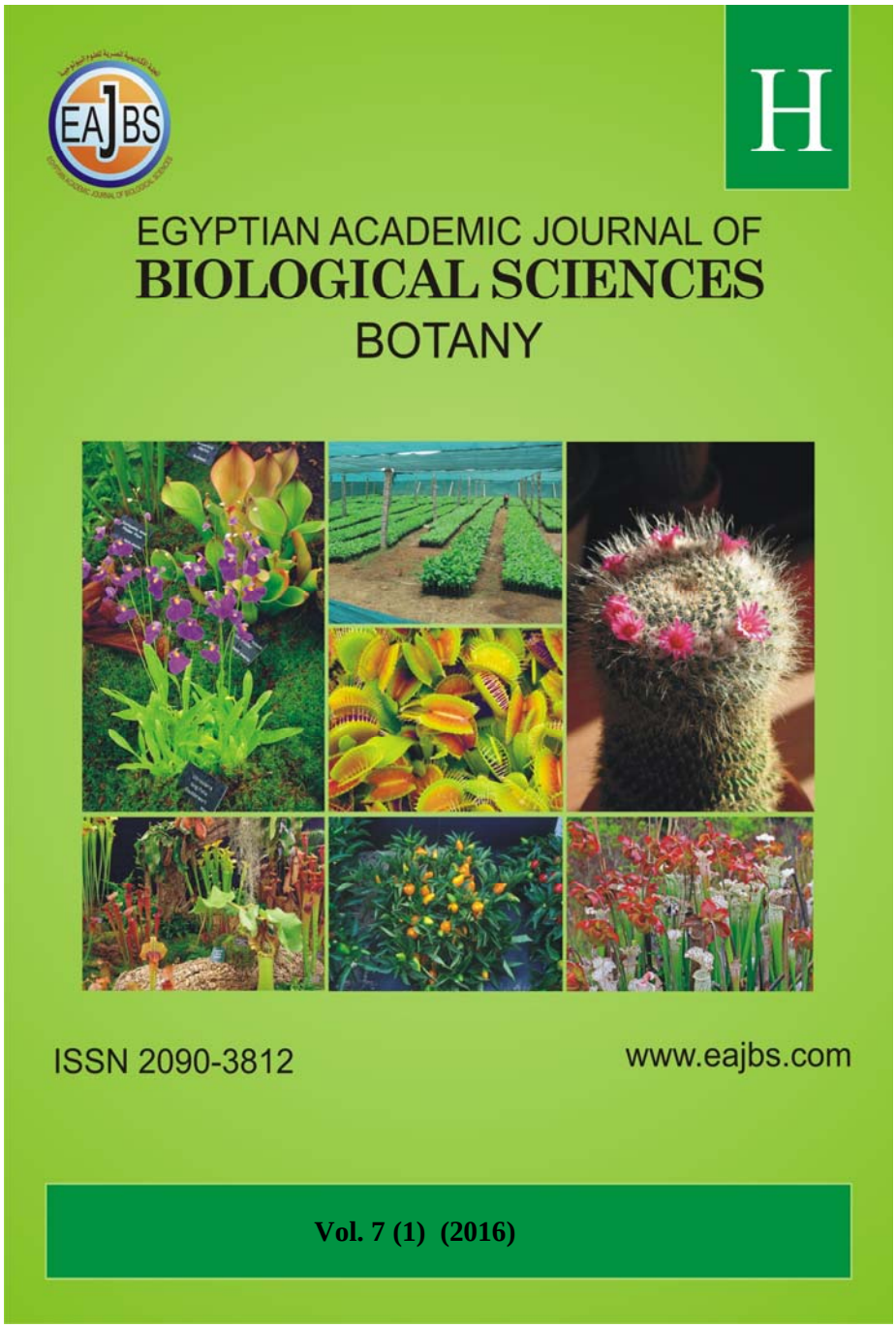


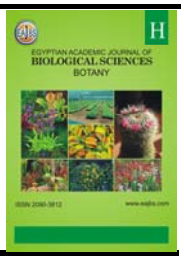
Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences , Department of Entomology ,Faculty of Sciences Ain Shams University .

The Botany Journal publishes original research papers and reviews from any botanical discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematic, morphology, evolution, control of herbs, arachnids, and general botany..

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci.,7(1): 1–7 (2016)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbs.eg.net



Utilization of Aquatic Plants Extracts as an Alternative to Plant Growth Regulators In Vitro Experiments

Ashwaq Shanan Abed*, Sameer Naji Mahmmoud and Eman N. Ismail
Biotechnology Research Center, Al-Nahrain University, Baghdad 10072, Iraq.
E.Mail: ashwaqbio@yahoo.com

ARTICLE INFO
Article History
Received: 21/3/2016
Accepted: 1/5/2016

***Keywords*:**
Aquatic plants
Ceratophyllum demersum
Egeria densa
plant growth regulators

ABSTRACT

In the current study, the levels of endogenous free and conjugated auxin (Indole-3-acetic acid, IAA), gibberellic acid (GA_3), and abscisic acid (ABA) were examined in two species of aquatic plants (*Ceratophyllum demersum* and *Egeria densa*). The comparison between the content of endogenous phytohormones in aquatic plants showed that *C. demersum*had highestlevels of total (IAA, GA_3 and ABA) than *E. densa.* Different concentrations of free phytohormones extracts were prepared (0, 25, 50, and 100µl/l), then added to (MS) culture medium as an alternative to plant growth regulators. Effects of these concentrations on callus productionof black henbane (*Hyoscyamusniger*) and potato propagation (*Solanum tuberosum*) were studied *in vitro*. Freephytohormones extracts with all concentrations encouraged production of callus tissue from leaves explant of black henbane compared with control treatment, also, these extracts promoted propagation of potatoby increasing number of nods and length of shoot.So, its appear that remnants of aquatic plants could be used successfully for agricultural improvement and another application of bioassay.

INTRODUCTION

Plant growth hormones (Indole-3-acetic acid IAA, Gibberellic acid GA_3 and Abscisic acid ABA) are specialized chemical substances or known as secondary metabolites, which are important biotechnological products, widely used in agricultural and horticultural applications.

These hormones are synthesized not only by higher plants, they have also been synthesized by Mosses, Lichens (Ergün *et al*., 2002), Fungi (Ünyayar *et al*., 1996; MacMillan, 2002; Rangaswamy, 2012), Bacteria (Karadeniz *et al*., 2006), Yeast (Tawfiq, 2010) and Algae (Jacobs *et al*., 1985). Hormones are synthesized in plant material at a very low concentration, together with many other compounds (Muller, 2011; Karadeniz *et al*., 2006) for this reason, be highcostin the market.

In this respect, were chosen two types of aquatic plants (*Ceratophyllum demersum* and *Egeria densa*) to estimating the content of IAA, GA_3 and ABA in their tissues and use it as substitute for plant growth regulators in plant tissue culture experience for employ cheap raw materials to rendering it economical.

Ceratophyllum demersum (hornwort) is a submersed member of the Ceratophyllaceae family and *Egeria densa* (Elodea) belonging to the family

Hydrocharitaceae. Both species have a similar distribution patterns in Iraq, they are widespread, Submersed and abundant plants (Al-Daody and Al-Mandeel, 2012; Bowmer *et al*., 1995; Aziz, 2009), can be found in sluggish lakes, ponds, and slow streams (Schmidt and Kannenburg, 1998).

Most of waterweed poses serious environmental problems when the dense growths are over 25% of the surface area. Aquatic plants can restrict swimming, boating, fishing and reduce water flow to irrigated regions. These plants provide a food source for many freshwater organisms, including aquatic insects, fish and aquatic invertebrates, plus help to stabilize bottom sediments (Helfrich *et al*., 2000).

Aquatic plants like *C. demersum* and *E. densa* can be used as biofilter for heavy metals such as Ni, Pb, and Cd (Módenes, 2009; Foroughi *et al*., 2011; Dhir, 2013). Many countries are currently utilizingaquatic plants for agricultural purposes as food for cattle and sheep, poultry, and as preparation for silage, because they are rich in protein, minerals and pigmentscarotenoids (Edwards, 1981).

The aim of our investigation was to determine IAA, GA_3 and ABA in the aquatic plants and use it as alternative to plant growth regulators which are widely used in plant tissue culture technique and many areas of horticulture including pomology and ornamental horticulture.

MATERIALS AND METHODS

Collection of samples

Two different samples of common Iraqi aquatic plants (*C. demersum* and *E. densa*) were collected on March and April in 2014fromstreamlet of Baghdad University, placed in plastic bags, and transferred immediately to the laboratory. Each plant was washed with fresh tap water and cleaned itoffimpurities and then oven-dried at 45ºC for 48 h.

Extraction of plant growth regulators IAA, GA_3 and ABA

One gram of dry tissueper sample was homogenized for 24h with 60 ml of mixter of solvents (methanol: chloroform: 2N ammonium hydroxide, 12:5:3 v/v/v) at 4ºC.Both combined extract (60 ml) was centrifugedfor 15 min at 2500 rpm/min, the supernant was treated with 25 ml of dissteled water. The other steps of free and bound hormones extraction were done according to Kelen *et al.,* 2004. The dried residues were dissolved in 10 ml of 70% methanol, then the Optima UV Spectro-photometer (Optima SP-3000nano, Japan) was used to estimate the content of free and conjugate forms of phytohormones using 222 nm and 280 nm wave lengths for IAA, 254 nm for $GA_3$and 263 nm for ABA. IAA standard reagent was obtained from Sigma Cemichal Co. (USA), GA_3 from Merck Co. (German) and ABA from Himedia Co. (India).

Phytohormones Bioassay

Free phytohormones extracts for both *C. demersum* and *E. densa* were tested *in vitro* on callus formation from leaves of *Hyoscyamusniger* (black henbane), and on propagation of potato (*Solanum tuberosum* L.). The culture medium used for all experiments as based on (MS) Murashige and Skoog's medium (Murashige and Skoog, 1962).

Leaves explant of black henbane (*H. niger*) was cultured for induction of callus using MS medium supplemented separately with 0, 25, 50 and 100 µl/l each of *C. demersum* and *E. densa* free phytohormones extracts. Fresh and dry weights of callus were recorded after 8 weeks of incubation at 25 ±2°C under a 16 h/day photoperiod.

Furthermore, single nods of potato explant was planted in MS medium supplemented separately with 0, 25, 50 and 100 µl/l of free phytohormones extracts. All cultures were incubated at 25 $\pm 2^0$C under 16h/day in photoperiod condition. Number of nods and length of plantlets were recorded after 4 weeks.

Experimental design

All experiments were done with minimum of 20 replicates per treatment. A one-way ANOVA with replication was done using Statistical Software-Minitab 11. Least significant differences (LSD) between means were calculated at $P \leq 0.05$.

RESULTS AND DISCUSSION

Phytohormones content

The IAA, GA_3 and ABA content of two aquatic plant samples were obtained by spectrophotometric method. Calibration curves of these three substances were prepared using analytical reagent grade standards. Linear regression data for IAA, $GA_3$and ABA are listed in Figure 1. The comparison between two species of aquatic plants showed that *C. demersum*had highestlevels of total phytohormones than *E. densa*.

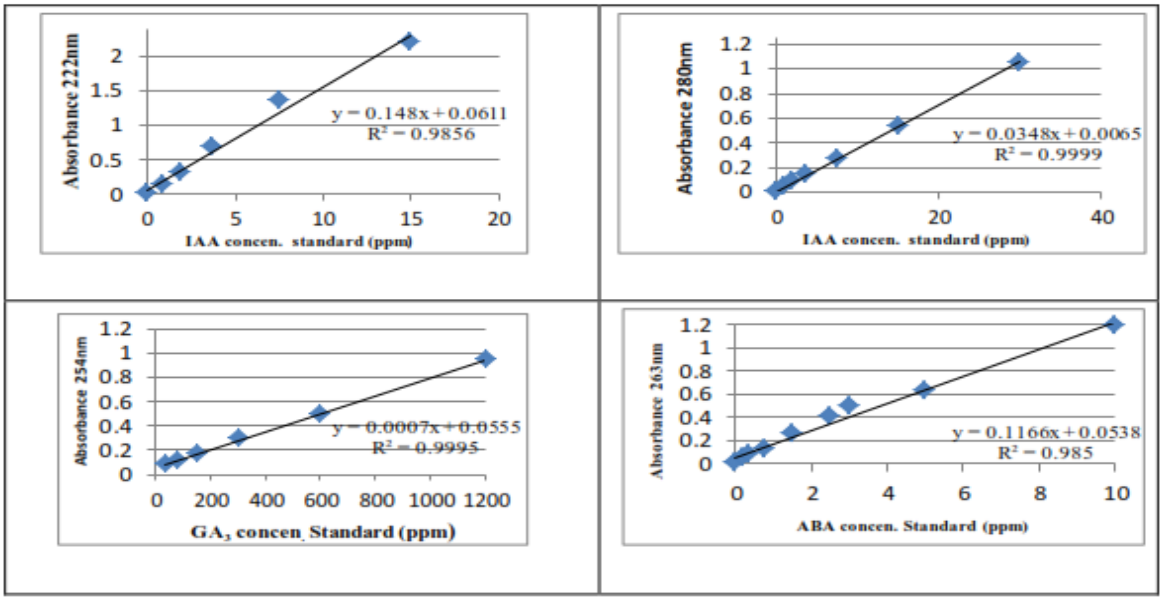


Fig. 1: The standard curvs for plant growth regulators using spectrophotometric method; IAA (222 and 280 nm), GA_3 (254nm) and ABA (263nm).

Total values of IAA in *C. demersum* were (53.49 and 132.17 ppm) at 222 and 280nm wavelengths respectively, while the total levels of this compound were found in *E. densa* (53.73 and 98.55 ppm) respectively. Also highest GA_3 and ABA concentrations were recorded in *C. demersum* (8614.28 and 77.135 ppm) respectively, and the lowest total levels of these compounds were determined in *E. densa* (5314.28 and 30.56 ppm) respectively. The amounts of IAA, GA_3 and ABA in tow species of aquatic plants are given in Table (1).

Table 1: Content of phytohormones (ppm) in two species of aquatic plants (*Ceratophyllumdemersum* and *Egeria densa*) using spectrophotometric method.

Phytohormones (ppm)		Aquatic plants species	
		Ceratophyllum demersum	*Egeria densa*
IAA 222nm	Free IAA	33.64	28.57
	Conjugate IAA	19.85	25.16
	Total IAA	53.49	53.73
IAA 280nm	Free IAA	73.99	51.86
	Conjugate IAA	58.18	46.69
	Total IAA	132.17	98.55
GA3 254 nm	Free GA3	4578.57	2050
	Conjugate GA3	4035.71	3264.28
	Total GA3	8614.28	5314.28
ABA 263 nm	Free ABA	55.506	16.74
	Conjugate ABA	21.629	13.82
	Total ABA	77.135	30.56

Phytohormones bioassay

Bioassay test was done only on free phytohormones form because the conjugate forms are generally considered inactive (Korasick *et al*., 2013). Effect of free phytohormones extracts on fresh and dry weights of callus production from leaves of black henbane (*H. niger*) was studied. Generally, the enhancement of mass callus production depended on the type plant extract and their concentration. The result shows that all treatments of phytohormonesextractssignificantly encouraged the induction of callus comparison with control.

C. demersum extractenhanced the fresh and dry weights of callus tissue more than *E. densa*, (Table 2). Furthermore, these phytohormones extracts promoted *in vitro* propagation of potato (*S. tuberosum*) by raising number of nodes more than control treatment. The results also showed the presence of significant differences in length of plant produced depending on the type of aquatic plant extract and their concentration, (Table 3).

Table 2: Beneficial effects of free phytohormones extracts added to MS medium on fresh and dry weights (mg) of callus production from leaves of (*Hyoscyamus niger*) after 8 weeks of incubation.

Amount of phytohormones extracts	*Ceratophyllum demersum*		*Egeria densa*	
	Fresh weight of callus	Dry weight of callus	Fresh weight of callus	Dry weight of callus
0.0(control)	—	—	—	—
25 µl/l of	1200.0	90.1	1000.5	76.36
50 µl/l of	1281.7	93.6	941.3	68.93
100 µl/l of	1280.8	99.2	893.5	69.97
Means	940.6	70.7	708.8	53.8
LSD at P ≤ 0.05	69.50	4.11	40.7	2.96

Table 3: Beneficial effects of free phytohormones extracts added to MS medium on potato (*Solanum tuberosum*) propagation after 4 weeks of incubation.

Amount of phytohormones extracts	*Ceratophyllum demersum*		*Egeria densa*	
	Number of nods	Length of plant (cm)	Number of nods	Length of plant (cm)
0.0 (control)	5.3	4.2	5.3	4.5
25 µl/l	7.0	6.1	6.3	4.5
50 µl/l	7.0	7.0	7.3	5.5
100 µl/l	6.5	6.2	7.6	6.0
Means	6.4	5.8	6.6	5.1
LSD at P ≤ 0.05	0.4	0.4	0.5	0.3

DISCUSSION

The comparison between the content of endogenous phytohormones in *C. demersum* and *E. densa* show variation at the levels of phytohormones between them. It is worth mention that the endogenous levels of several phytohormones in aquatic macrophytes are variable during the annual cycle of the plants (Best, 1982). Aquatic macrophytes are plants that have adapted to living in watery environments.In spite ofthe numerousenvironmental problemsca used by these plants, butthereare many solutions that make these plantseconomically beneficial. Of these solutionsisusedas feed forpoultry and livestock (Jassim *et al*., 2006;Easley and Shirley, 1974), and use it as fertilizer to increase the fertility of the land or to enhance the growth of plants, because several authors reported that aquatic plants considered as perfect sources for ash, proteins and other organic materials (Edwards, 1981).

In presented study, greater effectiveness of medium supplemented with aquatic plant extracts than un supplemented medium in promoting callus formation of black henbane and enhancing growth of potato could be explained that these plants have a good amounts of plant hormones. Thus, the addition of aquatic plant extracts to culture media is beneficial and suitable measure to improve *in vitro* culture media which used for commercial production.There are several reports on successful using plant extracts as an alternative to plant growth regulators.

One of these report is that of Ibrahim *et al*., 2008,who succeeded in growingsoya bean, potato and wheat plants in *in vitro* culture by adding the *Glycyrrhiza glabra*callus extracts to their culture medium as an alternative to plant growth regulators. Also, occurrence of auxin and gibberellin-like substances in coconut milk have been roported by (Dix and Van Staden, 1981). So, many researchers founed that coconut milk could be used to initiate and induce the growth *in vitro* plant tissue cultures(George, 200; Thorpe *et al*., 2008). A wide variety of organic extracts are now commonly added to culture media and are often reported to promote growth, these include tomato juice,ground banana, orange juice, carrot juice, malt extract, yeast extract, leaf extracts, casein hydrolysate, (Puchooa and Ramburn, 2004; Dodds and Roberts, 1985;Saad and Elshahed, 2012). likewise, Straus (Straus, 1960) has shown that complexorganic extracts function by supplying a form of organic nitrogen content (a mixture of amino acids.

In our study, the Analysis of phytohormones showed that IAA, $GA_3$and ABA are produced with good amounts in aquatic plants. So, it is believed that success of using these plants as an alternative to plant growth regulators could be reducing the cost of *in vitro*plant tissue cultures, or can be used as green manure or biofertilizers to add phytohormones and organic matters to thesoil.

ACKNOWLEDGEMENT

This research was carried out in biotechnology research center at Al Nahrain University. We thank our colleagues from plant biotechnology department who provided insight and experience that greatly supported the research.

REFERENCES

Al-Daody AC, and Al-Mandeel FA, (2012). A morphological and chemical study for *Ceratophyllum demersum* L. growing in some ponds near Mosul forests. Tikrit Journal of Pure Science، 17(3): 1-6.

Aziz KA, (2009). Effect of different levels of pH on growth parameters of Hornwort *Ceratophyllum demersum* L.Journal of Al-Qadisiyah for Pure Sci.,14(1): 1-10.

Best EP, (1982). Hormonal interactions in Ceratophyllum demersum. Aquatic Botany, 13: 87-95.

Bowmer kH, Jacobs SWL, and Sainty GR, (1995). Identification, biology and management of *Elodea canadensis*, Hydrocharitaceae. Aquatic Plant Management Society, Inc., 33:13-19.

Dhir B, (2013). Phytoremediation: Role of aquatic plants in environmental clean-up. Springer Science and Business Media, - Science, 21-54.

Dix L, and Van StadenJ, (1981). Auxin and gibberellin-like substances in coconut milk and malt extract. Plant Cell, Tissue and Organ Culture, 1(1): 239-246.

Dodds JH, and Roberts LW, (1985). Experiments in plant tissue culture. 2nd ed. Cambridge Univ. Press, New York, 40-42.

Easley JF, and Shirley RL, (1974). Nutrient elements for livestock in aquatic plants. Hyacinth Control Journal, 12: 82-84.

Edwards P, (1981). Food potential of aquatic macrophytes (as human food, livestock feed and fertilizer; study conducted in Thailand). *ICLARM* (International Center for Living Aquatic Resources Management) Studies and Reviews (Philippines). http://pdf.usaid.gov/pdf_docs/PNAAJ073.pdf

Ergün N, Topcuoúlu SF, and Yildiz A, (2002). Auxin (indole-3-acetic acid), gibberellic acid (GA3), abscisic acid (ABA) and cytokinin (zeatin) production by some species of mosses and lichens. Turkish J. Botany, 26(1):13-18.

Foroughi M, Najafi P, and Toghiani S, (2011). Trace elements removal from waste water by *Ceratophyllum demersum*. Journal of Applied Sciences and Environmental Management, 15(1): 197–201.

George EF, Hall M A, and De Klerk G J, (Eds.). (2008). Plant propagation by tissue culture. 3rd Ed. Vol.1. the background. Springer Sci. and Business Media. 15-20.

HelfrichL A, Neves RJ, Libey G, and Newcomb T, (2000). Control methods for aquatic plants in ponds and lakes.Virginia Cooperative Extension, Virginia Tech, Virginia State University. https://pubs.ext.vt.edu/420/420-251/420-251.html

Jacobs WP, Falkenstein K, and Hamilton RH,(1985). *Nature and amount of auxin in algae*. IAA from extracts of *Caulerpa paspaloides* (Siphonales). Plant Physiology, 78:844-848.

Jassim JM, Moss RK, and Abbas RJ, (2006). The response of broiler hybrid to replacement two types of aquatic plants (*Vallisneria spiralis* and *Bacopa monniera*) in diets - nutritive value, chemical composition of plants. Basrah Journal of Agricultural Sciences, 19(1): 1-11.

Ibrahim KM, Abd AS, Tawfiq AA, Al-Samarai KW, and Al Ani NK. (2008). The possibility of using *Glycyrrhiza glabra* callus extraction as an alternative to plant growth regulators in plant tissue culture experiments, Journal of Biotechnology Research Center, 2(1): 71-80.

Karadeniz AS, Topcuog˘lu F, and Inan S, (2006). Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World Journal of Microbiology and Biotechnology, 22(10): 1061–1064.

Kelen M, Demiralay EC, Sen S, and ¨Ozkan G¨, (2004). Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri x Vitis rupestris*) and rose oil (*Rosa damascena* Mill.) by reversed phase liquid chromatography. Turkish Journal of Chemistry, 28: 603-610.

Korasick DA, Enders TA, and Strader LC, (2013). Auxin biosynthesis and storage forms. Journal of Experimental Botany, 64(9): 2541–2555.

MacMillan J, (2002). Occurrence of gibberellins in vascular plants, fungi and bacteria. Journal of Plant Growth Regulation, 20: 387-442.

Módenes AN, de Abreu Pietrobelli JM, Espinoza-Quiñones FR, (2009). Cadmium biosorption by non-living aquatic macrophytes Egeria densa. Water Science and Technology, 60(2): 293-300.

Muller JL, (2011). Auxin conjugates: their role for plant development and in the evolution of land plants. Journal of Experimental Botany, 62(6): 1757–1773.

Murashige T, and Skoog F, (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum, 15(3): 473-497.

Puchooa D, and RamburnR,(2004). A Study on the use of carrot juice in the tissue culture of Daucus carota. African Journal of Biotechnology, 3(4): 248-252.

Rangaswamy V, (2012). Improved production of gibberellic acid by *Fusarium moniliforme.* Journal of Microbiology Research, 2(3): 51-55

Saad AIM, and Elshahed AM, (2012). Plant tissue culture media, recent advances. In plant *in vitro* culture, Dr. Annarita Leva (Ed.), ISBN: 978-953-51-0787-3, InTech, DOI: 10.5772/50569

Schmidt JC, and Kannenburg JR, (1998). How to identify and control water weeds and algae: 5th edition. Applied Biochemists. Germantown, WI. 132p. http://www.lonza.com/~/media/Files/water-treatment/Weedbook.ashx?la=en

Straus J, (1960). Maize endosperm tissue grown *in vitro*. III. Development of a synthetic medium. American Journal of Botany, 47(8): 641-647.

Tawfiq AA, (2010). Estimation levels of Indol acetic acid (IAA) and Gibberellic acid (GA_3) from dry bakery yeast *Saccharomyces cereviciae*. Journal Biotechnology Research Center, 4(2): 94-100.

Thorpe TA, Stasolla C, Yeung EC, de Klerk G-J, Roberts A, and George EF, (2008). The components of plant tissue culture media II: Organic additions, osmotic and pH effects, and support systems. In George, E.F., Hall, M.A., de Klerk G-J., eds., Plant Propagation by Tissue Culture, 3rd Edition, Vol. 1. Springer, Dordrecht, 115-173.

Ünyayar S, Topcuoglu SF, Ünyayar A, (1996). A modified method for extraction and identification of indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin produced by *Phanerochaete chrysosporium* ME446. Bulgarian Journal of Plant Physiology, 22(3–4): 105–110.

.