Effect of growth regulator in micropropagation of
*Catharanthus* roses in vitro

Sarab Abdulhadi Mohamed Hussain Al-Mukhtar ¹ and Moslim Abd Ali ²

¹Horticulture and landscape department, College of Agriculture, University of Kerbala,
²Horticulture and landscape department, College of Agriculture, University of Kufa

Abstract. The experiment was carried out in the plant tissue culture laboratory of the Horticulture and landscape department – College of Agriculture University of Kerbela during 2017-2018 to investigate the effect of growth regulators in the micropropagation of the *Catharanthus roseus* *iv vitro*. The culture of the branches of the *Catharanthus roseus* plants were obtained by cultivating the shoot tips of the branches of 1 cm length obtained from the developing plants growing in the field. After sterilization and culturing on MS medium supplied with different concentrations (0, 1, 2 and 3) of BA and Kin in separate experiment with steady concentration of 0.5 mg / l of IAA. The results showed a difference in the rate of studied characters according to the type and concentration of cytokines used. The BA in the concentration of 3 mg / L results were significantly higher in the studied traits, including the number and length of the vegetative branches and the soft and dry weight of the vegetative system, it achieved a rate of (21.66 branch / plantlet, 7.00 cm, 1.40 g, 0.71 g) respectively, while the Kin at the same concentration achieved the highest rate of studied traits of (15.66 branch / plantlet, 5.66 cm, 0.93 g, 0.40 g) respectively. The results of the study also showed a difference in the rate of studied traits, which included the number and length of roots and the soft and dry weight of the root total, depending on the type and concentration of auxien used. The IBA result was superior at 1.5 mg / L and with a constant concentration of 0.5 mg / l BA in achieving the highest rate of (36.33 root / shoot, 6.43 cm, 0.35 g, 0.23 g) respectively, while the NAA at the same concentration achieved the highest rate of studied traits (22.00 root / plant fraction, 2.53 cm, 0.21 g, 0.12 g), respectively, and the percentage of success of acclimation plants was 90%.

Introduction

The plant of *Catharanthus roseus* belongs to the Apocynaceae family (1), whose scientific name was *Vinca rosea*, then it was was replaced to what it is now by the botanical class George Don(2). It is called Vinca in Arabic and Periwinkle in English(3),Its native country is the state of Madagascar and its cultivation is successful in many tropical regions of the world and grows wildly in different parts of the Mediterranean countries and is a common ornamental plant cultivated in Iraq(4).The plant is classified as a perennial herbaceous group, planted in ponds and flower beds. It multiplies with seeds and stems and blooms in most months of the year, and the flowering colors range from white to red, with a height of 30-
The leaves are rectangular with a round top. The flowers are in the shape of the eye of the cat, hence the name of Al-venca. The fruit contains 10-20 seeds with a black cover length of 2-3 mm. The vinca plant has a great medical and economic importance as a source of production of more than 200 alkaloids of indole atropine alkaloids and their derivatives, the most important of which are Vincristine and Vinblastine compounds, which are important in the treatment of cancerous tumors, as well as its aesthetic value and as a flowering plant. Because of the importance of plant, many botanists have sought to provide the latest innovations in the field of propagation and laboratory techniques to improve agricultural production with new visions and innovative solutions, and in recent years, interest has increased in the practical applications of biotechnology. Tissue culture is one of the most important and most advanced technologies, which proved to be an effective and useful tool in the rapid production of these substances and compounds throughout the year without limiting the planting season, as well as reducing the areas required for cultivation as well as the high purity of the materials formed when compared to those manufactured. Plant growth regulators are used in propagation stages, which are natural or synthetic organic compounds that play a major role in determining the desired target of tissue culture, especially in vitro modulation processes, and are effective in very small quantities, the most common in tissue culture are Auxins and Cytokinines and to a lesser extent Gibberellins. The Auxins are an indoli acid with an unsaturated ring nucleus or they may be derivatives of these acids. Auxins are responsible for elongation of cells and formation of roots. Cytokinines are nitrogenous organic bases with high molecular weights that encourage cells to divide and differentiate and work better with Auxins. The growing plants in the tissue culture tubes are characterized by their feeding on media used and do not need photosynthesis process. Moreover, these plants are characterized by the reduction of the layer of cuticle and the absence of the pores in their natural function due to the growth of plants in a high humidity environment, as well as the lack of vascular association between vegetative and root systems. Overcoming these constraints requires the creation of certain conditions for the purpose of transition to free living conditions and self-nutrition and the development of the layer of cuticle and its integration on the leaves and stimulate the pores to carry out the process of opening and closing normally. Removing some leaves on the plant reduces the area exposed to air, as well as putting the transparent plastic covers that allow light and air to penetrate through small holes to maintain the moisture of the surrounding area. Therefore, the aim of this research was to study the effect of different types and concentration of growth regulators added to the MS medium for the propagation of the Periwinkle for its medicinal importance as well as being an ornamental plant used to decorate the gardens.

Materials and Methods

The experiment was carried out in the plant tissue laboratory of the Department of Horticulture and Landscape Engineering – College of Agriculture, University of Kerbala in 2017-2018. The shoot tips of the branches of a length of 1 cm obtained from the growing plants in the College field were used for the beginning of propagation. The explants were washed with flowing water several times, then with 70% alcohol for 2 minutes, then with distilled water, then the explants were sterilized in sodium hypochlorite solution at 1.5% concentration for 10 minutes. Followed by washing with distilled sterilized water for three times at a rate of 5 minutes / time to remove traces of sterile material, then they were placed in glass bottles containing MS medium with the concentrations of (0, 1, 2, 3) Mg / L of BA and Kin with a constant concentration of 0.5 mg / L of IAA in a separate experiment with 10 replicates per each concentration. The plants were incubated in the growth chamber at a temperature of 25 ° C ± 2 ° and the intensity of lighting 1000 lux for 16 hours / day. The study parameters included counting the number of branches and their length and the soft and dry weight of the vegetative group after four weeks of planting to know the best cytokinin and the best concentration for the emergence of branches from shoot tips. The multiplying branches were then transferred to the medium of the rooting, which included the MS medium.
(14), equipped with different concentrations of IBA (0.5, 1, 1.5) mg / L and NAA (0.5, 1, 1.5) Mg / BA in ten replicates per each concentration. The seedlings were incubated under the same conditions to encourage rooting. Data on the number and length of roots and the soft and dry weight of the root total after four weeks of planting were recorded.

Acclimation of plantlets
The plantlets were washed with water to remove the residues of the medium, which is a good source of microorganisms. The roots of the plants were soaked with Benlate 0.1% for 10 seconds to reduce the risk of fungal infections. The plantlets were placed in a solution containing a quarter of the strength of the MS salts for a period of seven days to hardening them, Then sterilize the mixed soil and the peatmoss in the autoclave for 20 minutes at a temperature of 121° c, cooled and mixed by (1:2). The plantlets were then planted in the pots and were washed with water and covered with glass coverings to maintain an appropriate level of moisture. The covers were taken away gradually, and observations about the success of acclimation of the plants after two weeks of planting were recorded.

Statistical Analysis
All experiments were performed using the Completely Randomized Design (CRD) . The results were analyzed using the statistical program (15). The means were compared using the Least Significant Difference (LSD) and the probability level was 0.05.

Results and discussion
Effect of Cytokinines concentrations in the rate of vegetative characteristics
The results of Table 1, 2, 3 and 4 indicate significant differences between the BA and Kin concentrations in the length and number of vegetative branches and the soft and dry weight of the vegetative group. The concentration gave 2 mg / L the highest weight of the mentioned traits, 7 cm and 21.6 branches, 40 and 0.71 g, respectively. The response was then decreased at the concentration of 3 mg / L, with a rate of 5 cm, 18.3 branch, 1.08 and 0.57 g respectively. The results showed that there was a significant increase in the rate of vegetative characteristics studied by increasing the concentration of Kin added to the MS medium for multiplication. The concentration of 3 mg / L was significantly higher in the length and number of branches, as well as in the soft and dry weight of the vegetative group of 4.8 cm, 15.6 branches, 0.93 and 0.41 g respectively, while the treatment of the comparison for both treatments showed the lowest rate of the same studied qualities amounted to 1.5 cm and 1 branch and 0.12 and 0.08 g, respectively. As observed from the results reported in tables 2 and 3, the concentrations of the BA are significantly higher in the above-mentioned traits on the Kin concentrations. The reason may be due to the structural nature of the BA, which is linked to its lateral chain, a benzyl ring with three double bonds, making it superior to the rest of the Cytokinines. The double bonds increase the efficiency and activity of the compound, thus making the BA more effective in dividing the cells, size and differentiation. On the process of growth and development, making it one of the most prominent cytokines used in propagating many plant species (16). Many researchers have reported that the use of Cytokinines, particularly BA, in tissue culture is due to the fact that they are stable compounds for not being easily decomposed and highly efficient in breaking capillary sovereignty by detecting and expanding vessels for both wood and bark, preventing chlorophyll degradation, and the stimulation of cells and the formation of nuclear acids (17, 18 and 19). This is in line with what (20) found in the development of Cichorium intybus plant plantations by cultivating the branches of sterile seedlings on the MS medium, which was synthesized with different combinations of growth regulators BA, Kin, TDZ, IAA, NAA and IBA. From 2 mg / 1 BA and 0.2 mg / 1 IAA, which gave more than 130 branches within 10 weeks. also agreed with (21) when they tested a number of growth regulators with different concentrations. They removed the shoot tips of the sterile Digitalis davisiana
seedlings and planted them on MS medium with different concentrations of 2,4-D, BA, Kin, and TDZ. In the light of combinations of growth regulators, the researchers found that the MS medium containing 2 mg / L BA with 0.2 mg / L 2.4-D was the best for shoots culture.

Table (1) Effect of BA and Kin concentrations (mg / l) and 0.5 mg / l IAA in branches length rate (cm) after 1 month of planting

| Men of treat. | 3   | 2   | 1   | 0   | Con. Treat. |
|---------------|-----|-----|-----|-----|-------------|
| 4.37          | 7.00| 5.00| 4.00| 1.50| BA          |
| 3.37          | 5.66| 3.66| 2.66| 1.50| Kin         |
| 0.61          | 1.22| 3.33| 1.50|     | L.S.D(0.05) |
| 6.33          | 4.33|     |     |     | Men of con. |
|               |     |     |     |     | L.S.D(0.05) |

Table (2) Effect of BA and Kin concentrations (mg / l) and 0.5 mg / l IAA in branches number rate after one month of planting

| Men of treat. | 3     | 2     | 1     | 0     | Con. Treat. |
|---------------|-------|-------|-------|-------|-------------|
| 13.9          | 21.66 | 18.33 | 14.66 | 1.00  | BA          |
| 10.42         | 15.66 | 14.00 | 11.00 | 1.00  | Kin         |
| 0.70          |       | 1.41  |       |       | L.S.D(0.05) |
| 18.66         | 16.17 | 12.83 | 1.00  |     | Men of con. |
|               |       |       |       |     | L.S.D(0.05) |

Table (3) Effect of BA and Kin concentrations (mg / L) and 0.5 mg / l IAA in wet weight rate (g) after 1 month of planting

| Men of treat. | 3  | 2  | 1  | 0  | Con. Treat. |
|---------------|---|----|----|----|-------------|
| 0.87          | 1.40| 1.08| 0.88| 0.12| BA          |
| 0.67          | 0.93| 0.86| 0.79| 0.12| Kin         |
| 0.06          | 0.13|    |    | 0.12| L.S.D(0.05) |
| 1.17          | 0.97| 0.83|    |    | Men of con. |
|               |    |    |    | 0.09| L.S.D(0.05) |

Table (4) Effect of BA and Kin concentrations (mg / L) and 0.5 mg / l IAA in dry weight rate (g) after 1 month of planting

| Men of treat. | 3  | 2  | 1  | 0  | Con. Treat. |
|---------------|---|----|----|----|-------------|
| 0.43          | 0.71| 0.56| 0.37| 0.08| BA          |
| 0.28          | 0.40| 0.36| 0.27| 0.08| Kin         |
| 0.03          | 0.06|    |    |    | L.S.D(0.05) |
| 0.55          | 0.47| 0.32|    | 0.08| Men of con. |
|               |    |    |    | 0.04| L.S.D(0.05) |
Effect of auxien concentrations in average Characteristics of total root.

The results of Table 6, 7, 8 and 9 indicate significant differences between IBA and NAA concentrations in the length and number of roots and the soft and dry weight of the root total. The concentration of 1.5 mg / L gave the highest weight of the studied characters with 6.43 cm, 36.3 root / plant, 0.35 and 0.22 g, respectively. As shown in Table 5 data, there was a significant increase in the rate of vegetative traits studied by increasing the concentrations of NAA added to the MS medium for propagation. The concentration of 1.5 mg / L was significantly higher in the length and number of roots as well as in the soft and dry weight of the root mass of 2.50 cm 22.00 root / plant and 0.21 and 0.11 g respectively, while the comparison treatment for both treatments had little response to the origin of the adventitious roots. As can be seen from above, IBA is superior to 1.5 mg / L in the above mentioned properties, the reason may be that IBA is one of the auxien cheerleader for division and elongation of cells, thus stimulating the formation of roots from the cut areas, and increasing concentration of growth regulators increases the number of roots and their lengths close to the optimum concentration (22). This is in line with (23) when cultivating the *Digitalis lanata* branches on MS medium with 1.5 mg / L IBA with 0.1 mg / L BA, the highest root count rate of 11.2 root / branch with a mean length of 10.24 cm after 12 weeks of agriculture were obtained, as well as with (24) when cultivating *Euonymus fortunie* branches on MS medium with 1.5 mg / L IBA with 0.2 mg / L BA resulted in the highest root rate of 3.6 root / branch and length 10.1 cm after four few weeks of culturing. Also with (25) when planting the shoot tip of the plant branches of *Stevia rebaudiana* on the MS medium, equipped with 1.5 mg / l IBA and 0.1 mg / l NAA gave the highest rate of root number of 35 root / branch and a length of 4.2 cm and a dry weight of 1.58 g after four weeks of planting. And with (26) when cultivating the shoot tip of *Ocimum basilicum* on MS medium supplemented with 1.5 mg / l IBA obtained root rate of 3.5 root / branch and length 2 cm after four weeks of planting. As well as with (27) when cultivating the branches of the *Toddalia asiatica* on the MS medium supplemented with 1.5 mg / L IBA obtained average number of roots was 28.5 root / branch and 3.5 cm length after four weeks of planting.

In addition, these data showed IBA superiority compared with NAA in the rate of qualities mentioned previously, the reason for this efficiency of IBA in the rooting process may be due to its relatively high stability due to its non-influence on the enzymes responsible for the destruction of the auxien (28). This is consistent with (29) when rooting the branches of the *mintha piperita*, and with (30) when rooting the branches of the plant *Digitalis purpurea*, and with (31) when rooting the branches of *Stevia rebaudiana*, and with (32) when rooting the vegetative branches of the medicinal plant *Toddalia asiatica*, and with (33) when rooting the shoot tip of branches of *Digitalis lanata*, who confirmed that the use of the IBA was better than the IAA and NAA.

**Table (5)** Effect of IBA and NAA concentrations (mg / l) with 0.5 mg / L BA in root length rate (cm) after 1 month of planting

| Men of treat. | 1.5 | 1.0 | 0.5 | 0       | Con. Treat. |
|--------------|-----|-----|-----|--------|-------------|
| 3.47         | 6.43| 4.63| 2.83| 0.00   | IBA         |
| 1.38         | 2.53| 1.83| 1.16| 0.00   | NAA         |
| 0.20         | 0.40|     |     | 0.28   | L.S.D(0.05) |
| 4.48         | 3.23|     |     | 0.00   | Men of con. |

L.S.D(0.05)
### Table 6
Effect of IBA and NAA concentrations (mg/l) with 0.5 mg/L BA in root number rate (cm) after 1 month of planting

| Men of treat. | 1.5  | 1.0  | 0.5  | 0.0  | Con. Treat. |
|---------------|------|------|------|------|-------------|
|               | 20.91| 36.33| 28.66| 18.66| 0.00        |
|               | 13.66| 22.00| 19.33| 13.33| 0.00        |
|               | 0.99 |      | 1.99 |      | L.S.D(0.05) |
|               |      | 29.16| 24.00| 16.00| 0.00        |
|               |      |      |      |      | Men of con. |
|               |      |      |      | 1.41 | L.S.D(0.05) |

### Table 7
Effect of IBA and NAA concentrations (mg/l) with 0.5 mg/L BA in wet weight rate (cm) after 1 month of planting

| Men of treat. | 1.5  | 1.0  | 0.5  | 0.0  | Con. Treat. |
|---------------|------|------|------|------|-------------|
|               | 0.18 | 0.35 | 0.22 | 0.18 | 0.00        |
|               | 0.13 | 0.21 | 0.18 | 0.12 | 0.00        |
|               | 0.01 |      | 0.03 |      | L.S.D(0.05) |
|               |      | 0.28 | 0.20 | 0.15 | 0.00        |
|               |      |      |      | 0.02 | L.S.D(0.05) |

### Table 8
Effect of IBA and NAA concentrations (mg/l) with 0.5 mg/L BA in dry weight rate (cm) after 1 month of planting

| Men of treat. | 1.5  | 1.0  | 0.5  | 0.0  | Con. Treat. |
|---------------|------|------|------|------|-------------|
|               | 0.11 | 0.23 | 0.14 | 0.09 | 0.00        |
|               | 0.08 | 0.12 | 0.08 | 0.07 | 0.00        |
|               | 0.01 |      | 0.03 |      | L.S.D(0.05) |
|               |      | 0.17 | 0.11 | 0.08 | 0.00        |
|               |      |      |      | 0.02 | L.S.D(0.05) |

**Acclimatization of plants**

The germination process was carried out in the MS medium containing 1.5 mg/L IBA for four weeks. The mix soil and the Patmos were sterilized in the autoclave and mixed in percentages with 1: 2. The plants were then planted in the pots and were irrigated with faucet water as in fig (1).
The method of acclimatization of the plants resulting from tissue culture and transfer to the soil proved the importance of washing them from the food medium under the water and immersed in the fungicide Benlate at a concentration of 2.5% for ten seconds to reduce the risk of pathogens, as that, putting the plants in a solution containing a quarter of the strength of MS salts for seven days had a significant role in the hardening of the plants before transferring them to the soil and thus reduce the losses, and that the coverage of the plants with a glass cover helped to maintain the required moisture within the surrounding cover with the gradual lifting and then coverage because it is characterized by the absence of the cuticle layer that covers the surface of the leaves, as well as the absence of holes function naturally for the growth of the plants in a high humidity environment, and after two weeks of cultivation, lifting the cover of the plants to live under the conditions of the field and the percentage of success of acclimatization amounted to 90%.

References

1) Fattorusso, E. and T. Scafati.(2008). Modern alkaloids structure. WILEY- VCH Verlage cmbH. and KGAA Co., Germany: 28-41.
2) Van Der Heijden, R.; D.I.Jacobs; W.Snoeijer; D. Hallard and R.Verpoorte.(2004). The Catharanthus alkaloids: pharmacognosy and biotechnology. Curr. Med. Chem., 11(5): 607-628.
3) Al-Nuaimi, Jabbar Hassan (2010). Treatment with trees, fruit bushes Forestry. Al-Hawraa Printing and Advertising House, Mutanabi Street, Baghdad.
4) BAKRUDEEN, A.A; G. S. Shanthi; T. Gouthaman; M.S.Kavitha and M.V.Rao. (2011). *In vitro* micropropagation of *Catharanthus roses* – an anticancer medicinal plant. Acta Botanica Hungarica 53(1–2), pp. 197–209.

5) Aslam, J.; H.K.Sheba; H.S.Zahid; F.Zohra; M.Mehipara and Mukthar. (2010). *Catharanthus roseus* Don. L an important Drug it is application and production, pharmacie global(IICP), 4:12.

6) Arab Organization for Agricultural Development - League of Arab States. (1988). Medicinal, aromatic and toxic plants in the Arab world, Khartoum.

7) Simpson, M.G. (2006). Plant systematic. Elsevier, Amsterdam, The Netherlands.

8) Lila, M. (2005). Valuable secondary production from in vitro culture. Chapter 24. Secondary production *in vitro*, CRC Press, LLC.

9) Aliyu, O.M. and J.A.Awopetu.(2005). *In vitro* Regeneration of Hybrid Plantlets of Cashew (*Anacarium occidental L.*) Through Embryo Culture, African J. Bioth, 4(6): 548- 553.

10) Mohammed, Abdul Muttilah Sayed and Omar, the missionary Saleh. (1990). The main concepts in the cultivation of cells, tissues and organs of the plant. Directorate of the House of Books for Printing and Publishing / University of Mosul, Iraq.

11) Mohamed, Abdel-Azim Kazem and Al-Yunis, Moayed Ahmed (1991). Fundamentals of Plant Physiology. Part III, Faculty of Agriculture, University of Baghdad.

12) Trigiano, R.N. and D.J. Gray.(1996). Plant Tissue Culture Concepts and Laboratory Exercises. CRC. Press. Inc.- PP:11-17.

13) Grout, B.W.W . and M.J. Aston.(1977).Transplanting of cauliflower plant regenerated from meristem culture.I.Water loss and water transfer related to changes in leaf wax and to xylem regeneration.Hort.Res.,17:1-7.

14) Murashige, T. and F. Skooge.(1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.

15) SAS.SAS/STAT Users Guide for personal compu-ter, SAS Institute Inc, Cary, N.C. USA (2002).

16) Abdul, Karim Saleh. (1987). Plant growth regulators. part One. University of Salahaddin. Ministry of Higher Education and Scientific Research. Iraq.

17) Abu Zaid, Al-Shahat Nasr (2000). Plant Hormones and Agricultural Applications, Dar Al Arabiya Publishing and Distribution, Second Edition, Nasr City, Egypt.

18) Mok, M.C.; R.C.Martin and W.S.Mok.(2000). Cytokinins: Biosynthesis. Metabolism and Perception. *In vitro* Cell.Dev.Biol. 36: 102-107.

19) Schmulling, T.(2004). Cytokinins in Encyclopedia of Biological Chemistry. Academic Press/ Elsevier Science.

20) Yucesan,B.; A.Turker and E.Gurel.(2007). BA-induced high frequency plant regeneration through multiple shoot formation in witloof chicory (*Cichorium intybus L.*). Plant Cell Tiss Organ Cult 91: 243–250.

21) Gurel, E.; B. Yucesan; E. Aglic; S. K. Verma and M. Sokmen. (2011). Regeneration and cardiotonic glycoside production in *Digitalis davissiana* Heywood (Alanya foxglove) . Plant Cell, Tiss. and Org. Culture. 104 217-225.

22) Hartmann, H.T.; D.E.Kester and R.L.Geneve.(2002). Plant Propagation Principles and Practices. 7th.ed. Perntice Hall. Inc. New Jersey, USA.

23) Fatima, Z.; Abdul Mujib; S. Fatima; A. Arshi and S. Umar (2009). Callus induction biomass growth and plant regeneration in *Digitalis lanata* Ehrh.: influence of plant growth regulators and carbohydrates. Turk J Bot 33. 393-405.

24) Aiqin, S.; S.Zhenyuan and Z.Liangun.(2009). High efficient regeneration *in vitro* hypocotyls of *Euonymus fortunei* var. radicans. Scientia Silvae Sinicae.45.(2).136-141.
25) Tawar, A.S.; D.S.Mukadam and S.D.Tawar.(2010). Comparative studies of in vitro and in vivo grown plants and callus of Stevia rebaudiana. International Jornal of Integrative Biology. Vol.9, No.1, 11.

26) Shahzad, A.; M. Faisal; N. Ahmad; M. A. A. Alatar and A. A. Hend. (2012). An efficient system for In vitro multiplication of Ocimum basilicum through node culture. African Journal of Biotecnology. 11(22): 6055-6059.

27) Praveena,C. and C.Veeresham.(2014). Multiple shoot regeneration and effect of sugars on growth and nitidine accumulation in shoot culture of Toddalia asiatica. Original article, Vol.10, Issue:39, Page: 480-486.

28) Nissen,S.J. and E.G.Sutter.(1990). Stability of IAA and IBA in nutrient medium to several tissue culture procedures. Hort Science. 25(7): 800-820.

29) Sunandakumari, C.; K.P.Martin and P.V.Madhuhsoodanan. (2004). Rapid axillary bud proliferation and ex vitro rooting of herbal spice. Mentha piperita L. Indian Jornal. Biotechnology. Vol.3, pp:108-112.

30) Awad, Z.J. and E.H.Al- Khatieb.(2005). Using tissue culture technique for the production of cardiac glycosides from root of Digitalis purpurea L. Plantlets. Department of pharmacognacy, college of pharmacy. University of Baghdad, Baghdad, Iraq.

31) Tawar, A.S.; D.S.Mukadam and S.D.Tawar.(2010). Comparative studies of in vitro and in vivo grown plants and callus of Stevia rebaudiana. International Jornal of Integrative Biology. Vol.9, No.1, 11.

32) Praveena,C. and C.Veeresham.(2014). Multiple shoot regeneration and effect of sugars on growth and nitidine accumulation in shoot culture of Toddalia asiatica. Original article, Vol.10, Issue:39, Page: 480-486.

33) Al-Mukhtar, S. Abdulhadi. (2015). Micro propagation and production of cardiac glycoside compounds from Digitalis lanata In vitro. P.H.D. Thesis , College of Agriculture, University of Baghdad.