Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

Callus Induction Increase of *Trigonella foenum-graecum* L. Biomass Accumulation Indifferent Medium by 2,4-D and BA

Masara D. J. M.*[1] , S. I. Neamah [2]
[1]Department of Field Crops, College of Agriculture, University of Anbar, Iraq.
[2] Department of Horticulture, College of Agriculture, University of Anbar, Iraq.

ABSTRACT

This investigation was carried out to determine the role of the plant growth regulators 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzyladenine (BA) added to the media culture LS and B5 in the biomass accumulation of induced callus culture from the cotyledonous of *Trigonella foenum-graecum* L. different concentrations of 2,4-D at 4.0, 8.0 and 12.0 µM and BA at 0.0, 2.0, 4.0, 6.0 and 8.0 µM were tested. The surface sterilization of seeds showed that it is possible to rely on a concentration of 2.0% of NaOCl for 5 min to obtain sterile seedlings. The results also indicated that there was a significant effect of the growth regulators 2,4-D and BA added to the LS medium, as the interaction between the two concentrations of 12.0 µM of 2,4-D and 4.0 µM of BA recorded the highest means value of callus formation response (CFR), the shortest duration for the Start callus induction (SCI) and complete callus formation (CCF). At the same time, the interaction between 8.0 µM of 2,4-D and 6.0 µM of BA recorded the highest fresh weight (FW), dry weight (DW) and relative humidity (RH) for calli. The B5 medium, the interaction between the two concentrations of 8.0 µM of 2,4-D and 2.0 µM of BA recorded the highest means value of CFR. In contrast, the treatment with a concentration of 12.0 µM of 2,4-D with the control treatment of BA showed the lowest duration of callus induction initiation.

Key words: *In vitro*, Plant growth regulators, Relative humidity.

Received: 3/11/2023, Accepted: 18/2/2024, Published: 30/6/2026

INTRODUCATION

Trigonella foenum-graecum L. is a herbaceous annual plant belonging to the Fabaceae family that has been used since ancient times as a type of spice (Aasim *et al*,2018). It is also used in many industrial and medical fields, as it is used in the manufacture of perfumes, beverages, and cosmetics (Djeridane *et al*,2006) It is characterized by its high content of secondary metabolites, including phenolic acid, alkaloids, saponins, and flavonoids [Kenny et al,2010, Omezzine *et al*,2014, Syed *et al*,2020, Wani and Kumar ,2018,Yasothai ,2021).]. These compounds derived their therapeutic properties as antioxidant, anticancer, blood sugar regulator (Ruwali *et al*,2022) and anti-inflammatory (Sindhu *et al*,2018), as well as their role in the treatment of liver disease, hypercholesterolemia, cardiovascular disease, and testosterone disorders (Aasim *et al*,2018, Park *et al*,2019). Plant tissue culture is one of the essential ways to develop agricultural production, as it can solve many problems that are difficult to solve with traditional agriculture. It is considered one of the means used in the development and cultivation of medicinal plant thus, the production of effective compounds. The compounds are available from their natural sources without adherence to the cultivation season and without being affected by environmental conditions and agricultural pests in small spaces inside the laboratory with the possibility of controlling the production

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

process of these compounds at a lower cost and higher purity than its counterpart field. Therefore, this technology was used to protect the diminishing wild plants known for their high content of secondary compounds and to develop strains characterized by their high content of these compounds, in addition to the possibility of producing these compounds based on the technique of culture of callus or cell suspensions (Wani and Kumar ,2018).). Plant growth regulators play an important role in the growth and development of plant cells through their regulation of many vital processes (Abu Zaid,2003).). Auxins, including (2,4-D) 2,4-Dichlorophenoxy acetic acid, contribute to stimulating the processes of cell division and elongation, as it takes place through the role of auxins in displacing the ions that support the cell wall, which gives it the softness and flexibility necessary for cell expansion. cytokinins, including 6-Benzyladenine (BA), also contribute to cell division, as it supports synthesis of proteins, nucleic acids and enzymes necessary for cell division (Taiz and Zeiger ,2010).). It should also be noted that the synergistic relationship is within safe limits for both Phyto regulators, which is positively reflected in the development of callus tissue, given its great importance as the cornerstone of tissue culture (Bhatla and Lal ,2018) The media culture intended to develop plants in vitro differ in their chemical components. Accordingly, they differ in their ability to establish tissue cultures, as many studies contributed to the formulation of different ideas about the role of these media with the elements and vitamins they contain in the development of plants (Ibrahim ,2017) Based on this, the study aimed to determine the ability of growth regulators 2,4-D and BA to improve the induction and accumulation of biomass for *T. foenum-graecum* callus cultures growing in two different nutrient mediums, LS and B5, by studying their effects on growth and physiological indicators, thus the possibility of exploiting them to increase biomass for calli cultures.

MATERIALS AND METHODS

Tools sterilization: The tools used in cultivation, distilled water, and media culture prepared for cultivation were sterilized using an Autoclave at 121 ˚C for 15 min and a pressure of 1.04 kg cm^2. The laminar airflow cabinet has been sterilized with a 70% Ethanol solution.

Surface Sterilization of Seeds: An experiment was conducted to sterilize the seeds after washing them under running tap water with detergent powder added for 30 min. Then, it was transferred to a laminar airflow cabinet to perform the surface sterilization process using sodium hypochlorite (NaOCl) at a concentration of 0, 2.0, 4.0, and 6.0% for 5, 10 and 15 min. Then, they were rinsed with sterile distilled water three consecutive times to remove traces of the sterilizer. Seeds per vials were cultured in MS medium. The media was prepared by dissolving 4.43 g L^{-1} MS and 30 g L^{-1} sucrose. Then, the pH was adjusted to 5.7 ± 0.1, and 7.0 g L^{-1} agar was added. Vials were placed in a growth chamber at 25±1 $^{\circ}$C under a light intensity of 1000 lux and 16-h photoperiod with white fluorescent light.

Induction of callus culture: Ready-made, Caisson-produced LS and B5 media culture devoid of growth regulators. The dietary media used in the study were prepared independently by adding 4.73 and 3.21 g L^{-1} to LS and B5 media, respectively. Also, 20 g L^{-1} sucrose was added. 2,4-D was added at a concentration of 4.0, 8.0, and 12.0 µM. The growth regulator BA of 0, 2.0, 4.0, 6.0, and 8.0 µM was added, after which the pH was adjusted to 5.6. Then, it was added agar at 7.0 g L^{-1}. The media was sterilized according to the method described in instrument sterilization. The cotyledon were taken from sterile seedlings at the age of 10 days by cutting a part of them with a length of 1 cm using the surgical blade with scratches to be cultured in the two prepared media. The cultures were incubated under the same previous physical conditions.

Experimental Design and Statistical Analysis: All experiments were conducted using a completely randomized design (CRD) with ten replications. Data were analyzed statistically by two-way analysis of variance using GenStat version 12 Differences between mean values were calculated using LSD at 5% level of significance.

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

RESULTS AND DISCUSSION

Surface Sterilization of Seeds: The results in Figure 1 show that there was a significant differences ($p < 0.05$) among treatments with NaOCl and the sterilization period to obtain sterile seedlings, as the concentration achieved 2.0% of NaOCl at 5 or 10 min, an average of 100% of the trait. The concentration 6.0% when treated for 10 or 15 min at an mean value of 100%. The concentration achieved 4.0% NaOCl for 15 min with an mean value of 100% for the trait, while the lowest average appeared when the seeds did not treated with a 50% NaOCl solution for 5 min.

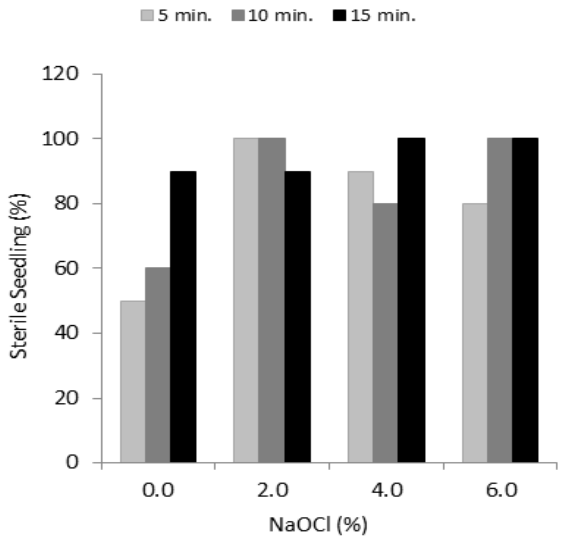


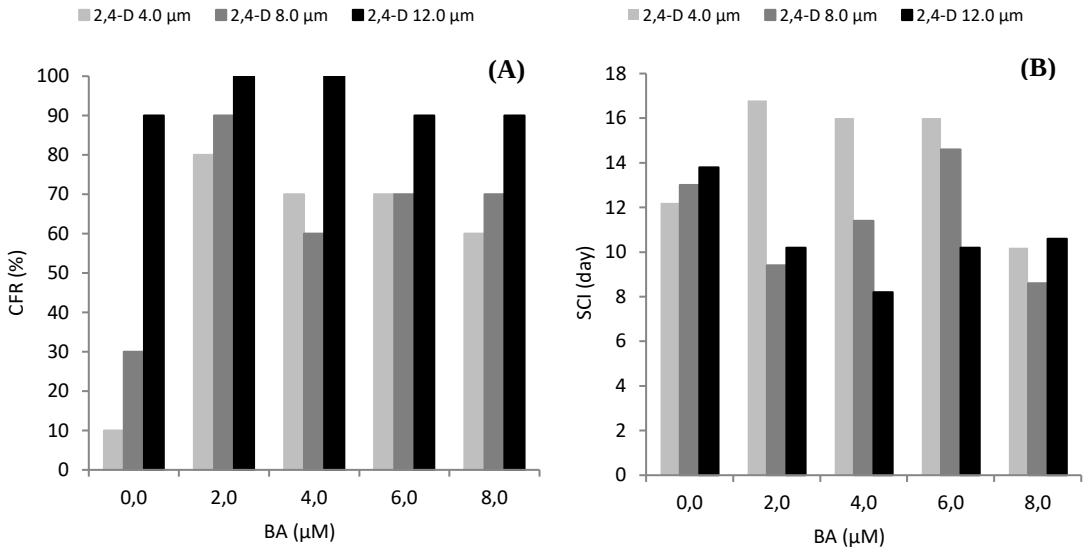
Figure 1. Surface sterilization of *T. foenum-graecum* L. seeds treated with 0, 2, 4 for 6% NaOCl and 5, 10 and 15 min

Induction and development of cultured biomass in LS medium: The effects of treatment with 2,4-D and BA on biomass accumulation of *T. foenum-graecum* L. cultures grown in LS medium were evaluated by determining changes in some growth and physiological parameters (Figure 2). The results of Figure 2 show a significant effect of concentrations of 2,4-D and BA on the callus formation response (CFR), as the combination (12.0 µM of 2,4-D+2.0 or 4.0 µM of BA) recorded the highest stimulation of callus tissue at 100%. In contrast, the concentration of 4.0 µM of 2,4-D with the control treatment of BA show the lowest induction of callus tissue at 10%. The study factors had a significant effect on the Start callus induction (SCI), as the concentration of 8.0 µM for each of 2,4-D and BA recorded the lowest duration for the SCI was 8.60 days. In comparison, the highest duration for the SCI appeared at the combination of 4.0 µM of 2 ,4-D with 2.0 µM of BA, reaching 16.80 days. The period required for complete callus formation (CCF), the combination (12.0+4.0 µM) for each of 2,4-D and BA, respectively, achieved the lowest period for CCF, reaching 21.60 days. In comparison, the highest period for CCF reached 32.20 days when the combination 4.0 µM of 2,4-D and 2.0 µM of BA (Figure 2C). There was a significant difference ($p < 0.05$) as a result of the treatment with 2,4-D and BA in the fresh weight (FW) of the induced callus culture, as the concentration of 8.0 µM of 2,4-D+6.0 µM of BA achieved the highest tissue FW. The induced callus was 545 mg. While the lowest mean value of the induced callus FW was 118 mg when the 4.0 µM mixture of 2,4-D was obtained with the comparison treatment of BA (Figure 2D).The dry weight (DW) of study factors affected it significantly, as the combination between 2,4-D and BA was achieved under the two concentrations of 8.0+6.0 µM, respectively. The highest DW of the induced callus culture was 46.8 mg, while lower back, the mean DW of the induced callus was 5.3 mg at the low concentration of both regulators (Figure 2E). It is reveal from the results in Figure 2F that there is a significant difference in the interaction between the concentrations of 2,4-D and BA in the relative humidity (RH) of the induced callus culture, as the treatment recorded 8.0 µM of 2,4-D and 6.0 µM of BA, the highest mean value of RH amounted to 92.82%, While, the lowest mean value was 79.78% when the combination included the lower levels of growth regulators.

Induction and development of biomass cultured in B5 medium: The effects of treatment with 2,4-D and BA on biomass accumulation of *T. foenum-graecum* L. cultures grown in B5 medium were evaluated by determining changes in some growth and physiological parameters (Figure 3). The results show that there are significant differences for the study in the CFR, as the concentration achieved (4.0 µM of BA+6.0 or 8.0 µM of 2,4-D) or (8.0 µM of 2,4-D+2.0 µM

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

of BA) or (12.0 µM of 2,4-D+6.0 µM of BA) had the highest CFR reaching 80% for each of the previous combinations. While the lowest CFR appeared at a concentration of 4.0 µM of 2,4-D, and the comparison treatment of BA was 30%. (Figure 3A). The highest reduction was observed in the SCI when treated with a concentration of 8.0 µM for each of 2,4-D and BA, which reached 5.40 days. While, the highest time needed to SCI appeared at a concentration of 8.0 µM of 2,4-D mixed with a concentration of 6.0 µM of BA, which amounted to 15.20 days (Figure 3B). The results show a significant differences among concentrations of 2,4-D and BA in the CCF, as the concentration of 12.0 µM of 2,4-D with 4.0 µM of BA achieved the lowest duration for CCF of 22.00 days. While, the most extended period for CCF appeared when the interaction between the two treatments was 0.0 µM+4.0 µM, and the highest or lowest concentrations of growth regulators 2,4-D and BA, respectively, reached 29.40 days (Figure 3C). The highest FW of callus culture observed when treated with 8.0 µM of 2,4-D with 6.0 µM of BA was 587 mg. While the lowest mean values of FW appeared when the concentration of 4.0 µM of 2,4-D overlapped with the comparison treatment of BA amounted to 177 mg (Figure 3D). The highest significant DW of callus culture was obtained when treated with 8.0 µM of 2,4-D with 6.0 µM of BA of 38.3 mg. The lowest DW of induced callus appeared when the concentration was 4.0 µM of 2,4-D with the comparison treatment of BA, which amounted to 10.2 µM (Figure 3E). The highest mean RH values resulted from the treatment with a concentration of 8.0 µM for both growth regulators, which amounted to 90.58%. In contrast, the lowest mean values of induced callus appeared at the lowest concentration of 2,4-D, and BA amounted to 77.16% (Figure 3F). The production of sterile seedlings to eradicate the appropriate explant is the first step in developing tissue cultures in pollution-free conditions. NaOCl is usually used in specific concentrations and durations for surface



Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

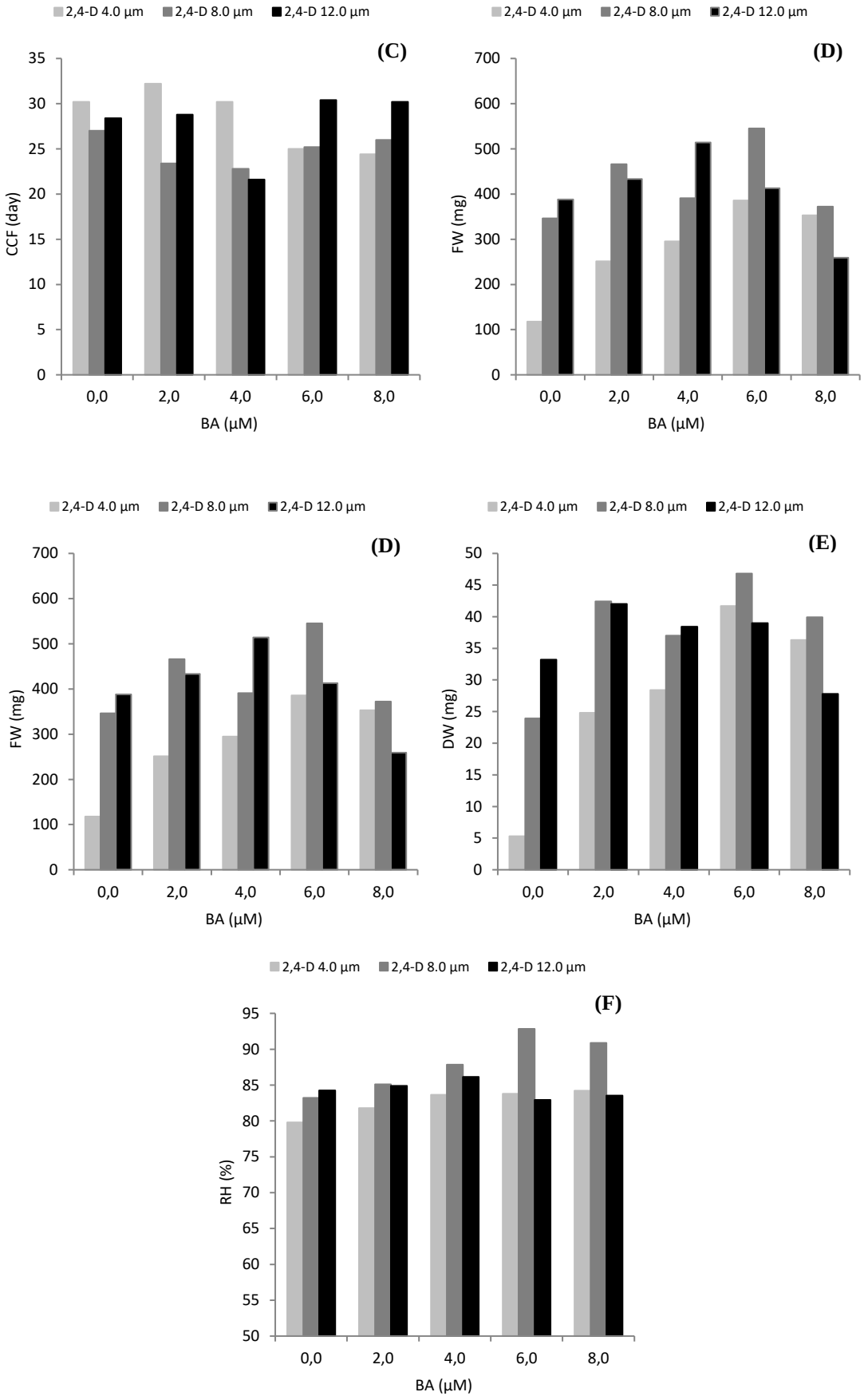


Figure 2. CFR (A) SCI (B) CCF (C) FW (D) DW (E) RH (F) in a *T. foenum-graecum* L. calli cultured in LS media treated with 4, 8 and 12 µM 2,4-D and 0, 2, 4, 6 and 8 µM BA.

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

sterilization of seeds. The increase in the concentration and duration of treatment with NaOCl reflected a positive effect on obtaining sterile seedlings, as more than one combination appeared 100%. However, in our subsequent experiments, we relied on the combination (2.0%×5 min) to consider the economic feasibility (Figure 1). The effect of NaOCl as a sterilizer for plant tissues is due to hypochlorous acid (HOCl), which is a strong oxidant. This acid is produced from the dissolution of chlorine with water (Ramawat KG,2004) These are similar results for Al-Oubaidi (2021). on *Physalis peruviana* Plant growth regulators (2,4-D and BA) contributed to the improvement of growth, and physiological indicators of callus tissue, as high concentrations of 2,4-D had a high affinity for improving callus induction with 2.0 µM of BA. This could be observed in the LS media (Figure 2A). The treatments reduced the CSI, as it is a primary aim in its development. However, the onset of callus induction cannot be considered an indicator of its formation's completion. The dose could be induce callus, but it cannot continue growth, which causes an increase in the CCF (Figure 3B, C). The response of the explant to the stimulation and formation of callus culture depends mainly on the plant regulator's type and concentration and the explant hormonal level, leading to the highest response to the formation of callus tissue and its continued growth. The cotyledon as the explant from which callus was induced in our study, has a high ability to form callus tissue, as callus usually arises due to the multiple divisions of the plant cell. As for the decrease in the level of CFR under high concentrations of 2,4-D and BA, it may be an indicator of the toxicity suffered by the cultivated explant, as increasing the concentration leads to an opposite condition, causing inhibition of the vital pathways responsible for inducing callus from the explant (Hayat *et al* 2007) The middle levels of 2,4-D and BA for both media contributed to improving FW, DW and RH. The increases in FW and DW of calli cultures reflects the different metabolic accumulations of the cells, which depends on the chemical components of the medium, including plant growth regulators. In general, the division of callus cells is accompanied by an increase in proteins, nucleic acids, and essential enzymes to sustain division and increase growth revealed Kasprzyk-Pawelec *et al*,2015, Saini HK *et al*,2010, Serma et al,2014) This was observed in the treatment of a concentration of 8.0 µM 2,4-D with 6.0 µM BA in obtaining the highest mean values of FW and DW of the induced callus (Figure 2,3-DE). The high levels of auxins in the treatment with a concentration of 12.0 µM 2,4-D and cytokinins with a 8.0 µM of BA, they cause a decrease in the growth and division rates of cells due to the disturbance of vital processes within the tissues, as a result of hormonal imbalance, which leads to the formation of ethylene, which In turn, it inhibits metabolic activities and decreases the rates of division and growth (Devlin and Witham ,1983). The medium cultures used in this study differed relatively in stimulating the growth of callus tissue due to the concentrations of 2,4-D and BA. This indicates the difference in the compatibility of growth regulators with these media components in biomass accumulation. In general, FW is the main criterion in determining the biomass of callus tissue, and it was affected by some parameters, especially DW and RH. The results reveal did not such a strong relationship with other indicators, including CFR, SCI and CCF, especially in the B5 medium. Based on this, it can be said that the combination needed to stimulate callus cultures is not necessarily appropriate for the development of biomass of callus tissue in the fenugreek.

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

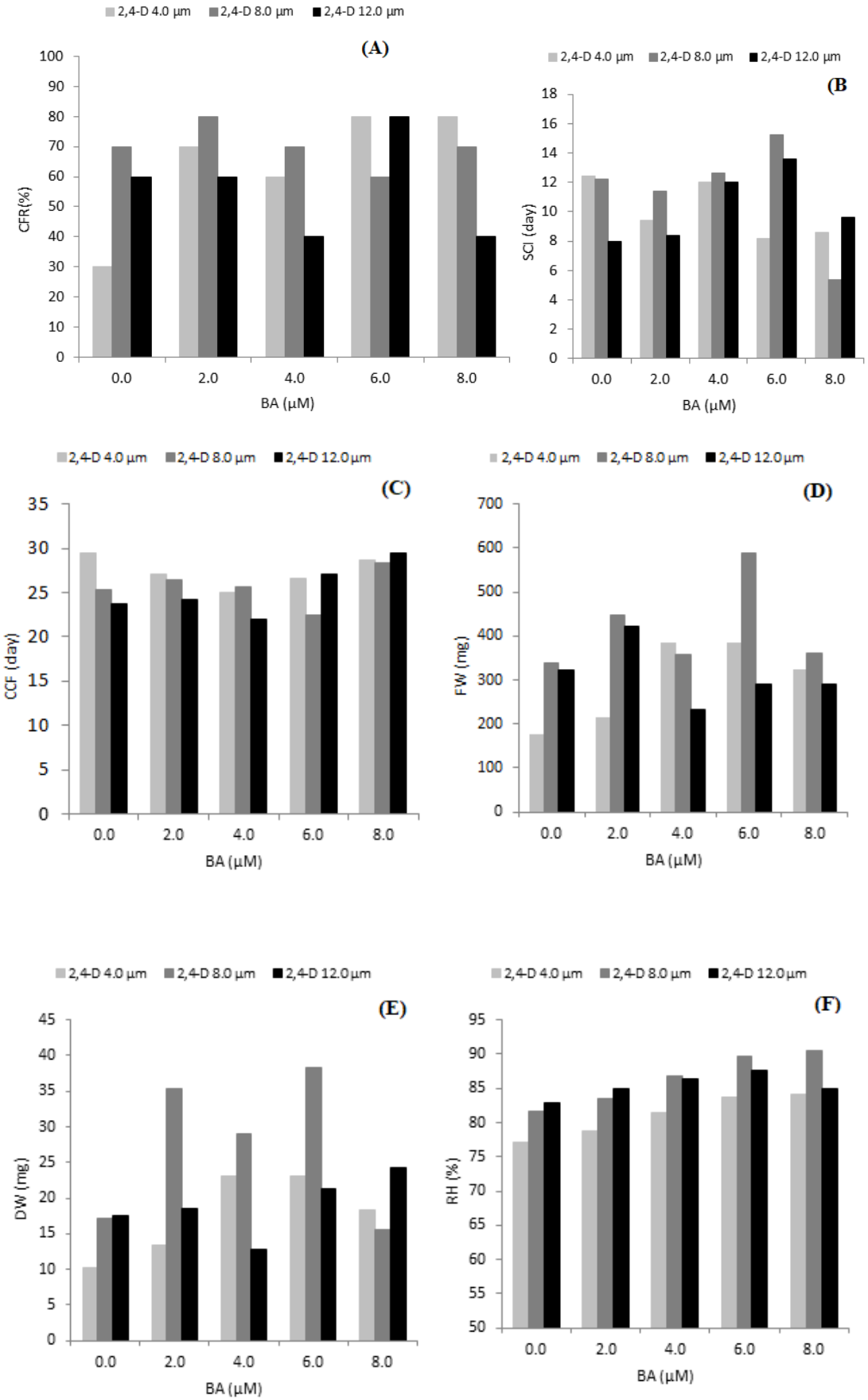


Figure 3. CFR (A) SCI (B) CCF (C) FW (D) DW (E) RH (F) in a *T. foenum-graecum* L. calli cultured in B5 media treated with 4, 8 and 12 µM 2,4-D and 0, 2, 4, 6 and 8 µM BA.

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

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.

REFERENCES

Aasim, M.; Baloch F. S., Nadeem M. A., Bakhsh A., Sameeullah M & Day S. (2018). Fenugreek (*Trigonella foenum-graecum* L.): An underutilized edible plant of modern world. Global Perspectives on Underutilized Crops, Springer, pp. 381–408.

Abu Zaid, A. N. (2003). Plant Hormones and Agricultural Applications, Third Edition. Arab House of Publishing and Distribution, Arab Republic of Egypt, pp. 351.

Al-Oubaidi, M.H.A. (2021). In vitro *Physalis peruviana* Response To Nanoparticles And Their Effects On Secondary Metabolite And Genetic Variation. M.Sc. thesis, University of Baghdad, Baghdad, Iraqi. pp: 148.

Bhatla, S. C & Lal, M. A. (2018). Plant Physiology, Development and Metabolism. Springer, pp. 1237.

Devlin, R. M & Witham, F. H. (1983). Plant Physiology, (Fourth ed). Wadsworth Publishing Company Belmont California. pp: 340.

Djeridane, A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, & Vidal N. (2006). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chemistry, 97: 654–660. https://doi.org/10.1016/j.foodchem.2005.04.028

Hayat, Q, Hayat S, Irfan M, & Ahmad A. (2010). Effect of exogenous salicylic acid under changing environment: a review. Environ Exp Bot., 68:14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005

Ibrahim, K.M. (2017). Applications in Plant Biotechnology. College of Biotechnology, Al-Nahrain University, pp. 680.

Kasprzyk-Pawelec A, Pietrusiewicz J, & Szczuka E. (2015). In vitro regeneration induced in leaf explants of *Citrus limon* L. Burm cv.'Primofiore'. Acta Sci Pol Hortoru., 14(4): 143-153.

Kenny ,O., Smyth T.; Hewage C.M & Brunton N.P. (2013). Antioxidant properties and quantitative UPLC–MS analysis of phenolic compounds from extracts of fenugreek (*Trigonella foenum-graecum*) seeds and bitter melon (*Momordica charantia*) fruit. Food Chem., 141: 4295–4302. https://doi.org/10.1016/j.foodchem.2013.07.016

Omezzine, F.; Bouaziz M.; Daami-Remadi M.; Simmonds M.S.J & Haouala R. (2014). Chemical composition and antifungal activity of *Trigonella foenum-graecum* L. varied with plant ploidy level and developmental stage. Arab J Chem., 10(2): 3622-3631. https://doi.org/10.1016/j.arabjc.2014.03.013

Park, H.J., Lee, K.S., Lee E.K., & Park N.C. (2019). Efficacy and safety of a mixed extract of *Trigonella Foenum-graecum* seed and *Lespedeza cuneate* in the treatment of testosterone deficiency syndrome: A randomized, double-blind, placebo-controlled trial. J. Urol., 201. https://doi.org/10.5534/wjmh.170004

Ramawat, K.G. (2004). Plant Biotechnology. S. Chand and Company LTD, Ram Nagar, New Delhi. India. pp. 50-62.

Ruwali, P., Pandey, N., Jindal, K., & Singh R.V. (2022). Fenugreek (*Trigonella foenum-graecum*): nutraceutical values, phytochemical, ethnomedicinal and pharmacological overview. S Afr J Bot., 151: 423-431. https://doi.org/10.1016/j.sajb.2022.04.014

Saini, H. K, Gill, M. S, & Gill, M. I. S. (2010). Direct shoot organogenesis and plant regeneration in rough lemon (*Citrus jambhiri* Lush.). Ind. J. Biotech., 9: 419:423.

Serma, C., Borthakur, A., Singh ,S., Modi, M.K, & Sen, P. (2011). Efficient in vitro plant regeneration from cotyledonary explants of *Citrus reticulata* L. Blanco. Ann Biolo Res., 2(6): 341-348. http://dx.doi.org/10.13140/2.1.3560.2560

Sindhu, G., Shyni, G.L., Pushpan, C. K., Nambisan, B., & Helen, A. (2018). Evaluation of anti-arthritic potential of *Trigonella foenum-gracum* L. (Fenugreek) mucilage against rheumatoid arthritis. Prostag Oth Lipid M., 138: 48-53. https://doi.org/10.1016/j.prostaglandins.2018.08.002

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

Syed, O.A., Rashid, Z., Ahmad, M.H., Shukat, R., Ishaq, A., Muhammad, N., & Rahman, H.U. (2020). Nutritional and therapeutic properties of fenugreek (*Trigonella foenum-graecum*): a review. Int J Food Proper., 23 (1): 1777–1791. https://doi.org/10.1080/10942912.2020.1825482

Taiz, L., & Zeiger, E. (2010). Plant Physiology, fifeth ed. Sinauer Associates, Inc., publishers, Sunderland, Massachusetts, U.S.A.

Vinay, S and Afroz ,A. (2015). Plant Tissue Culture. Springer, pp. 529.

Wani, S.A & Kumar, P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. J. Saudi Society Agri. Sci., 17: 97-106. https://doi.org/10.1016/j.jssas.2016.01.007

Yasothai, R. (2021). Chemical composition of fenugreek (*Trigonella foenum-graecum* L.) seed and galactomannan depleted fenugreek residue. The Pharma Innovation J., 10(7): 944-947. 10.22271/tpi.2021.v10.i7t.7105

Iraqi Journal of Agricultural Sciences –2026:57(6):1797-1806
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fxvhcc92

زيادة استحثاث مزارع الكالس وتراكم الكتلة الحيوية لنبات *Trigonella foenum-graecum* L في أوساط مختلفة باستخدام 2,4-D وBA

مسرة ظافر جاسم ، شامل إسماعيل نعمة

قسم المحاصيل الحقلية، كلية الزراعة، جامعة الانبار، العراق. قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة الانبار، العراق.

المستخلص

أجري هذا البحث لتحديد دور منظمات نمو النبات2 ، 4-D و BA المضافين إلى الوسط الزرعي LS و B5 في تراكم الكتلة الحيوية لمزارع الكالس المستحثة. من الورقة الفلقية لنبات *Trigonella foenum-graecum* L. من خلال اختبار تراكيز مختلفة من 2,4-D عند 4.0 و8.0 و12.0 ميكرومتر و BAعند 0.0 و2.0 و4.0 و6.0 و8.0 ميكرومتر. أظهر التعقيم السطحي للبذور أنه من الممكن الاعتماد على تركيز 2.0% من NaOCl لمدة 5 دقائق للحصول على بادرات معقمة. أشارت النتائج أيضًا إلى وجود تأثير معنوي لمنظمات النمو 2,4-D و BA المضافة إلى الوسط LS ، إذ سجل التداخل بين التركيزين 12.0 ميكرومتر من 2,4-D و 4.0 ميكرومتر من BA أعلى متوسط قيمة استجابة لتكوين الكالس وأقصر مدة لبدء تكوين الكالس واكتمال تكوين الكالس في نفس الوقت سجل التداخل بين 8.0 ميكرومتر من 2,4-D و 6.0 ميكرومتر من BA أعلى وزن طري ووزن جاف ورطوبة نسبية لنسيج الكالس. أما بالنسبة لنتائج الوسط B5 فقد سجل التداخل بين التركيزين 8.0 ميكرومتر من 2,4-D و 2.0 ميكرومتر من BA أعلى متوسط لاستجابة تكوين الكالس.

الكلمات المفتاحية: الحلبة، خارج الجسم الحي، منظمات نمو النبات، الرطوبة النسبية.