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Effect of Different Plant Growth Regulators on the *In Vitro* Induction and Maintenance of Callus from Different Explants of *Hyoscyamus muticus* L.

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ABSTRACT

The effects of Murashige and Skoog basal medium even alone or supplemented with different concentrations and combinations of plant growth regulator on the maximum callus induction *in vitro* from different explants of *Hyoscyamous muticus* L. (family Solanaceae) were studied. After two months 6-benzylaminopurine in combination with α-naphthalene acetic acid recorded to be the most effective for *in vitro* callus production in this study. Root explants recorded to be the most effective for the maximum callus production *in vitro* followed by leaf explant. Maximum mean fresh weight of callus formed per explant (31.56 mg) with a maximum dry weight (1.04 mg) were obtained on MS medium supplemented with 1.0 mg/l 6-benzylaminopurine in combination with 0.5 mg/l α-naphthalene acetic acid using root explants after two months.
KEYWORDS: Solanaceae, *Hyoscyamus muticus* L., *in vitro*, callus induction, plant growth regulators, explant.

INTRODUCTION

H. muticus L. is one of the medicinal herbs grown in Egypt (family Solanaceae). Solanaceous plants, such as genus Hyoscyamus were regarded as rich sources of natural secondary metabolites that are called tropane alkaloids. All parts of the plant under investigation contain tropane alkaloids (hyoscyamine, and traces of hyoscine and atropine) and these alkaloids have a good deal of medicinal importance **[1].**

Callus induction and production of secondary metabolites are considered to be one of the most important applications of plant tissue culture *in vitro* and to be an effective alternative method for production of phytochemical compounds **[2-5].**

Plant growth regulators and type of explant play an important role in the process of callus induction *in vitro* because each species and each explant requires it is own combination and concentration of exogenous application of plant growth regulators for *in vitro* callus production **[6-11].**

In the present study, an attempt is done to investigate the effect of different combinations and concentrations of plant growth regulators for the optimal callus production from different explants of the *H. muticus* L. *in vitro,* in preparation for use in cell suspension culture and production of secondary metabolites.

MATERIAL AND METHOD

Plant material: seeds of *H. muticus* L. seeds were obtained from Alazhar University, Faculty of Agriculture. Firstly, seeds were thoroughly washed and left for an hour under running water to remove dirt. Seeds were treated within laminar cabinets with 1.5% sodium hypochlorite for 10 minute then washed with sterilized distilled water for 6-7 times **[12]**. Serialized seeds were cultured on MS medium without any additional plant growth regulators and incubated for germination under controlled conditions. The 30 days old seedlings (fig.1) were used for explant preparation.

Callus production: Callus cells have been initiated from different explant types (leaf, nodal segment and root) using MS **[13]** basal media even alone or fortified with different concentrations and combinations of auxins (NAA & 2,4-Dichlorophenoxy Acetic Acid (2,4-D)) and cytokinins (BAP& KIN). The 30 days old seedlings were cut into 1 cm segments leaf, nodal segment and root explants, which were then transferred to the culture media. Three explants per jar were grown on the used MS solid media. Media were solidified using phytagel and PH value was adjusted to 5.7± 0.1. Results were

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taken after two menthes to show percentage of callus induction, mean fresh weight of callus per explant, and mean dry weight of callus per explant. Data were analyzed using analysis ANOVA. The differences among means for all treatments were tested for significance at 5% level by using **[14]** new multiple range tests as described by **[15]**. Means followed by the same letter are not significantly different at $p \leq 0.05$.

Fig.1: 30 days old seedlings

RESULTS AND DISCUSSION

Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transferring onto growth medium supplemented with suitable hormones **[16-17].** Different growth hormones are used to promote callus induction and development from young tissue explants.

1-Effect of BAP and NAA on callus induction from different explants (leaf, nodal segment and root):

In this experiment callus induction from different explants (leaf, nodal segment and root) were tested by inoculating them on MS medium supplemented with different concentrations of BAP (0.5, 1.0, 2.0 and 3.0) even alone or in combination with different concentrations of NAA (0.5, 1.0, 2.0 and 3.0) along with MS media free of plant growth regulators as control. Data were recorded after two months as showing in table; 1, 2 and 3. To determine the optimal concentration of plant growth regulators for the highest production of callus, various combinations of auxin: cytokinin were tested, among all the tested combinations, BAP and NAA were better in terms of high frequency to induce callus production from different explants under investigation. On the other hand, MS media supplement with BAP alone either or free of plant growth regulators cannot induce any callus formation with leaf or nodal segment explants. Root explants showed callus formation on MS media supplemented with BAP alone or in combination with NAA and cannot produce callus on MS media free of plant growth regulators.

The most effective results were obtained with root explants, after two months. The highest mean fresh weight of callus (31.56 mg/explant) with the highest mean dry weight of callus (1.04 mg/explant) were obtained from root explants on MS medium fortified with 1.0 mg/l BAP in combination with 0.5 mg/l NAA (fig. 2, C) followed by those cultured on MS media fortified with 0.5 mg/l BAP and 0.5 mg/l NAA which produced (28.48 mg/explant) mean fresh weight with (0.79 mg/explant) mean dry weight. On the same time, MS medium supplemented with 1mg/l BAP in combination with 1mg/l NAA showing an increase in the mean fresh and dry weight of callus formation/explant using leaf explant (20.11 and 0.67 respectively) (fig. 2, A), while MS medium fortified with 1mg/l BAP in combination with 2 mg/l NAA showed to be the most effective for callus production using nodal segment explants, it is able to produce 21.81 mg mean fresh weight of callus/explant with 0.9 mg mean dry weight (fig. 2, B).

From the obtained data we can noted that, MS medium free of plant growth regulators did not induce any callus formation and. On the same time, MS medium supplemented with BAP in addition to NAA were found to be suitable for callus production from different explants of *H. muticus* L. than those which supplemented with BAP alone. Also, root explants were found to be the best explant for callus production in this experiment.

Table (1) Effect of BAP and NAA on callus formation from leaf explants of *H. muticus* L. after two months.

Growth regulators concentration(mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
BAP	NAA			
0	0	0 b	0 g	0 d
1	0	0 b	0 g	0 d
2	0	0 b	0 g	0 d
3	0	0 b	0 g	0 d
0.5	0.5	100% a	15.28 c	0.61 ab
0.5	1	100% a	14.08 e	0.62 ab
0.5	2	100% a	13.64 f	0.48 c
0.5	3	100% a	15.19 c	0.50 c
1	1	100% a	20.11 a	0.67 a
2	1	100% a	14.58 d	0.56 b
3	1	100% a	17.07 b	0.58 b

Table (2) Effect of BAP and NAA on callus formation from nodal segment explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
BAP	NAA			
0	0	0 b	0 f	0 f
1	0	0 b	0 f	0 f
2	0	0 b	0 f	0 f
3	0	0 b	0 f	0 f
0.5	0.5	100% a	16.27 b	0.64 b
0.5	1	100% a	12.30 d	0.40 d
0.5	2	100% a	7.40 e	0.31 e
0.5	3	100% a	13.84 c	0.49 c
1	1	100% a	12.54 d	0.40 d
2	1	100% a	21.81 a	0.90 a
3	1	100% a	8.10 e	0.25 e

Table (3) Effect of BAP and NAA on callus formation from root explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
BAP	NAA			
0	0	0% c	0 j	0 h
1	0	80% ab	2.13 i	0.12 g
2	0	100% a	6.88 h	0.24 f
3	0	73.34% b	1.47 i	0.08 g
0.5	0.5	100% a	28.48 b	0.79 b
0.5	1	100% a	31.56 a	1.04 a
0.5	2	100% a	13.52 g	0.34 e
0.5	3	100% a	18.04 e	0.55 d
1	1	100% a	24.50 c	0.73 c
2	1	100% a	19.04 d	0.52 d
3	1	100% a	14.79 f	0.50 d

2-Effect of KIN and 2,4-Dichlorophenoxy Acetic Acid (2,4-D) on callus induction from different explants (leaf, nodal segment and root):

Data which represented in table; 4, 5 & 6 clarified that, MS media free of plant growth regulators did not induce any callus formation for the three used explants. Also, MS medium supplemented with KIN alone cannot induce any callus formation from leaf or nodal segment explants and they can stimulate callus formation using root explants but in small amount (MS medium supplemented with 3.0 mg/l KIN showed 60% of callus induction from root explants with 1.34 mg mean fresh weight and 0.08 mg mean dry weight of callus formed/explant). On the other hand, MS

medium supplemented with KIN in combination with 2,4-D were found to be effective for callus induction than that which containing KIN alone in this experiment. All leaf explants those were cultured on MS medium supplemented with 1.0 mg/l KIN and 0.5 mg/l 2,4-D showed moderate callus formation with 5.89 and 0.28 mg for mean fresh and dry weight of callus formed/explant respectively. Also, combination of KIN and 2,4-D showed the lowest callus formation from nodal segment explants. The highest mean fresh weight of callus formed from nodal segment explant (1.07 mg/explant with a mean dry weight 0.012 mg/explant) were recorded on MS medium fortified with 1.0 mg/l KIN and 1.0 mg/l 2,4-D followed by that containing 0.5 mg/l KIN and 2.0 mg/l 2,4-D (0.9 mg/explant mean fresh weight and 0.026 mg/explant mean dry weight). On the other hand, the highest mean fresh weight of callus formed/explant (6.76 mg) with a mean dry weight (0.22 mg) were obtained from root explants on MS medium fortified with 0.5 mg/l KIN and 0.5 mg/l 2,4-D. All results recorded after two months.

Table (4) Effect of KIN and 2,4-Dichlorophenoxy Acetic Acid (2,4-D) on callus formation from leaf explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
KIN	2,4-D			
0	0	0 b	0 f	0 e
1	0	0 b	0 f	0 e
2	0	0 b	0 f	0 e
3	0	0 b	0 f	0 e
0.5	0.5	100% a	5.23 b	0.21 b
0.5	1	100% a	2.12 d	0.12 c
0.5	2	100% a	1.28 e	0.08 d
1	0.5	100% a	5.89 a	0.28 a
1	1	100% a	5.11 b	0.22 b
1	2	100% a	3.38 c	0.12 c

Table (5) Effect of KIN and 2,4-Dichlorophenoxy Acetic Acid (2,4-D) on callus formation from nodal segment explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
KIN	2, 4-D			
0	0	0 b	0 e	0 c
1	0	0 b	0 e	0 c
2	0	0 b	0 e	0 c
3	0	0 b	0 e	0 c
0.5	0.5	100% a	0.53 d	0.014 b
0.5	1	100% a	0.76 c	0.02 ab
0.5	2	100% a	0.90 b	0.026 a
1	0.5	0 b	0 e	0 c
1	1	100% a	1.07 a	0.012 b
1	2	100% a	0.74 c	0.018 ab

Table (6) Effect of KIN and 2,4-Dichlorophenoxy Acetic Acid (2,4-D) on callus formation from root explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
KIN	2, 4-D			
0	0	0 c	0 g	0 b
1	0	0 c	0 g	0 b
2	0	13.33% c	0.04 g	0.01 b
3	0	60% b	1.34 fg	0.08 b
0.5	0.5	100% a	6.76 a	0.22 a
0.5	1	100% a	4.63 bc	0.21 a
0.5	2	80% ab	5.63 b	0.21 a
1	0.5	100% a	3.73 cd	0.23 a
1	1	60% b	2.71 de	0.10 b
1	2	60% b	2.12 ef	0.10 b

3-Effect of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) and NAA on callus induction from different explants (leaf, nodal segment and root):

In these experiment the effect of MS medium supplemented with 2,4-D even alone or in combination with NAA on the callus induction from different explants of *H. muticus* L. were studied along with that free of plant growth regulators as control. MS media fortified with 2,4-D even alone or in combination with NAA could be introduced moderate callus formation from different used explants. High concentrations of 2, 4-D and NAA cannot induce callus induction either on leaf or root explants (table; 7, 8 & 9). Moderate callus formation were be noticed from leaf explant on MS media fortified with 0.5 mg/l 2,4-D in combination with 1.0 mg/l NAA (mean fresh weight of callus formed/explant; 6.18 mg and mean dry weight callus formed/explant; 0.28 mg) followed by nodal segment (100% of explant formed callus, 3.70 mg mean fresh weight and 0.16 mg mean dry weight of callus formed/explant on MS medium fortified with 1.0 mg/l 2,4-D in combination with 1.0 mg/l NAA) and root explant (80% of explant formed callus, 4.20 mg mean fresh weight and 0.18 mg mean dry weight of callus formed/explant on MS medium fortified with 0.5 mg/l 2,4-D in combination with 1.0 mg/l NAA).

Table (7) Effect of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) and NAA on callus formation from leaf explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
2,4-D	NAA			
0	0	0 b	0 g	0 d
1	0	100% a	4.36 b	0.21 b
2	0	100% a	0.97 f	0.04 d
3	0	100% a	1.11 f	0.001 d
0.5	0.5	100% a	3.13 c	0.16 c
1	0.5	100% a	2.23 e	0.14 c
2	0.5	100% a	1.03 f	0.052 d
0.5	1	100% a	6.18 a	0.28 a
1	1	100% a	2.49 d	0.15 c
2	1	0 b	0 g	0 d

Table (8) Effect of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) and NAA on callus formation from nodal segment explants of *H. muticus* L. after two months.

Growth regulators concentration (mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
2,4-D	NAA			
0	0	0 b	0 g	0 e
1	0	100% a	0.47 f	0.02 de
2	0	100% a	1.04 e	0.034 cd
3	0	100% a	1.54 d	0.04 cd
0.5	0.5	100% a	2.79 b	0.12 b
1	0.5	100% a	2.45 c	0.06 c
2	0.5	100% a	2.43 c	0.04 cd
0.5	1	100% a	2.23 c	0.03 cd
1	1	100% a	3.70 a	0.16 a
2	1	100% a	0.74 f	0.01 de

Table (9) Effect of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) and NAA on callus formation from root explants of *H. muticus* L. after two months.

Growth regulators concentration(mg/l)		% of callus induction	MFW of callus (mg/Exp.)	MDW of callus (mg/Exp.)
2,4-D	NAA			
0	0	0% b	0 c	0 d
1	0	60% a	1.14 b	0.06 bc
2	0	60% a	0.94 bc	0.04 bcd
3	0	60% a	0.46 bc	0.03 cd
0.5	0.5	73.34% a	1.13 b	0.06 bc
1	0.5	80% a	1.11 b	0.04 bcd
2	0.5	0% b	0 c	0 d
0.5	1	80% a	4.20 a	0.18 a
1	1	100% a	1.50 b	0.08 b
2	1	0% b	0 c	0 d

Generally results were found to be in harmony with **[18-23]**. They showed that auxin and cytokinins have a significant effect on callus induction and MS medium free of plant growth regulators did not show any positive effect on callus formation in most of the Solanaceae family members**.** MS medium containing BAP along with NAA the most effective for callus induction from leaf explants of *H. niger* L. and *Datura metal* **[21]**. Also, the highest percentage of callus induction was observed in MS medium fortified with BAP and NAA from leaf explants of *Solanum nigrum* L. **[25]**. MS medium fortified with BAP and NAA capable of producing callus from leaf explants of *Atropa acuminate* after 62 days of inoculation **[26]**. All MS media supplanted with BAP in addition to NAA a positive response in callus formation from leaf explants of *Withania somnifera* L. **[27].** Similar results were reported in *Stevia rebaudiana* **[28]** and *Biophytum sensitivum* **[29].**

On the other hand, MS medium supplemented with KIN in combination with 2,4-D were suitable for maximal callus induction from leaf explants of *D. stramonium* L. and *W. somnifera* L. **[30, 31]**. Combination of 2,4-D and BAP was effective for callus induction from *Solanum tuberosum* **[22, 24].**

Finally, callus is an unorganized mass of cells started from the cut portions of the explant in which cells undergoing mitosis, which leads to the formation of callus. Among the different concentrations and combinations of the tested plant growth regulators, different combinations of BAP and NAA proved to be the best for maximum callus production from different explants of *H. muticus* L. and root explants were found to be the most effective explant which can produce the maximum mean fresh and dry weight of callus/explant followed by leaf and nodal segment explants. MS media free of plant growth regulators cannot induce any callus formation. On the same time, MS media fortified with 2,4-D in combination with KIN or NAA also can introduce callus induction but it is not effective for mass production.

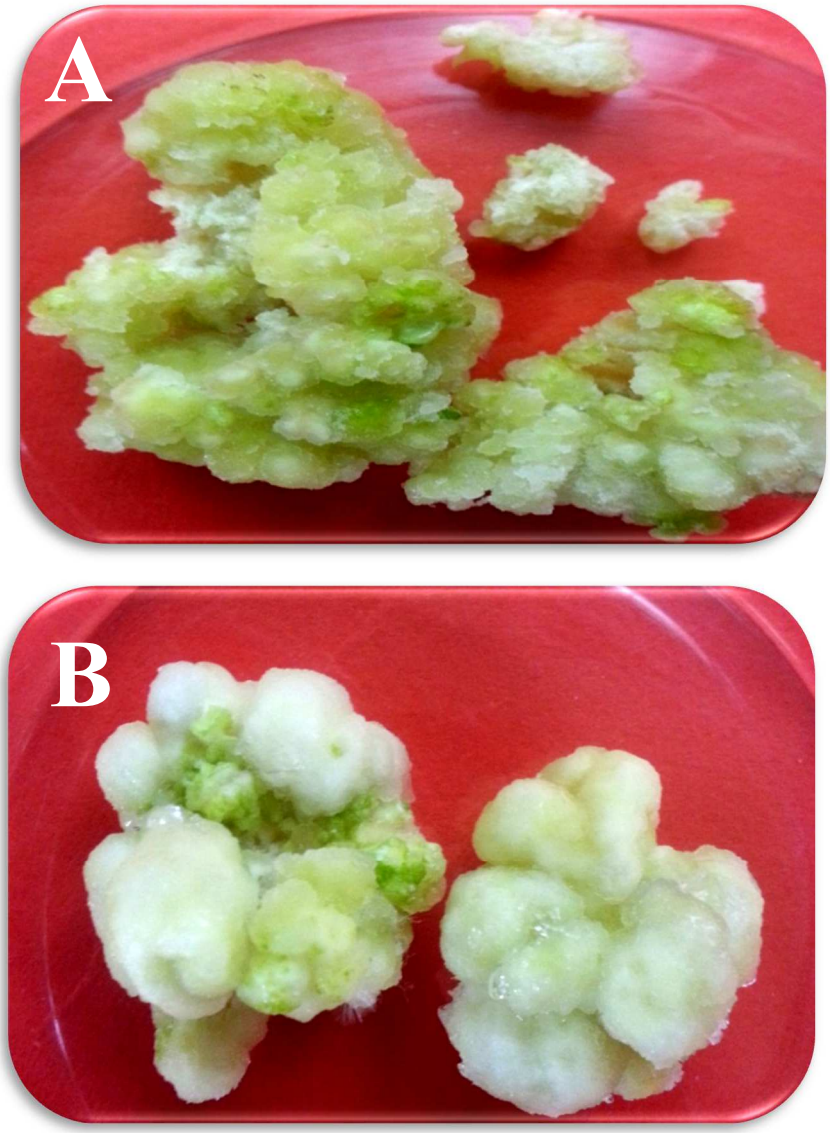

Fig. 2:

A) Callus formation from leaf explants on MS medium supplemented with 1.0 mg/l BAP in combination with 1.0 mg/l NAA after two months.

B) Callus formation from nodal segment explants on MS medium supplemented with 2.0 mg/l BAP in combination with 1.0 mg/l NAA after two months.

C) Callus formation from root explants on MS medium supplemented with 0.5 mg/l BAP in combination with 1.0 mg/l NAA after two months.

ABBREVIATION

MFW: Mean Fresh Weight; **MDW:** Mean Dry Weight; **NAA:** α-Naphthalene acetic acid; **BAP:** 6-benzylaminopurine; **KIN:** kinetin; **MS:** Murashige and Skoog basal medium.

REFERENCES

1. **Khater M.A. and M.M.A. Elashtokhy, 2015.** Effect of growth regulators on *in vitro* production of *Hyoscyamus aureus* L. and tropane alkaloids. International journal of ChemTech Research. Vol.8, No.11, p. 113-119.
2. **Bahorun, T., F. Trotin and J. Vasseux, 1994.** Comparative polyphenolic productions in Crataegusmonogyna callus cultures, Phytochemistry, 37: 1273-1276.
3. **Balz, J.P., D. Courtois, J. Drieu, K. Drieu, J.P. Reynoird, C. Sohier, B.P. Teng, A. Touche and V. Petard, 1999.** Production of ginkgolides and bilobalide by Ginkgo biloba plants and tissue culture, PlantaMedica, 65:620–626.
4. **El Bahr, M.K., S.A. Ghanem, M.M. El-Missiry and M.M. El-Nasr, 1997.** Production of tropane alkaloids in tissue cultures of *Hyoscyamus muticus*. Fitoterapia., 68(5): 423-428.
5. **Zhentian, L., J. Jervis and R.F. Helm, 1999.** C-glycosidic ellagitannins from white oak heartwood and callus tissues, Phytochemistry, 51: 751-756.
6. **Li, R., Reed, D.W., Liu, E., Nowak, J., Pelcher, L.E., Page, J.E., and Covello, P.S., 2006.** Functional genomic analysis of alkaloid biosynthesis in *Hyoscyamus niger*reveals a cytochrome P450 involved in littorine rearrangement. Chem. Biol. 13: 513–520.
7. **Oto, G. H, Ozdemir. B, Yaren. Y, Yetkin. A, Tas and P, Tantitanir, 2013.**Antinociceptiveactivity of methanol extract of *Hyoscyamus reticulatus*L. in mice .American Journal of Phytomedicine and Clinical Therapeutics. 177-123.
8. **Rasheed M, Jaskani MJ, Rasheed M, Iqbal MS, Hasan SZU, Rafique R, Mushta S and Iqbal M. , 2013.** Regeneration of Plantlets from various Explants of Tetraploid watermelon. J Bio. Agri&Healtcare. 3(1): 27-29.

9. **Majid, I., J. J. Muhammad, R. Rizwan, Z. U. Syed, S. I. Muhammad, R. Misbah and M. Salman, 2014.** Effect of Plant Growth Regulators on Callus Formation in Potato. Journal of Agri-Food and Applied Sciences. Vol. 2(3), pp. 77-81.
10. **Shah, S. H., S. Ali, S. A. Jan, J. Din and G. M. Ali, 2015.** Callus induction, in vitro shoot regeneration and hairy root formation by the assessment of various plant growth regulators in tomato (Solanum lycopersicum Mill.). The Journal of Animal & Plant Sciences, 25(2): Page: 528-538.
11. **Aminnejad M., B. Hosseini and A. Qaderi, 2015.** Effect of plant growth regulators and explant types on in vitro direct plant regeneration *of Hyoscyamus reticulatus*L. International Journal of Advanced Research, 3 (3), 457-462.
12. **Walla M. Abed Elmaksood, Fawzia A. Ebad and Hussein A. Bosila,2016.** *In vitro* Propagation of the Endangered Medicinal Plant *Hyoscyamus muticus* L. (Egyptian henbane). J. Appl. Environ. Biol. Sci., 6(4)1-1.
13. **Murashige, T. and F. Skoog, 1962.** A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497**.**
14. **Duncan, D.B. 1955.** Multiple Range and Multiple "F" test. Biometrics, 11: 1-42.
15. **Snedecor, G.W. and W.G. Cocharn, 1967**. Statistical Methods. 6 Ed. Iowa State Univ. Press, Ames, Iowa, U.S.A.
16. **Bhojwani, S.S. and M.K. Razdan, 1996.** Plant tissue culture: Theory and Practice: Developments in crop science Vol. 5. Elsevier, Amsterdam.
17. **Fowler, M.R., F.W. Rayns and C.F. Hunter, 1993.** The language and aims of plant cell and tissue culture. In Vitro Cultivation of Plant Cells, Butterworth-Heinemann Ltd, Oxford, Page 1-18.
18. **Aljibouri, A. M. J., K.W. Al-samarraei, A.S. Abd, D.M. Mageed, and A.A. Ali, 2012.** Alkaloids Production from Callus of Hyoscyamus niger L .in Vitro. Journal of Life Sciences 6. 874-882.
19. **Ibrahim, A.I., M. Abd El Kawi, A. Nower, A. Abdel Motaal and A. Abdel Aal, 2009.** Alkaloids production and organogenesis from callus of Hyoscyamus muticus L. In vitro, J. of Applied Sciences Research 5: 382-392.
20. **Iranbakhsh, A.R., M.A. Oshagi and M. Ebadi, 2007.** Growth and production optimization of tropane alkaloids in Datura stramonium cell suspension culture, Pakistan Journal of Biological Sciences 10 1236-1242.
21. **Abd-Rahman, R., E.H. El-Din, A. El-Said and H.D. Khlifa, 2008.** Production of scopolamine and hyoscyamine in callus and regenerate cultures of Datura metel (L.), J. of Applied Science Research 4. 1858-1866.
22. **Mutasim, M. K., G.A.E. Khadiga and S.M. Rasheid, 2010.** "Callus formation and organogenesis of potato (Solanum tuberosum L.) cultivaralmera", Journal of Phytology,Vol. 2(5),pp.40 – 46.
23. **Nistor, A., G.H. Campeanu, C. Nicoleta and K.C. Diana, 2009.** "Effect of auxin and cytokinin on callus induction in potato (Solanum tuberosum L.) explants", Agricultura Stiintasipractica, Vol. 12(69-70),pp.47 -50.
24. **Swarnkar, P.L., S.P. Bohra and N. Chandra, 1986.** "Biochemical changes during growth and differentiation of the callus of Solanum surattense",Journal of Plant Physiology,Vol.126,pp.75 – 81.
25. **Yogananth, N., R. Bhakyaraj, A. Chanthuru, S. Parvathi and S. Palanivel, 2009.** Comparative analysis of solasodine from in vitro and in vivo cultures of Solanum nigrum Linn. Kathmandu university journal of science, engineering and technology vol. 5, No. I, pp 99- 103.
26. **Farhana, M., S. Seema, A.K. Zahoor, J. Mahroofa and M. Maryum, 2014.** Callus induction and shoot regeneration of Atropa acuminate Royle-a critically endangered medicinal

plant species growing in Kashmir Himalaya**.** Journal of Scientific and Innovative Research; 3(3): 332-336.

27. **Adhikari, S. R. and B. Pant, 2013:** Induction and Proliferation of in vitro Mass of Callus of Withania somnifera (L .) Dunal. Research in Plant Sciences, Vol. 1, No. 3, 58-61.
28. **Preethi, M., Sridhar, T. M., Naidu, C.V. 2011.**Efficient protocol for indirect shoot regeneration from leaf explants of *Stevia rebaudiana* (Bert.) – An important calorie free biosweetner. Journal of Phytology, 3(5): 56-60.
29. **Shivanna, M.B., Vasanthakumara, M.M., Mangala, C. 2009.** Regeneration of Biophytum sensitivum (Linn.) DC. Through organogenesis and somatic embryogenesis. Indian Journal of Biotechnology, 8: 127-131.
30. **Amiri, S., S.K. Kazemitabar, G.A. Ranjbar and M. Azadbakht, 2011:** In vitro propagation and whole plant regeneration from callus in Datura (Datura stramonium. L)**.** African Journal of Biotechnology Vol. 10(3), pp. 442-448.
31. **Gita, R., G. S. Virk and N. Avinash, 2003:** Callus induction and plantlet regeneration in Withania somnifera (L.) Dunal. In Vitro Cellular and Developmental Biology Plant 39(5): 468-474.