Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

POTENTIAL OF CHEMICAL ELICITORS TO ENHANCE PRODUCTION SECONDARY METABOLITES IN INDUCED CALLUS FROM HYOSCYAMUS NIGER LEAVES

M. A. Khalaf[1], A. J. Taha[1], A. S. Abed*[2]
[1] Department Biology., College of Science, Mustansiryiah University, Iraq.
*[2] Department of Plant Biotech., Biotech. Res. Center, Al-Nahrain University, Iraq.
*Corresponding author: E-mail: ashwaqbio@nahrainuniv.edu.iq

ABSTRACT

Hyoscyamus niger **have the main pharmaceutical tropane alkaloids and glycosides in their tissues. The study aimed to enhance the biosynthesis of these compounds by tissue culture techniques. Callus cultures were established from leaf explants on MS medium. Three chemical salts were added separately in different concentrations to the culture medium to evaluate their effects on callus growth and content of pharmaceutical compounds. The results demonstrate that copper (Cu) and cadmium (Cd) decreased callus weights, while the aluminum (Al) did not exhibit any significant effects, compared to the control treatment. However, the HPLC assay proved that the chemical stress caused beneficial effects in promoting the production of alkaloid and glycoside compounds in callus cultures. Succinctly, the highest concentration of Cu (36 mg/L) caused a significant increasing in the content of hyoscyamine, scopolamine, and hyoscyammoside-A. Whereas, Al and Cd at low concentration (5 mg/L) produced the varying significant increasing in the substances of tropane alkaloids and glycosides, compared to the other treatments and the control. These finding concludes that application of chemical elicitation in certain concentrations offer advantageous visions for developing the conditions that related with biosynthesis of medicinal compounds in *H. niger*.**

Key words: Black henbane, glycosides, HPLC, tropane alkaloids.

Copyright© 2025. The Author (s). Published by College of Agricultural Engineering Sciences, University of Baghdad. This is an open-access article distributed under the term of the Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cite.

Received: 12/8/2023, Accepted: 27/12/2023, Published: 26/1/2026

INTRODUCTION

Many types of tropane alkaloids, glycosides, phytosterols, and phenolics were detected in the *Hyoscyamus niger* (*H. niger*), which are used for diverse medicinal purposes (Rajput et al., 2024). This shrub is small to medium in size, growing in two forms (annual or biennial), leaves are dark green, flowers are pale yellow or white in color, while the fruits are tiny, deadly blackberry, dense with gray-black seeds (Azhar and Mustehasan, 2020). It is native at Europe and Asia (Alamholo *et al.*, 2019). Plants produce organic compounds in response to environmental stress or the application of biotic and abiotic stimulants, and these factors are used in the production of medicines, tastes, and other essential biochemical components (Naik and Al-Khayri, 2016) Plant tissue cultures and chemical elicitation techniques are the processes of exposing plant cells to elicitors, which are compounds that act as extracellular signaling molecules and cause or activate plant defense responses and the synthesis of natural secondary products which are mostly higher than plant grown in the *in vivo* conditions (Al-Khayri and Naik, 2020). Inducing callus from various plant sources and parts required various combinations of plant growth regulators or growth media (Sidik *et al.*, 2024).

Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

Generally, the callus-extracted metabolic chemicals are highly pure (Surchy *et al.*, 2025). HPLC technology is often used in quantitative and qualitative screening of active compounds found in many cultures (Giglou *et al.*, 2025). In previous studies, it has been shown that $CuSO_4$ was the most efficient chemical for raising the concentration of phenolic compounds in *Artemisia annua* callus cultures (Zarad *et al.*, 2021). Another study demonstrated that $CuSO_4$ promoted shoot cultures of *Ocimum basilicum* and increased the production of eugenol, cineole, and linalool contents (Trettel *et al.*, 2018). In addition, the elicitation of $AlCl_3$ was shown to boost secondary metabolites (SM) output in cultured tissues of *Rauvolfia serpentina* (Zafar *et al.*, 2017). Increase the accumulation of tropane alkaloids in micropropagated plantlets of *Datura innoxia* was detected with reducing the weights of the shoot cultures (Karimi and Khataee, 2011). In a study conducted by Sivanandhan *et al.*(2014), it was observed that increasing levels of $AlCl_3$ (5-15 mg/L) stimulated the accumulation of withanolide compounds while maintaining a consistent growth rate in cell cultures of *Withania somnifera*. According to Rao *et al.* (2021), $CdCl_2$ at 1 mM of concentration enhanced the production of stigmasterol (2.59-fold) compared to the control in the cell cultures of *Abutilon indicum*. But the high concentrations of $CdCl_2$ (0.20 mM) inhibited callus growth and decreased the production of alkaloids in *Rauvolfia serpentina* (Zafar *et al.*, 2020). The aims of this experiment are to determine the influence of $CuSO_4$, $AlCl_3$, and $CdCl_2$ on the callus growth, alkaloid and glycoside concentrations in the callus cultures formed from *H. niger* leaf explants.

MATERIALS AND METHODS

Callus formation and select maintenance medium

The experiment was conducted in "Biotechnology Research Center, Al-Nahrain University, Iraq". The dried seeds of *H. niger* were provided from "Plant Genetic, Resources Division, Botany Directorate, Abu-Ghraib, Iraq". In vitro seedlings were utilized to generate juvenile leaves, which served as a source for explants. Callus formation was initiated from leaves cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), which was enriched with a combination of 2 mg/L benzyladenine (BA) and 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) (Khalaf and Taha, 2023). The callus cultures were maintained under incubator conditions with a photoperiod of 16 h light and 8 h dark. This specific treatment was selected to initiate callus formation and subsequently support the ongoing growth of the callus cultures.

Elicitation of secondary metabolites

After obtaining a suitable amount of initiated callus, the callus was cut into equal sections and planted on the same medium with different stimuli, including $CuSO_4.5H_2O$ with concentrations of (0, 12, 24, and 36 mg/L), $AlCl_3$ with concentrations of (0, 5, 10, and 20 mg/L), and $CdCl_3$ concentrations of (0, 5, 10, and 15 mg/L). Following a 4-week interval, the precise measurement of fresh sample weights was conducted using an analytical balance. Subsequently, the samples were exposed to a 24-hour drying process in a lab electric oven set at 45 °C for recording the dry weights (Soni *et al.*, 2018).

Extraction of alkaloids and glycosides

The tropane alkaloid extraction was extracted from plant leaves (grown in field) and callus cultures according to Kamada *et al.* (1986) with minor modification. While the glycosides were extracted according to Rai and Han (2022).

Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

HPLC Analysis

The extraction methods of total alkaloids and glycosides used in this study have been described by High-Performance Liquid Chromatography (HPLC) was used to measure

$$\text{Conce. of sample } (\mu g/ml) = \frac{\text{Area of sample}}{\text{Area of standard}} x \text{ Conce. of standard } X \text{ dilution factor}$$

the content and quality of secondary metabolites in methanolic extracts of leaves of the mother plant grown in the field and different treatments of callus cultures. By comparison the peak area and retention time (RT) of the authentic standards to those of the analyzed samples. The concentration of *H. niger* compounds in the samples was determined using the formula below (Mohammed, 2019):

Statistical analysis

To compare the experimental treatments statistically, a data analysis was presented using "Duncan's Multiple Range test" at a significance level of 5% ($P<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of chemical treatments on the callus weights : The impact of chemical abiotic elicitors at various concentrations on the growth of callus cultures is illustrated in **Figure (1)**, while the morphology and size of elicited callus are shown in **Figure (2)**.

Effect of $CuSO_4$ on the callus growth

The findings in **Figure (1, a and b)** demonstrate that as the $CuSO_4$ concentrations increased, there was a notable decrease in callus weight, both in fresh weight (FW) and dry weight (DW). Specifically, at a concentration of 36 mg/L, the FW and DW of callus decreased to 951.5 and 84.1 mg, respectively, compared to the control treatment, which exhibited the highest values of weights of 1842.1 and 170.6 mg, respectively. The morphology and size of the elicited callus are illustrated in **Figure (2)**. Copper (Cu) is a micronutrient that is essential for plant growth and development. However, an excessive amount of copper could be a toxic effect on plants (Prażak and Molas, 2015). In a study by Al-mayahi (2014), it was found that prolonged exposure to even relatively low levels of Cu could have far-reaching negative effects on *in vitro-* grown plants, including a reduction in photosynthesis, a disruption of pigment synthesis, and damage to plasma membrane permeability.

Effect of Al on the callus growth

The **Figure (1, a and b)** shows that there was a non-significant tendency for a decrease in callus weights across all concentrations of $AlCl_3$ when compared to the control treatment. The highest FW and DW weights (1787.2 and 165.8 mg, respectively) of callus biomass were obtained when cultured on MS medium supplemented with 5 mg/L of $AlCl_3$, whereas the highest concentration 20 mg/L of $AlCl_3$ resulted in the lowest biomass yields (1735.6 and 161.1 mg) for FW and DW, respectively. Conspicuously, the decline in weight did not statistically significant among the examined concentrations of $AlCl_3$. Aluminum (Al) is known as an abiotic stress factor that can cause damage to various cellular components such as cell membranes, nucleic acids, proteins, and lipids. Most of this damage is caused by more reactive oxygen species (ROS), like superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Zheng *et al.*, 2025). In response to this oxidative stress, plants activate their antioxidative enzymatic systems, including superoxide dismutase and peroxidase, which act as the first line of defense (Panda *et al.*, 2024). Interestingly, when exposed to moderate doses of the metal, the activity of these enzymes increases, but at higher concentrations, their activity decreases. The reduction in enzyme activity under high concentrations of heavy metals may be associated with the elevation in

Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

ROS or H_2O_2 amounts inside various cellular parts (Anjitha *et al.*, 2021).

Influence of Cd on the callus development

The **Figure 1 (a, and b)** illustrates that control treatment which did not contain any dose of chemical elicitors, showed highest average mean of fresh and dry callus weight (1842.1 mg FW, and 170.6 mg DW). Significance differences in the callus growth were detected when $CdCl_2$ was applied at different doses, contrasted with the control. The reduction of weights ranging from 1206 to 1226 mg for FW and 110.5 to 119.3 mg for DW. The negative effects of Cd on plant cells were confirmed by the previous investigations. Zafar *et al.* (2020) observed similar decrease in callus weigh, when adding Cd to the culture medium. They attributed the reason to the role of Cd in delaying cell division, disrupting the cell cycle, and interference with the normal mitotic continuance. Additionally, the rise in oxidative damage, metabolism disorder, and reduce of the normal plant physiologic process were demonstrated by Aslam *et al.* (2023). These factors together cause callus development decline down when exposed to different salts of Cd.

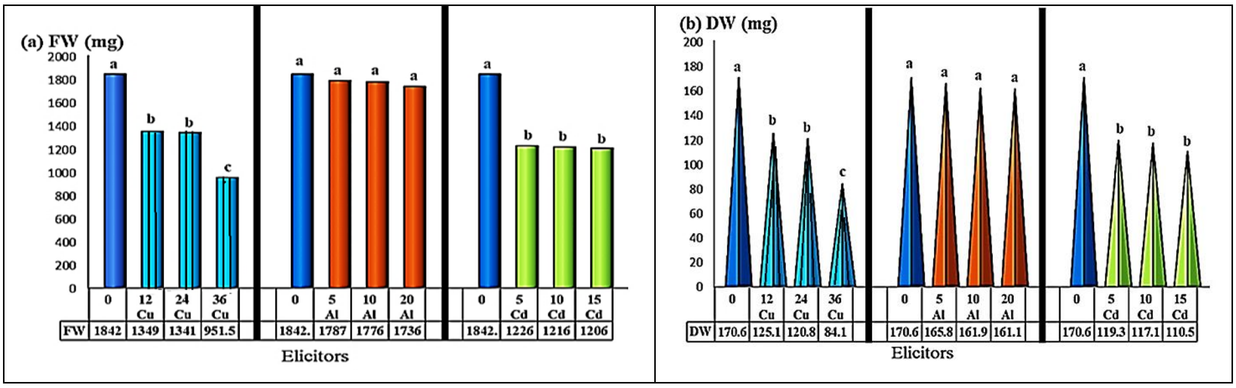


Figure 1. Comparison of the effect of different concentrations of Cu, Al, and Cd (mg/L) added to the maintenance medium on the (a) fresh weight (FW) and (b) dry weight (DW) of callus cultures (mg). Dissimilar letters are used to indicate statistically significant differences within each compound, using Duncan's test at P≤ 5%.

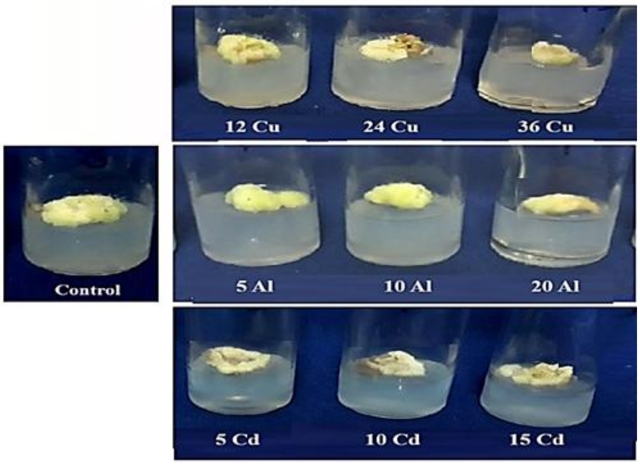


Figure 2. Morphology of *H. niger* callus cultures after treatment with different concentrations of Cu, Al, and Cd.

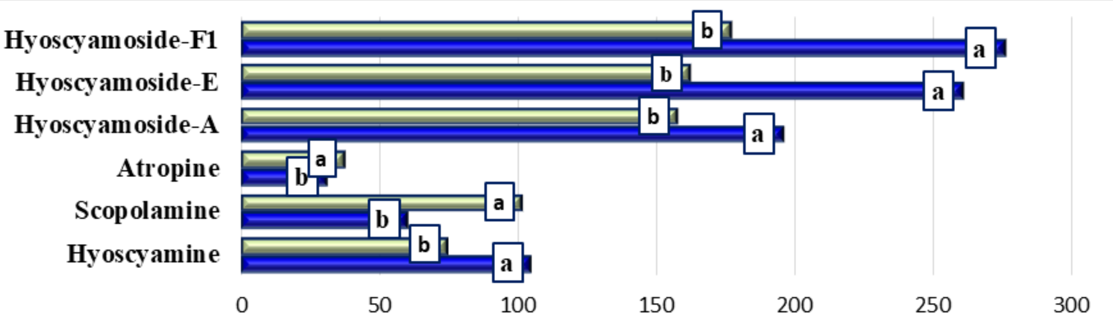
Estimation of certain phytochemical compounds in the callus and mother plant of *H. niger* using HPLC analysis

Figure (3) shows the biosynthesis of secondary metabolites (SM) shows significant increases in the mother plant when compared to the callus. These increases were particularly

Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

remarkable for glycosides (hyoscyamoside-A, E, and F1) and alkaloids (hyoscyamine).

In contrast, there was a notable rise in the concentrations of scopolamine and atropine in the callus tissue as compared to the original plant. Specifically, the levels of scopolamine reached 101.1 µg/ml, while the levels of atropine reached 37 µg/ml. The synthesis of SM compounds can be regulated by various stresses that affect the mother plant, consequently impacting gene expression and subsequent production of these chemicals (Bakhtiari and Golkar, 2022). The usage of growth regulators during the establishment and maintenance of callus cultures possibly speeds up the production of specific secondary metabolites, leading to an augmentation in the concentration of bioactive substances (Hemmati *et al.*, 2020)**.** According to another study, a variation in phytochemical content was observed between field and *in vitro* plants, which depended on the type of compound and their metabolic pathways (Salih and Al Dabagh, 2021).



	Hyoscyamine	Scopolamine	Atropine	Hyoscyamoside-A	Hyoscyamoside-E	Hyoscyamoside-F1
Callus	74.2	101.1	37	157.1	161.7	176.3
Mother plant	104.3	59.8	30.5	195.2	260.1	275.3

Figure 3. Comparison of secondary metabolite content in the leaves of mother plant and callus cultures. Dissimilar letters (a–b) are used to indicate statistically significant differences within each compound, using Duncan's test at P≤ 5%.

Impacts of Cu elicitation on the content of certain phytochemical compounds

The data in **Table (1)** presents the effect of varying Cu treatments on the concentrations of compounds in *H. niger* callus cultures. The results reveal that two alkaloids, namely hyoscyamine and scopolamine, and the glycoside hyoscyamoside-A, exhibited a positive response to copper concentrations. Their concentrations significantly increased with higher Cu concentrations, reaching 118.2 µg/ml, 124.7 µg/ml, and 750.2 µg/ml, respectively, at 36 mg/L Cu, in contrast to the control. While the other glycosides, like hyoscyamoside-E, significantly increased at 12 mg/L Cu (547.8 µg/ml), whereas hyoscyamoside-F1 showed a significant increase (427.3 µg/ml) at 24 mg/L Cu. All Cu treatments caused significant decrease in atropine compound compared to the control group (37.0 µg/ml). These results reveal the potential regulating function of Cu in the biosynthesis of secondary metabolites in *H. niger* and may have impacts for its actual use in the therapeutic sector. In this regard, the application of copper sulfate can lead to an increase in the production of secondary metabolites in plants through multiple mechanisms, including stress response and regulation of enzyme activity and gene expression (Senekoviˇ, 2025)**.** The Cu effective in the increasing of some secondary metabolites compounds because it had a feature that destroys biofilms or Cu have

Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

property to the optional permeability of the salts. The integrity of cell membranes is one physiological mechanism that Cu may disrupting the plant's ion balance and causing oxidative stress, this stress response can trigger the synthesis of SM such as terpenoids and phenolics (Akhtar *et al.*, 2026; Liu *et al.*, 2024).

Table 1. Evaluating the influence of several concentrations of Cu (mg/L) on the amount of some medicinal compounds (µg/ml) in the *H. niger* callus cultures.

Compounds (µg/ml)	Concentration of $CuSO_4$ (mg/L)			
	0	12	24	36
Hyoscyamine	74.2 ^{b}	73.0 ^{b}	62.6 ^{c}	118.2^{a}
Scopolamine	101.1^{c}	83.6 ^{d}	109.3^{b}	124.7^{a}
Atropine	37.0 ^{a}	31.2 ^{c}	25.9 ^{d}	34.1 ^{b}
Hyoscyamoside-A	157.1^{d}	331.7^{c}	561.4^{b}	750.2^{a}
Hyoscyamoside-E	161.7^{c}	547.8^{a}	475.5^{b}	475.6^{b}
Hyoscyamoside-F_1	176.3^{c}	340.8^{b}	427.3^{a}	123.9^{d}
Total yield of compounds	707.4	1408.1	1662	1626.7

Different letters indicate significant differences between compounds within each column, using Duncan's test at P≤ 5%.

Impacts of Al elicitation on the content of certain phytochemical compounds

Table (2) shows that when compared to the control treatment and the other doses utilized, all glycoside contents considerably increased significantly at a concentration of 5 mg/L $ALCl_3$, reaching (262.7, 233.8, and 207.6 µg/ml) for hyoscyamoside-A, E and F_1 respectively. Correspondingly, the highest value of hyoscyamine and atropine reached to 78 and 39.5 µg/ml at 20 mg/L concentration. The alkaloid scopolamine exhibited its highest content (101.1 µg/ml) significantly in the control treatment, surpassing the concentrations observed in other experimental treatments.The outcomes of this experiment can be analyzed in terms of the impact of the element Al on the concentration of the studied compounds, either increasing or decreasing it. The presence of Al appears to be beneficial in exerting stress on cells and tissues, which could lead to disrupting the plant's ion balance and causing oxidative stress and higher production of secondary metabolites such as flavonoids, phenolic compounds, and alkaloids in plant cultures (Khan *et al.*, 2021). Moreover, many investigators proved that Al is considered a necessary element for various developmental and physiological activities (Chauhan *et al.*, 2021). Also, it plays a role in controlling the nutrients transport and regulating several gene expression (Ofoe *et al.*, 2023).

Table 2. Evaluating the influence of several concentrations of the Al (mg/L) on the amount of some medicinal compounds (µg/ml) in the *H. niger* callus cultures.

Compounds (µg/ml)	Concentration of $AlCl_3$ (mg/L)			
	0	5	10	20
Hyoscyamine	74.2 ^{b}	55.3^{d}	64.6 ^{c}	78.0 ^{a}
Scopolamine	101.1^{a}	92.8^{b}	69.7^{d}	78.9 ^{c}
Atropine	37.0 ^{ab}	35.0 ^{b}	27.5 ^{c}	39.5^{a}
Hyoscyamoside-A	157.1^{c}	262.7^{a}	184.9 ^{b}	142.0^{d}
Hyoscyamoside-E	161.7^{b}	233.8^{a}	98.4^{d}	116.0^{c}
Hyoscyamoside-F_1	176.3^{b}	207.6^{a}	121.8^{c}	85.2^{d}
Total yield of compounds	707.4	887.2	566.9	539.6

Different letters indicate significant differences between compounds within each column, using Duncan's test at P≤ 5%.

Iraqi Journal of Agricultural Sciences –2026:57(1):92-104
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/h8r61b57

Impacts of Cd elicitation on the content of certain phytochemical compounds

The HPLC evaluation confirmed that the valuable metabolism compounds responded differently to the $CdCl_2$ concentrations used, as viewed in **Table (3).** At 10 mg/L Cd, Hyoscyamine's content increased significantly (80.2 µg/ml), over the control treatment (74.2 µg/ml). Further, atropine, hyoscyamoside A, and F_1, demonstrated a significant response to Cd treatment. The highest amounts (43.5, 248.7, and 198.4 µg/ml, respectively) were detected at 5 mg/L Cd and had been considerably more than the control. In contrast, scopolamine and hyoscyamoside-E exhibited a decreasing trend with increasing $CdCl_2$ levels, reaching their highest contents (101.1 and 161.7 µg/ml, respectively) in the control group. In this regard, various heavy metals and salts, like cadmium and others, have been proven to elicit the generation of SM in different plants; this effect may be attributed to damage or disruption of cell membranes, allowing the signal transmission to increase enzymatic activity and metabolism (Vats, 2018). In general, the application of heavy metal stress on plants has upregulated sugar metabolism, increased amino acid proline concentrations, and increased enzymatic activity related to ROS scavenger pathways; thus, these interactions, in turn, lead to an increase in the biosynthesis of secondary compounds as a defense against abiotic stress (Zandalinas *et al.*, 2020).

Table 3. Evaluating the influence of several concentrations of the Cd (mg/L) on the amount of some medicinal compounds (µg/ml) in the *H. niger* callus cultures

Compounds (µg/ml)	Concentration of $CdCl_2$ (mg/L)			
	0	5	10	15
Hyoscyamine	74.2 ^{b}	58.2 ^{c}	80.2 ^{a}	57.9 ^{c}
Scopolamine	101.1^{a}	89.6 ^{b}	81.6 ^{c}	78.2 ^{c}
Atropine	37.0 ^{b}	43.5 ^{a}	36.4 ^{b}	22.6 ^{c}
Hyoscyamoside-A	157.1^{d}	248.7 ^{a}	179.3^{b}	167.2^{c}
Hyoscyamoside-E	161.7^{a}	1403 ^{b}	117.2^{c}	78.8 ^{d}
Hyoscyamoside-F_1	176.3^{b}	198.4 ^{a}	145.7^{c}	133.1^{c}
Total yield of compounds	707.4	778.7	640.4	537.8

Different letters indicate significant differences between compounds within each column, using Duncan's test at P≤ 5%.

Comparison of the Yield of SM in Callus Cultures Treated with Different Chemical Stimuli : Based on **Figure (4)**, the total yield of SM in the callus culture varied depending on the type of chemicals and doses used. The findings indicated that Cu elicitation was much more effective than other elicitors. It led to a significant raise in the quantities of valuable phytochemical compounds, in particular at high concentrations (24 and 32 mg/L), approximately 130% increase. While the lowest concentration of Al and Cd (5 mg/L) had a stronger impact on biosynthesis of beneficial photochemical constituents. These observations point out that Cu elicitation could possess a bigger influence on the secondary metabolism than other metals used in this investigation. Various heavy metals have been

P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI:

widely applied as abiotic elicitors in different plant cultures to stimulate the growth, increase phytochemical production, and boost antioxidant activities (Zandalinas *et al.*, 2020). Nevertheless, these elements are believed to be useful as elicitors, their use can also cause toxic effects under inappropriate conditions (Khan *et al.*, 2021). The main factors that determine the effectiveness of chemical treatments are the type of element, its concentration, and exposure time (Abed *et al.*, 2023). It is necessary to understand how abiotic stress affect the biosynthesis of SM compounds like worthy alkaloids and glycosides in different *H. niger* cultures, in order to improve their production (Khalaf and Taha, 2023).

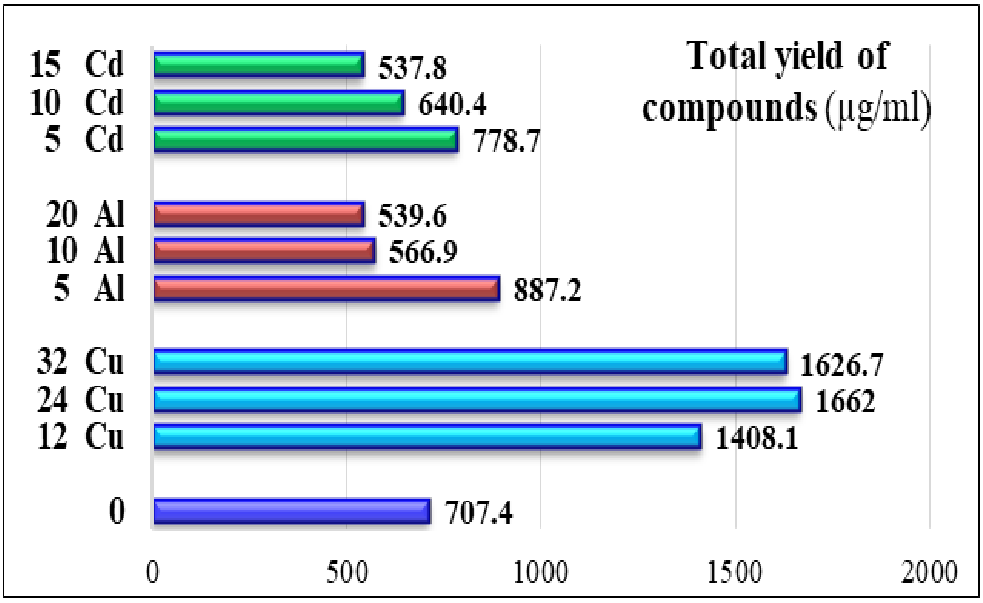


Figure 4. Contrasting the total amount of some medicinal compounds (µg/ml) in the *H. niger* callus cultures treated with various concentrations of Cu, Al, and Cd salts.

CONCLUSIONS

The heavy metals at appropriate concentrations may resulting desirable influences on the callus growth developments in *H. niger* cultures with elevation the production of medicinal secondary metabolites. More studies are needed to determine out which factors are actually goes on underlying these noticed phenomena.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support provided by the Biotechnology Research Center, Al-Nahrain University, Iraq, for providing laboratory facilities and technical assistance.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

AUTHOR/S DECLARATION

All authors confirm that all Figures and Tables in the manuscript are original to us.

AUTHOR'S CONTRIBUTION STATEMENT

A. J. Taha designed the study and supervised the experimental works, M. A. Khalaf carried out the tissue culture experiments and data collection, A. S. Abed performed the statistical analysis. All authors contributed to manuscript writing.

REFERENCES

- Abed, A. S., Ismail, E. N., Majeed, D. M., and Al-Jibouri, A. M. J. (2023). Increasing amounts of secondary metabolites and medicinal compounds in callus culture of Moringa oleifera (Lam.) using abiotic elicitors. Iraqi Journal of Science, 64(8), 3902–3913.

Iraqi Journal of Agricultural Sciences –2026:57(1):1-18
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI:

https://doi.org/https://doi.org/10.24996/ijs.2023.64.8.17

- Akhtar, M. T., Lu, Z., Ren, S., Zou, H., Noor, I., and Jin, B. (2026). Copper homeostasis: Crosstalk with plant secondary metabolism and stress responses. Plant Science, 362(January). https://doi.org/https://doi.org/10.1016/j.plantsci.2025.112795
- Al-Khayri, J. M., and Naik, P. M. (2020). Elicitor-induced production of biomass and pharmaceutical phenolic compounds in cell suspension culture of date palm (Phoenix dactylifera L.). Molecules, 25(4669). https://doi.org/10.3390/molecules25204669
- Al-mayahi, A. M. W. (2014). Effect of copper sulphate and cobalt chloride on growth of the in vitro culture tissues for date palm (Phoenix dactylifera L.) cv. Ashgar. American Journal of Agricultural and Biological Sciences, 9(1), 6–18. https://doi.org/10.3844/ajabssp.2014.6.18
- Alamholo, M., Katayounchah, R. N., and Soltani, J. (2019). Hyoscyamine and scopolamine production in induced callus and hairy roots of Hyoscyamus niger. Academia Journal of Biotechnology, 7(12), 207–215. https://doi.org/10.15413/ajb.2019.0114
- Anjitha, K. S., Sameena, P. P., and Puthur, J. T. (2021). Plant stress functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. Plant Stress, 2(100038), 1–14. https://doi.org/https://doi.org/10.1016/j.stress.2021.100038
- Aslam, M. M., Okal, E. J., and Waseem, M. (2023). Cadmium toxicity impacts plant growth and plant remediation strategies. Plant Growth Regul, 99, 397–41 2. https://doi.org/https://doi.org/1 0.1 007/s10725-022-0091 7-7
- Azhar, M., and Mustehasan. (2020). Phytopharmacology of an important Unani drug bazr-ul-banj (Hyoscyamus niger Linn.) – Review. Asian J Pharm Clin Res, 13(9), 28–32. https://doi.org/https://doi.org/10.22159/ajpcr.2020.v13i9.38166
- Bakhtiari, M. A., and Golkar, P. (2022). The effects of callus elicitation on lepidine, phenolic content, and antioxidant activity of Lepidium sativum L.: chitosan and gibberellic acid. Journal of Plant Growth Regulation, 41, 1 1 48–1 1 60. https://doi.org/https://doi.org/10.1007/s00344-021-10368-5
- Chauhan, D. K., Yadav, V., Vaculík, M., Gassmann, W., Pike, S., and Arif, N. (2021). Aluminum toxicity and aluminum stress-induced physiological tolerance responses in higher plants. Critical Reviews in Biotechnology, 41(5). https://doi.org/https://doi.org/10.1080/07388551.2021.1874282
- Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11(1), 1–42.
- Giglou, R. H., Giglou, M. T., Sobhanizadeh, A., and Szumny, A. (2025). Enhancing the accumulation of scopolamine and atropine in Hyoscyamus niger L. callus by LED light and glycine-a cheap method. BMC Plant Biology, 25(1162), 1–14. https://doi.org/https://doi.org/10.1186/s12870-025-07062-z
- Hemmati, N., Cheniany, M., and Ganjeali, A. (2020). Effect of plant growth regulators and explants on callus induction and study of antioxidant potentials and phenolic metabolites in Salvia tebesana Bunge. Botanica Serbica, 44(2), 163–173. https://doi.org/https://doi.org/10.2298/BOTSERB2002163H

P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI:

- Kamada, H., Okamura, N., Satake, M., Harada, H., and Shimomura, K. (1986). Alkaloid production by hairy root cultures in Atropa belladonna. Plant Cell Reports, 5(4), 239–242. https://doi.org/10.1007/BF00269811
- Karimi, F., and Khataee, E. (2011). Aluminum elicits tropane alkaloid production and antioxidant system activity in micro propagated Datura innoxia plantlets. Acta Physiologiae Plantarum, 34(3). https://doi.org/10.1007/s11738-011-0900-z
- Khalaf, M. A., and Taha, A. J. (2023). Photoperiod and UV light influence secondary metabolites of Hyoscyamus niger callus induced from leaves. Journal of Biotechnology Research Center, 17(2), 16–26. https://doi.org/https://doi.org/10.24126/jobrc.2023.17.2.684
- Khan, H., Khan, T., Ahmad, N., Zaman, G., Khan, T., Ahmad, W., Batool, S., Hussain, Z., Drouet, S., Hano, C., and Abbasi, B. H. (2021). Chemical elicitors-induced variation in cellular biomass, biosynthesis of secondary cell products, and antioxidant system in callus cultures of Fagonia indica. Molecules, 26(6340). https://doi.org/10.3390/molecules26216340
- Liu, J., Al-namazi, A. A., and Wan, J. S. H. (2024). Plants generally suffer less enemy damage and are more defended in a copper mine than in a closely adjacent site. South African Journal of Botany, 171(August), 164–172. https://doi.org/10.1016/j.sajb.2024.06.010
- Mohammed, Z. H. (2019). Determination of nicotine extracted from eggplant and green pepper by HPLC. Baghdad Science Journal, 16(1), 61–67. https://doi.org/10.21123/bsj.2019.16.1.0061
- Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with Tobacco tissue cultures. Physiologia Plantarum, 15, 473–497. https://doi.org/https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
- Naik, P. M., and Al-Khayri, J. M. (2016). Impact of abiotic elicitors on in vitro production of plant secondary metabolites: A review. Journal of Advanced Research in Biotechnology, 1(2), 1–7. https://doi.org/10.15226/2475-4714/1/2/00102
- Ofoe, R., Thomas, R. H., Asiedu, S. K., Wang-Pruski, G., Fofana, B., and Abbey, Lord. (2023). Aluminum in plant: Benefits, toxicity and tolerance mechanisms. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.1085998
- Panda, S. K., Gupta, D., Patel, M., Vyver, C. Van Der, and Koyama, H. (2024). Functionality of reactive oxygen species (ROS) in plants: Toxicity and control in Poaceae crops exposed to abiotic stress. Plants, 13(2071). https://doi.org/10.3390/plants13152071
- Prażak, R., and Molas, J. (2015). Effect of copper concentration on micropropagation and accumulation of some metals in the Dendrobium kingianum Bidwill Orchid. Journal of Elementology, 20(3), 693–703. https://doi.org/10.5601/jelem.2014.19.4.748
- Rai, A., and Han, S. (2022). Critical review on key approaches to enhance synthesis and production of Steviol glycosides: A blueprint for zero-calorie sweetener. Applied Sciences, 12(8640). https://doi.org/https://doi.org/10.3390/app12178640
- Rajput, V., Rani, K. S., & Patan, P. (2024). A review on exploring the medicinal value of Zingiber officinale and Hyoscyamus niger to prevent or overcome seasickness. Journal of Population Therapeutics and Clinical Pharmacology, 31(11), 1140–1150.

Iraqi Journal of Agricultural Sciences –2026:57(1):1-18
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI:

https://doi.org/https://doi.org/10.53555/1pszpb02

- Rao, K., Chodisett, B., Gandi, S., Giri, A., and Kishor, P. B. K. (2021). Cadmium chloride elicitation of Abutilon indicum cell suspension cultures for enhanced stigmasterol production. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology. 156(3), 613–618. https://doi.org/https://doi.org/10.1080/11263504.2021.1891151
- Salih, M. I., and Al Dabagh, F. M. K. (2021). Comparative analysis of some phenolic acids of in vitro and in vivo grown plant leaves of Salvia hispanica. Iraqi Journal of Agricultural Sciences, 52(1), 189–195. https://doi.org/10.36103/IJAS.V52I1.1250
- Senekoviˇ, J. (2025). Copper sulfate elicitation effect on biomass production, phenolic compounds accumulation, and antioxidant activity of Morus nigra L. stem node culture. Plants, 14(5) (766). https://doi.org/https://doi.org/10.3390/plants14050766
- Sidik, N. J., Agha, H. M., Alkamil, A. A., Alsayadi, M. M. S., and Mohammed, A. A. (2024). A mini review of plant tissue culture: The role of media optimization, growth regulators in modern agriculture, callus induction and the applications. AUIQ Complementary Biological System, 1(2024), 96–109. https://doi.org/10.70176/3007-973X.1019
- Sivanandhan, G., Selvaraj, N., Ganapathi, A., and Manickavasagam, M. (2014). Enhanced biosynthesis of withanolides by elicitation and precursor feeding in cell suspension culture of Withania somnifera (L.) Dunal in shake-flask culture and bioreactor. PLoS ONE, 9(8). https://doi.org/10.1371/journal.pone.0104005
- Soni, S., Chawra, H. S., Sharma, R. K., and Garg, R. (2018). Development of drying protocol for Withania somnifera roots. Asian Journal of Pharmaceutical and Clinical Research, 11(10), 86–92. https://doi.org/10.22159/ajpcr.2018.v11i10.28402
- Surchy, S. M., Alzebari, I. N., Saeed, S. M., and Essa, I. M. (2025). Callus culture as a tool for producing phytochemicals in Glycyrrhiza glabra: A medicinal plant study. Qalaai Zanist Scientific Journal, 10(3), 1272–1293. https://doi.org/10.25212/lfu.qzj.10.3.49
- Trettel, J. R., Gazim, Z. C., Gonçalves, J. E., Stracieri, J., and Magalhães, H. M. (2018). Effects of copper sulphate (CuSO4) elicitation on the chemical constitution of volatile compounds and the in vitro development of Basil. Scientia Horticulturae, 234(19–26). https://doi.org/10.1016/j.scienta.2018.01.062
- Vats, S. (2018). Biotic and abiotic stress tolerance in plants. Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-10-9029-5
- Zafar, N., Mujib, A., Ali, M., Tonk, D., and Gulzar, B. (2017). Aluminum chloride elicitation (amendment) improves callus biomass growth and reserpine yield in Rauvolfia serpentina leaf callus. Plant Cell, Tissue and Organ Culture (PCTOC), 130, 357–368. https://doi.org/10.1007/s11240-017-1230-7
- Zafar, N., Mujib, A., Ali, M., Tonk, D., Gulzar, B., Malik, M. Q., Mamgain, J., and Sayeed, R. (2020). Cadmium chloride ($CdCl_2$) elicitation improves reserpine and ajmalicine yield in Rauvolfia serpentina as revealed by high-performance thin-layer chromatography (HPTLC). 3 Biotech, 10(344).

Iraqi Journal of Agricultural Sciences –2026:57(1):1-18
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI:

https://doi.org/https://doi.org/10.1007/s13205-020-02339-6

- Zandalinas, S. I., Fritschi, F. B., and Mittler, R. (2020). Signal transduction networks during stress combination. Journal of Experimental Botany, 71(5), 1734–1741. https://doi.org/10.1093/jxb/erz486
- Zarad, M. M., Toaima, N. M., Refaey, K. A., Atta, R. F., and Elateeq, A. A. (2021). Copper sulfate and Cobalt chloride effect on total phenolics accumulation and antioxidant activity of Artemisia annua L. callus cultures. Al-Azhar Journal of Agricultural Research, 46(2), 26–40. https://doi.org/10.21608/ajar.2021.245610
- Zheng, C., Chen, J., Wang, X., and Li, P. (2025). Reactive oxygen species in plants: Metabolism, signaling, and oxidative modifications. Antioxidants, 14(617), 1–17. https://doi.org/10.3390/antiox14060617

Iraqi Journal of Agricultural Sciences –2026:57(1):1-18
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI:

قدرة المستحثات الكيميائية على تعزيز إنتاج المستقلبات الثانوية في الكالس المستحث من أوراق البنج الاسود

محمد عبد الكريم خلف[1] الاء جبار طه[1] اشواق شنان عبد[2]

[1] قسم علوم الحياة، كلية العلوم، جامعة المستنصرية، العراق.

[2] قسم التقنيات الاحيائية النباتية، مركز بحوث التقنيات الاحيائية، جامعة النهرين، العراق.

المستخلص

يحتوي البنج الاسود على قلويدات التروبان والجلوكوسيدات الصيدلانية الرئيسية في انسجته. هدفت الدراسة الى تحسين التخليق الحيوي لهذه المركبات بوساطة تقنيات زراعة الانسجة. تم انشاء مزارع الكالس من مزدرعات الاوراق على وسط MS. ثلاثة من الاملاح الكيميائية تم اضافتها بصورة منفصلة وبتراكيز مختلفة الى الوسط الزرعي لتقييم تاثيراتها على نمو الكالس ومحتوى المركبات الصيدلانية. توضح النتائج ان النحاس والكادميوم قلل اوزان الكالس، في حين لم يظهر الالمنيوم اي تاثيرات معنوية مقارنة مع معاملة السيطرة. الا ان اختبار HPLC اثبت ان الاجهاد الكيميائي سبب تاثيرات مفيدة في رفع انتاج مركبات القلويدات والجلوكوسيدات في مزارع الكالس. باختصار، التركيز الاعلى من النحاس (36 ملغم/لتر) سبب ارتفاعاً معنوياً في محتوى الهايوسيامين والسكوبولامين والهايوسياموسايد-أ. بينما انتج الالمنيوم والكادميوم عند التركيز الاوطأ (5 ملغم/لتر) ارتفاعاً معنوياً متبايناً في المواد القلويدية والجليكوسيدية مقارنة مع سائر المعاملات والسيطرة. هذه النتائج تخلص الى ان تطبيق الاستنباط الكيميائي عند تراكيز معينة يقدم رؤى مفيدة في تطوير الظروف المتعلقة بالتخلق الحيوي للمركبات الطبية في البنج الاسود.

الكلمات المفتاحية: البنج الاسود، الجليكوسيدات، HPLC، قلويدات التروبان.