamin A 0.1g Thiamine 0.23g Riboflavin 0.9g of niacin, 113mg of ascorbic acid (Ismail, 2004), that eating broccoli tablets more than once a week can reduce the risk of prostate cancer by 45% [5].

Plant tissue culture was employed using nanomaterials, as nanotechnology is one of the modern technologies that have proven its importance in agricultural sciences and related industries towards solving problems and shortcomings in many fields of science and other technologies. The term nano is derived from the Greek root dwarf which means short height and refers to dimensions equal to one billionth of each quantity [7].

In particular, nanotechnology has the potential to provide effective solutions to many areas related to agricultural problems, and to bridge the gap between large-sized materials and atomic or molecular compounds, nanoparticles offer great scientific benefit, and over the past two decades, there has been a large number of research carried out on the technology of Nanotechnology and focus on its many applications in the agricultural sectors [9].

The studied traits included:
1-Reaching the best conditions for plant propagation by tissue culture in terms of the type of plant part and the studied growth regulators.
2-Studying the effect of some auxins from (2-4.D and BA) on callus development and its subsequent growth.

2. MATERIALS AND METHOD

All experiments were carried out in the Cell and Plant Tissue Culture Laboratory of the Department of Life Sciences at the College of Education for Pure Sciences at the University of Diyala. 121 ºC and a pressure of 1.04 kg/cm² for 30 minutes. In addition to using ethyl alcohol at a concentration of 95% with burning, and using the laminar air flow cabinate, in culturing plant tissues after sterilizing them with UV rays for 30 minutes and wiping them with ethyl alcohol at a concentration of 70% [1]. The samples were kept in the growth room at a temperature of ±25 2 ºC and in the alternating light and dark regime of 16 hours light/8 hours of darkness [12]. The vegetative-derived callus was induced and multiplied on normal MS medium as stems and petioles of sterilized leaves were cut into pieces of length 1.0 cm2.Piece-1, the pieces were transferred at a rate of one piece for each 120 ml glass vial containing 20 ml of solid MS medium free of growth regulators, followed by control treatment, and another to MS medium at a concentration of 0.0, 2.00. 0,0.5,1.00,1.5 mg.L-1,2.4-D mixed with .0.5 mg.L-1 of Benzyl adenine (BA) to study the effect of growth regulators and the effect of plant part type on callus induction. The samples were incubated under the aforementioned preservation conditions, and the changes in the plant part were followed up until it changed

its shape and formation of the callus. The callus cultures were maintained by taking pieces weighing 1.00 g and replanting them on the same medium for 30 days and recording the data of the studied traits. Whereby, pieces weighing 1.00 g were taken from the callus of the plant parts and grown on MS medium supplemented with nano iron oxide Fe2O3 and the best concentrations of growth regulators, (2.00 mg.l-1,4-D intermingled with 0.5 mg.l-1 of BA) for 30 days. Day and record the data of the studied traits.

The samples were under the aforementioned preservation conditions. The growth of the nodal parts was followed up after 30 days, and the data of the studied characteristics were recorded. As for the physical examinations of iron oxide nanoparticles, they include:-

2.1 X-ray diffraction

X-ray diffraction technique is used to know the nature of the crystal structure, the main crystal phases and the prevailing trend of the prepared crystals under certain conditions, as well as to identify some structural parameters such as crystalline size, lattice constants, and the width of the curve at the middle of the top.

2.2 Scanning field-emitting electron microscope

(Field Emmision Scanning Electron Microscopy (FESEM)

The examinations were carried out using a scanning electron microscope at Kashan University / Iran. The scanning electron microscope has a magnification power ranging from 25-250,000 times. The sample is visualized by scanning it with beams of high-energy electrons. The electrons collide with the sample's atoms, thus producing signals (containing On X-rays, secondary electrons, and light) that includes information about the structure of the surface. Thus, the scanning electron microscope can produce images with a very high analysis of the surface of the sample and show minute details that may reach a size ranging from 1-5 nanometers. Moreover, these microscopic images are three-dimensional and help This helps to understand the surface structure of the sample [8]. As for the experiment of inducing callus on normal and nano-enriched MS medium, the average wet weight of the growing callus on the normal and nano-enriched MS medium was calculated by calculating the weight difference of the glass bottles and their contents for calluses.

3. Results and discussion

The results of Table (1) show that the stems grown on MS medium supplemented with growth regulators have superiority and ability to induce callus compared to their peers in the control treatment. It was found that the MS medium supplemented with concentrations of (1.5, 2.00) mg.L-1 of 2,4-D + (0.5) mg.L-1 of BA stimulated callus induction and achieved the highest value of 100% compared to the control treatment whose induction reached 0%, and the induction percentage decreased to 50% in the treatment with a concentration of (0.5,1.00) mg.l ¬¬-1 of 2,4-D + 0.5) (mg.l-1 of BA). The results also indicate that the highest mean was recorded The fresh weight of callus when treated (1.5, 2.00 mg.l ¬¬-1 of growth regulators, which amounted to 1.166, 2.343)) g.piece -1 in a row, outperforming the treatment with a concentration of (0.5,1.00) mg.l-1 of 2,4-D + (0.5) mg.L-1 of BA, whose value started to decrease to 0.313 and 0.860 g.Piece-1, respectively.While the plant parts of the comparison treatment showed a slight swelling (A9), it became a cut shape Stems grown on MS medium fortified with a concentration of (2.0) mg.L-1 of 2,4-D dumbly, as the callus tissue in the cut areas led to the parts losing their entire shape and turning them into a mass of callus, which was characterized by its fragile texture and light green color (E9) The directional shanks were taken, shifting the positions of the The callus was cut into a piece of callus after 30 days of cultivation at a concentration of (0.5, 1.00, 1.5 0.0) mg.l-1 of 2,4-D + (0.5) mg.l-1 of BA. The callus was distinguished in these concentrations by its green color. Light and solid texture, Figure (D,C, B).

The results in Table No. (2) Figure (1) indicate that the stem responded to the induction more than the leaf

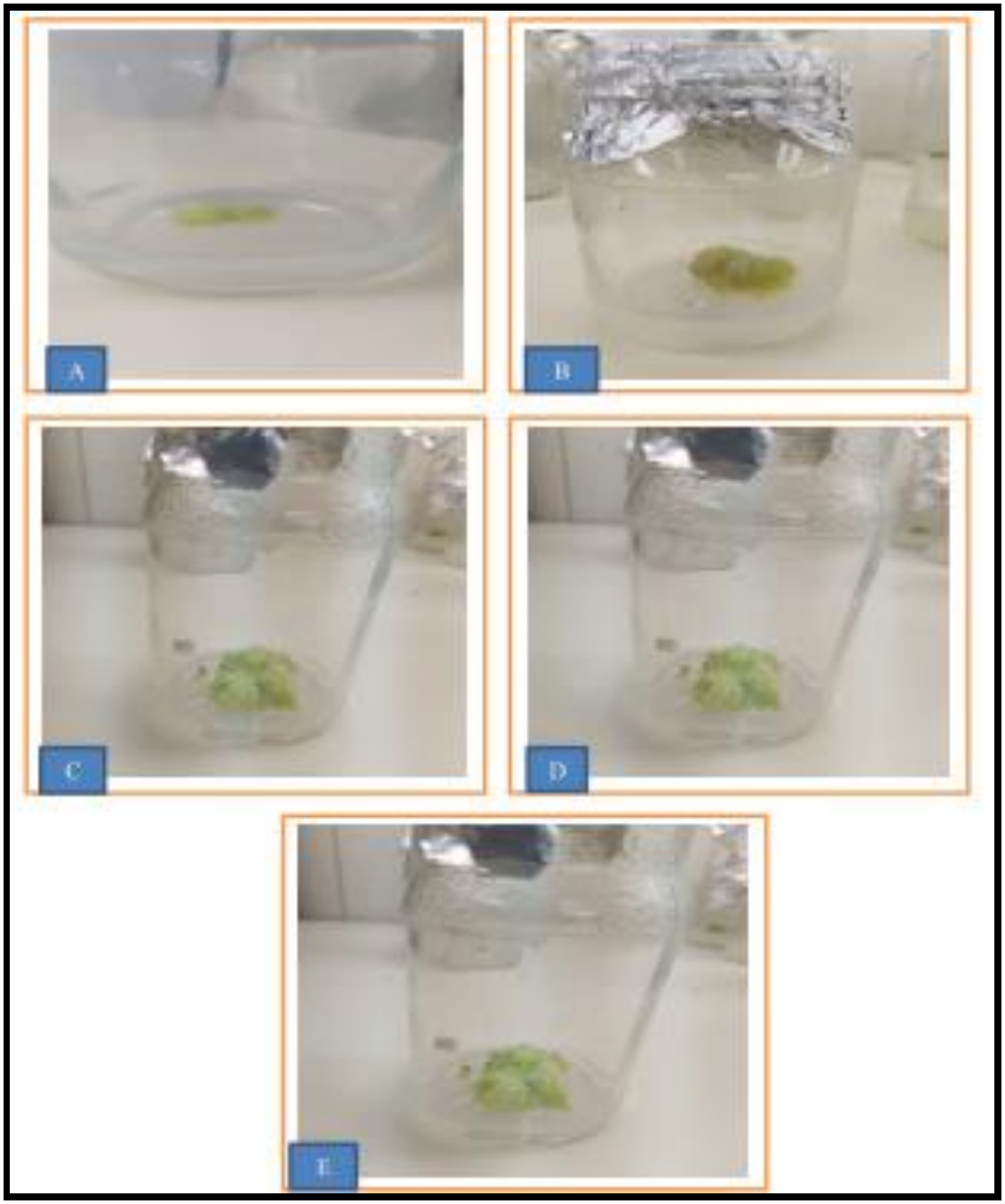
neck, as the highest value of the leaf neck wet weight was recorded (2.176) g at a concentration of (2.00) mg. Liter 1- of 2,4-D and the value decreased to (0.356,0590) for the two concentrations (0.0 and 0.5), respectively, for the wet weight of the leaf neck. Cytokinins have a major role in increasing the soft weight of the callus through their important effects in increasing cell division, as they work to increase the division of meristematic cells and parenchymal cells that have lost their specialization and transformed into meristematic cells, which leads to an increase in the size of the different tissues of plant organs, whether they are connected to the mother plant or separated and grown in sterile food media [13].

Table (1) The percentage of callus induction derived from sterilized and separated stems of broccoli and its average fresh weights in MS medium supplemented with different concentrations of 2,4-D + BA

callus size	*Average Soft Weight (gm.pc-1(*	*Induction*	*Growth regulators concentrations(mg.l-1)*	
			BA	*2,4-D*
+	*C 0.176*	*0*	*0. 5*	*0.0*
++	*C0.313*	*50*		*0.5*
++	*b 0.860*	*50*		*1.0*
+++	*b 1.166*	*100*		*1.5*
+++	*a 2.343*	*100*		*2.0*

uced callus from cut stems of the broccoli plant. By adding different concentrations of the growth regulator 2,4-D to which BA (A) was added: comparison treatment (B): concentration of 0.5 mg.L-1,2,4-D + 0.5 mg.L-1 BA (C): concentration of 1.00 mg L-1 2,4-D + 0.5 mg.L-1 BA (D): Concentration 1.5 mg.L-1 2,4-D + 0.5 mg.L-1 BA (E): Concentration of 2.00 mg. L-1 2,4-D + 0.5 mg.L-1 BA

ISSN: 03875547
Volume 45, Issue 02, April, 2022



uced callus from cut stems of the broccoli plant. By adding different concentrations of the growth regulator 2,4-D to which BA (A) was added: comparison treatment (B): concentration of 0.5 mg.L-1,2,4-D + 0.5 mg.L-1 BA (C): concentration of 1.00 mg L-1 2,4-D + 0.5 mg.L-1 BA (D): Concentration 1.5 mg.L-1 2,4-D + 0.5 mg.L-1 BA (E): Concentration of 2.00 mg. L-1 2,4-D + 0.5 mg.L-1 BA

Table (2) The percentage of callus induction derived from sterilized and separated leaves of broccoli plant and its average fresh weights in MS medium supplemented with different concentrations of 2,4-D + BA

callus size	***Average Soft Weight (gm.pc-1(***	***(%) induction***	***Growth regulators concentrations(mg.l-1)***

			BA	*2,4-D*
+	*d 0.356*	*0*		*0.0*
++	*d 0.590*	*50*		*0.5*
++	*C 1.510*	*50*	*0. 5*	*1.0*
+++	*b 1.831*	*100*		*1.5*
+++	*a 2.176*	*100*		*2.0*

he number of repetitions is 10 plant pieces. Treatment-1.

The averages that have similar letters have no significant statistically significant differences between them

The results of the two tables (3'4) Figure (2) showed that the concentration exceeded 2.00 mg. L-1 out of 2,4-D was significantly higher than the rest of the concentrations, as it recorded the highest mean of 4.453 g in comparison with the control treatment of 2.343 g. for the stem and it was 4.333 gm compared to the control treatment, which amounted to 2.650 gm for the leaf

Table (3) The percentage of callus induction derived from sterile and separated stems of broccoli and its average fresh weight in MS nanomedium supplemented with different concentrations of 2,4-D +BA

sample average	nanomaterial concentration	hormone concentrations	
		BA	2.4.D
c 2.343	0.0	0.0	0.0
b 2.883	50	0.5	2.00
b 3.190	100	0.5	2.00
a 4.453	150	0.5	2.00

Table (4) The percentage of callus induction derived from sterilized and separated leaves of broccoli plant and its average fresh weight in MS nanomedium supplemented with different concentrations of 2,4-D +BA.

sample average	nanomaterial concentration	hormone concentrations	
		2.4.D	2.4.D
C 2.650	0.0	0.0	0.0
b 3.563	50	0.5	2.00
b 3.640	100	0.5	2.00

ISSN: 03875547
Volume 45, Issue 02, April, 2022

a 4.333	150	0.5	2.00

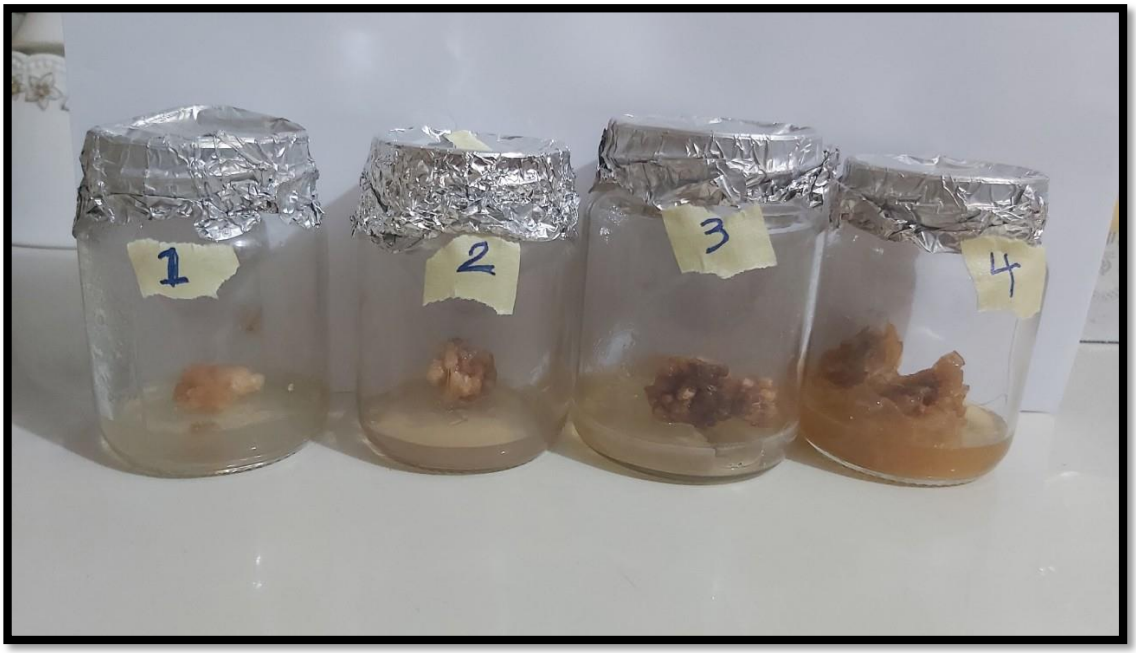


Figure (3) shows the percentage of callus induction derived from sterilized and separated leaves of broccoli plant and its average fresh weights in MS nanomedium supplemented with different concentrations of 2,4-D +BA.

Control1-
2-Concentration of 2.00 mg.l-1 of 2-4D +0.5 BA + 50% of nano iron oxide
3-Concentration of 2.00 mg.l-1 of 2-4D +0.5 BA +100% of nano iron oxide
4-Concentration of 2.00 mg.L-1 of 2-4D +0.5 BA +150% of nano iron oxide
The results of Table (5) indicate a significant superiority of the concentration of 2.4 D in the vegetative dry weight of the stems compared to the control treatment, and in the direction taken by all the traits, the treatments of MS medium supplemented with concentrations of 0.5 mg were recorded. L-1 BA + 0.0, 0.5, 1.00, 1.5, 2.00 mg. Liter-1 2.4D, where the averages reached 0.080, 0.156, 0.426, 1.170 g, respectively, and these values declined to the lowest in the comparison treatment, which amounted to 0.080 g.

As for the paper Table No. (6), the concentration of 2.00 mg exceeded. L-1 of 2.4 D was significant over the rest of the treatments, which amounted to 1.086 g and decreased to 0.170 g in relation to the control treatment

Table No. (5) Effect of different concentrations of 2.4D with BA on the dry weight of broccoli stems grown on normal MS medium

Average Soft Weight (gm.pc-1)(	***Concentrations of growth regulator(mg.l-)***	
	BA	***2,4-D***
C 0.080	*0. 5*	*0.0*

C 0.156		*0.5*
b 0.426		*1.00*
b 0,583		*1.5*
A 1.170		*2.0*

Table No. (6): Effect of different concentrations of 2.4D with BA on dry weight of broccoli leaves grown on normal MS medium:

Average dry weight)(gm.pc-1)	***Growth regulators)concentrations (mg.l-1)***	
	BA	***2,4-D***
d 0.170	*0. 5*	*0.0*
d 0.283		*0.5*
C 0.753		*1.00*
b 0.903		*1.5*
a 1.086		*2.0*

The results in the two tables (7,8) show that the highest value of dry weight of stems of plants grown on MS nanomedium was 2.210 g for 150% concentration of nano iron oxide Fe203, and it was significantly superior to the rest of the treatments which amounted to 1.170, 1.436, 1.593 g, respectively, as for leaves The treatment was significantly superior to the nano iron oxide by 150% than the rest of the treatments, which amounted to 2.166 gm compared to the control treatment, which amounted to 1.323 gm [14].

Table (7) Effect of different concentrations of 2.4D with BA on the dry weight of broccoli stems grown on MS nanomedium

sample average	**nanomaterial concentrations**	**hormone concentrations**	
		BA	**2.4.D**
c 1.170	0.0	0.0	0.0
b 1.436	50	0.5	2.00
b 1.593	100	0.5	2.00
a 2.210	150	0.5	2.00

Table (8) Effect of different concentrations of 2.4D with BA on dry weight of broccoli leaves grown on MS

nanomedium

sample average	Concentrations	hormone concentrations	
		BA	2.4.D
c 1.323	0.0	0.0	0.0
b 1.793	50	0.5	2.00
b 1.816	100	0.5	2.00
a 2.166	150	0.5	2.00

Values with similar letters do not differ significantly from each other according to Duncan test at the 0.05. probability level

4. References

[1] Salman, Muhammad Abbas (1988). Fundamentals of plant tissue and cell culture, Dar Al-Kutub for Printing and Publishing, University of Mosul, Iraq.

[2] Delloloio, R. (2007). Cytokininus determine Arabidopsis root- merestem size by controlling cell differentiation. Current Biology, (17): 678-682.

[3] smail, Abdel Moneim Ahmed. 2004. Production of non-traditional vegetable crops for export, Central Laboratory for Agricultural Climate - Agricultural Research Center, Bulletin No. 10, Republic of Egypt.

[4] Hassan, Ahmed Abdel Moneim. 2004. Secondary and non-traditional vegetable production, first part, first edition, Arab House for Publishing and Distribution

[5] Gad, N., & Abd El-Moez, M. R. (2011). Broccoli growth, yield quantity and quality as affected by cobalt nutrition. Agric. Biol. J. N. Am. 2, 226-231.

[6] Hanson, R. 2000. Colon cancer curbed by High Selenium Broccoli. Agricul- tural research. Human Nutrition research center P.O. Box 9034.

[7] Warad, C. and Dutta, J. (2006). Nanotechnology for Agriculture and Food Systems. Journal of Nanoparticle Research, 5(3): 29-38.

[8] Saleh, Mahmoud Mohamed Selim. 2015. Nanotechnology and a new scientific age. King Fahd National Library. Riyadh. Saudi Arabia. p 152

[9] Lv, M.; Liu, Y.; Geng, J.H.; Kou, X.H.; Xin, Z.H. and Yang, D.Y. (2018). Engin- eering nanomaterials-based biosensors for food safety detection. Biosens. Bioelectron,106: 122–128

[10] Ouda, B.A. and A.Y. Mahadeen, 2008. Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (Brassica oleracea var.italica). Int. J. Agric. Biol., 10: 627-632 .

[11] Mr., Syed Fathy .2009. Technology of producing warm season vegetables in desert lands. Egyptian Library. Egypt.555 p

[12] Nader, Duha Sabah (2018). Genesis of Physalis angulate cell suspensions and detection of physalin complex. Master Thesis, College. of Education for Pure Sciences, University of Diyala, Iraq

[13] Al-Shahat, Abu Zaid Nasr (2003). Plant hormones and agricultural applications. Third Edition. Arab House for Publishing and Distribution, Egypt

[14] Zuverza-Mena, N.D. Martínez-Fernández , W. Du et al. 2017. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review," Plant Physiology and Biochemistry. vol. 110. pp. 236-264.