IMPACT OF IRON OXIDE ON CAPSAICIN ALKALOID PRODUCTION FROM *Capsicum annuum* L. *IN VITRO*

E. H. Jassim [1] * **R. K. Mohammed [2]**
Assist. Prof. **Assist. Prof.**
[1] Inst. Genet. Engin. Biot. Post. Stud., University of Baghdad.
[2] Biol. Dept., Coll. Sci. , University of Baghdad.
*** Email: emadhamdi@ige.uobaghdad.edu.iq**

ABSTRACT

The chili pepper plant *Capsicum annuum* L. is used in processed foods and pharmaceuticals given the importance of Capsaicin; it has been produced through cell cultures of chili pepper obtained from a media known as Murashige and Skoog (MS), which is supplied by the best concentration1.5 and 0.3 mg/L of 2,4-Dichlorophenoxyacetic acid (2,4-D) and kinetin (kin.), respectively, gave highest fresh and dry weights of callus, reaching (368.06,29.40) mg respectively which was significantly different from other treatments. When determining the alkaloid compound capsaicin by HPLC, Its amount in the callus was found to be 3.8 µg/g after being treated with a nano dose of 0 mg/L. an increase of 1.5 fold over the quantity in the leaves of the plant growing in the field, which reached 2.5 µg/g. In order to increase the synthesis of active compound studied stimulated by iron oxide nanoparticles at concentrations (0, 2, 4, 6, 8, and 10) mg/L, the findings indicated that 6 mg/L caused a highly significant increase in Capsaicin, reaching 6.2 µg/g by the amount 1.6 fold compared to 3.8 µg/g in the treatment free the nanocatalyst. While the concentration of 6 mg/L achieved a 2.5-fold increase in its presence in the chili pepper intact leaf sample, the results show that Fe_3O_4NPs were effective in stimulating the accumulation of capsaicin compound.

Keywords: active compounds, chili pepper , abiotic catalysts, plant tissue culture.

تأثير أكسيد الحديد على إنتاج قلويد الكابسيسين من *Capsicum annuum* L. خارج الجسم الحي

عماد حمدي جاسم [1] **رشا كريم محمد [2]**
أستاذ مساعد **أستاذ مساعد**
[1] معهد الهندسة الوراثية والتقنيات الأحيائية للدراسات العليا , جامعة بغداد
[2] قسم علوم الحياة ,كلية العلوم جامعة بغداد

المستخلص

نبات الفلفل الحار *Capsicum annuum* L. يستخدم في الأغذية المصنعة والمستحضرات الصيدلانية ولأهمية الكابسيسين، تم إنتاجه من خلال مزارع الخلايا من الفلفل الحار, تم الحصول عليها بواسطة وسط موراشيج وسكوج المضاف إليه حمض ثنائي كلورو فينوكسي أسيتيك (2,4-D) والكينيتين (kin.) بتركيز 1.5 و 0.3 ملغم /لتر على التوالي، والتي أعطت أعلى وزن طازج وجاف للكالس، حيث بلغ (368.06، 29.40) ملغم على الترتيب ، وهو مختلفاً معنويا عن بقية المعاملات الأخرى. وعند تحديد المركب القلويدي الكابسيسين بواسطة HPLC وجد أن كميته في الكالس المعامل بتركيز 0 ملغم/لتر من النانو كانت 3.8 ميكروغم/غم بزيادة قدرها 1.5 ضعف عن كميته في أوراق النبات النامية في الحقل، والتي بلغت 2.5 ميكروغم/غم، ولغرض زيادة كمية المركب الفعال المدروس المحفز بواسطة جسيمات أكسيد الحديد النانوية عند تراكيز (0، 2، 4، 6، 8 و10) ملغم/لتر، أظهرت النتائج أن تركيز 6 ملغم/لتر تسبب في زيادة معنوية عالية في مركب الكابسيسين حيث وصل إلى 6.2 ميكروغرام/غرام أي بمقدار 1.6 ضعف مقارنة بكميته البالغة 3.8 ميكروجرام/جرام في معاملة الكالس خالية من المحفز النانوي، بينما حقق تركيز 6 ملغم/لتر زيادة بمقدار 2.4 ضعف وجوده في عينة أوراق الفلفل الحقلي ، ويتضح من النتائج أن أكسيد الحديد النانوي كان مؤثرا في تحفيز تراكم مركب الكابسيسين.

الكلمات المفتاحية: مركبات فعالة ، الفلفل الحار، المحفزات غير الحيوية، زراعة الأنسجة النباتية.

Received:12/9/2024, Accepted:15/1/2025

INTRODUCTION

Medicinal plants have been used since ancient times to treat a variety of illness (16) and in the development of synthetic drugs as they are considered to have therapeutic value (29,35).These plants contain natural compounds,such as phenolic compounds terpens and alkaloids (31), and can treat many diseases (6,12).Among these plants is the chili pepper (*Capsicum annuum*), which belongs to the nightshade family and is commercially important and grown all over the world. It is known for its pungent taste with a chemical content known as capsaicinoids, 6-zonisamide is amember of 8-methyl-N-vanillyl and dihydrocapsaicin derived from capsaicinoids. (26) Capsaicin isalkaloids a crystalline, aromatic, colorless, hydrophobic. Additionally, capsaicinoids are an active component in personal defense and riot control sprays. Comes into contact with the skin, especially the eyes(23). Hot peppers are used as spices in cooking, and they have a high concentration of vitamins, including folic acid, thiamine, calcium, vitamins A, C, and B6, ect.(15) Active compounds such as capsaicin are also a treatment for coughs, toothaches, parasitic infections, and rheumatism, along with obesity and type 2 diabetes, and halting the incidence of prostate cancer and as an anti-arthritic, antibacterial, anti-rhinitis, pain reliever, and antifungal (8,10) for headaches, irregular heartbeat, inflammation, rheumatism, joint stiffness, and bronchitis. It is safe to use, beneficial and has no side effects compared to chemically manufactured drugs. Currently, plants used for treatment grow either naturally or by cultivation (4). Converting them into a pharmaceutical project requires expanding their cultivation in the field, and therefore, we need to cultivate larger areas at the expense of other critical economic crops; in addition to fertilization, the problem of water for irrigation, the cost of labor, and the length of the season from planting seeds to the stage of fruit formation from which the required medicine is extracted. Despite all this, their production is limited and in quantities that are not sufficient to meet the needs of the pharmaceutical market (21). Nanotechnology has emerged as a promising topic of research in all fields and it relies on the use of ultra-fine particles , ranging in size from 1 to 100 nanometers(2).Therefore, we resort to using plant biotechnologies to produce alkaloids represented by cultivating plant tissue cells in the laboratory , which gives the production of the same compound in the naturally grown plant, but in larger quantities and a smaller area with a shorter period via employing nanotechnology in the laboratory with adding Iron oxide nanoparticles as a atalyst to increase the production of economically valuable capsaicin in plant cells.

MATERIALS AND METHODS

Sterilized seeds and germination

Seeds of *Capsicum annuum* L. were purchased from the local market in Baghdad and then disinfected with alcohol Seventy percent at 60 seconds and sodium hypochlorite 3% for 2 minutes following a sterile distilled water wash, then sown on essential MS medium. With thirty grams of sucrose and seven grams of agar at pH 5.5. They were kept under Light and dark, correspondingly, at (16/ 8) hours (22).

Initiation of culture: After the emergence of seedlings, small leaves were selected and cut into suitable sizes 1 cm to grow in MS medium with Kin. (0, 0.3, 0.6) mg/l and 2,4-D (0, 0.5, 1, 1.5, and 2) mg/l to initiate callus, cultured transfer to a grow room with ideal conditions 25 ± 1°C , after one month, the induced callus. Afterward, To capture dry weight data, weigh the fresh callus and dry it in an oven set at 45 °C for two days (32). with a sensitive balance to determine the best combination of hormones. Cultivation operations were carried out in the same medium, which gave the highest callus weight so could obtain more callus for the followingexperiments.

Elicitation treatments : Iron oxide nanoparticles (Fe_3O_4NPs) were dissolved in distilled water to make a stock, then employed at various doses (0, 2, 4, 6, 8, or 10 mg/L). From Sigma USA, the size of NPs was 20 nm were added to the basal medium comprising, respectively, (1.5, 0.3) mg/L of 2,4-D and Kin. which gave the best result, 150 mg of callus were placed on the medium for each concentration for thirty days ideally, at 25 ± 1 °C, in the absence of Light, then the data were collected for the fresh and dry weighing

of callus, for each consecration was analyzed by HPLC to record the values of capsaicin alkaloid.

Estimation of capsaicin from callus

The capsaicin alkaloid was quantitatively estimated to a weight of 150 mg of induced callus cells from the leaves were taken for each treatment and then compared with 2 grams of plant leaves planted in the field according to the method callus extraction of Chanthai *et al.* (9) utilizing Solid-Phase Extraction (SPE). Two milliliters of methanol and two milliliters of deionized water were used to condition the solid-phase cartridge. After being diluted with 600 µL of deionized water, the 400 µL of extracted material was put into the conditioned cartridge. Two milliliters of methanol were used to elute the capsaicin twice, and the final volume was adjusted to 10 milliliters .

High performance liquid chromatography conditions : The HPLC model (SYKAM) from Germany was used to examine the sample under high-performance liquid chromatography (HPLC) conditions. The column separation was C18 – ODS (25 cm * 4.6 mm), the detector was UV – 220 nm, and the mobile phase was MeOH: D.W = (80: 20). The flow rate was 1.0 ml/min.

Statistical analysis: Experiments were conducted employing SAS (2018) to perform both analyses of variance and mean in a completely randomized design (CRD).(34) use ten replicates and a 5% significance level ($P < 0.05$) for the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Callus induction: The results in both Tables (1) and (2) demonstrated that the average had nutrient medium compared to the treatment free of this auxin; since the maximum average rate of 243.07 mg clearly increased significantly. Fresh weight of callus within all concentrations of 2,4-D added to the was obtained at 1.5 mg/L since the weight decreased at the concentration of 2 mg/L less than the previous concentration, but it remained significantly higher than the comparison treatment. It is also noted that the comparison treatment of 2,4-D did not show any response to callus induction, which indicates the importance of auxin in stimulating callus formation. 2,4-D can make plant cells into an undifferentiated state and start dividing rapidly (13,28). The same two tables also show that the addition of Kin. This drove to a significant increased in fresh weight of the callus, as it reached the highest rate (192.96) mg at 0.3 mg/L, which differed significantly from the rest of the concentrations, and the lowest weight of the callus was observed in the control at 164.43 mg. Cytokinin plays a role in cell division by encouraging the conversion from the G1 to the G2 phase, leading to the production of proteins or enzymes necessary for the mitotic phase (27). As shown in Table 1,2, the data indicate that the 1.5 mg/L concentration of auxin 2,4-D and 0.3 mg/L of kinetin, a growth regulator belonging to the group of cytokinin, were effective in inducing callus formation from *C. annuum* leaves, giving the highest rate For the weights (368.06, 29.40) mg respectively (Figure 1, a and b) Which morally outperformed all other treatments. Otherwise, 0.5 mg/L 2,4-D without Kin showed lowest fresh weight callus. That amounted (119.26, 9.76) mg. The appropriate doses will induce callus cell proliferation and promote cell division from explants (30). At the same time, the treatment free of 2,4-D did not show any induction of callus with all Kin. Concentrations. Both regulators work well together, and kinetin is frequently employed to promote cell proliferation in plant tissue culture (14).

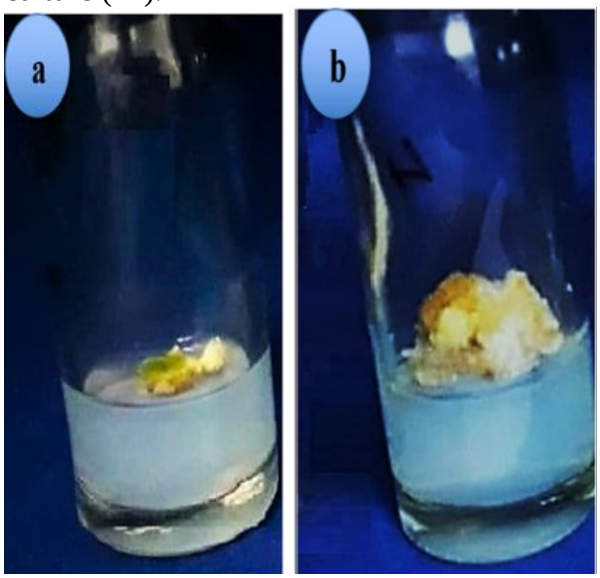

Figure 1. Comparison of the callus morphology induced from leaves on the control (a) and the optimal callus induction treatment (b).

Table 1. Impacts 2, 4-D, and Kin. on the fresh weight (mg) of callus formed from *C. annuum* leaves.

2,4- D (mg/L)	Kin. (mg/L)			Average
	0	0.3	0.6	
0	0	0	0	0
0.5	119.26	185.23	168.43	157.64
1.0	150.96	198.40	188.23	179.28
1.5	164.43	368.06	196.40	243.07
2.0	177.36	212.86	222.36	204.20
Average	122.47	192.96	155.08	--------
L.S.D. 0.05	2,4- D: 4.33 * ,	Kin.: 3.35* ,	2,4- D x Kin.: 7.50*	

Table 2. Effect of Kin and 2, 4-D on callus dry weight (mg)induced from*C. annuum* leaves

2,4- D (mg/L)	Kin. (mg/L)			Average
	0	0.3	0.6	
0	0	0	0	0
0.5	9.76	14.70	13.43	12.63
1.0	12.06	15.86	15.06	14.33
1.5	13.13	29.40	15.70	19.41
2.0	14.13	17.03	17.76	16.31
Average	9.82	15.40	12.39	----------
L.S.D. 0.05	2,4- D: 0.35 * ,	Kin.: 0.27* ,	2,4- D x Kin.: 0.60 *	

***Capsicum annuum* callus and leaves contain secondary metabolites**

Data presented in Table (3) and (Figures 2,3and4),clearly demonstrated that the active metabolites evaluated by HPLC in the callus samples without Fe_3O_4NPs recorded the highest amount of Capsaicin 3.8 µg/0.15g. Compared to the same compound found in the tissues of plant leaves grown under field conditions, it reached 2.5 µg/2g. The alkaloid compound was not determined by HPLC when weights of less than 2 grams of leaves were taken for examination by the device. Consequently, the callus exhibited a respective rise of Capsaicin 1.5 fold compared to *C. annuum* leaves. Because the plant chemical compounds are found in tiny quantities in plant tissues(11) , this was confirmed by many researchers whose studies were in the same direction through the results reached by Jasim and Habeeb (20) and Alwash *et al.* (3) where they indicated that plant leaves generally produce less secondary metabolism than callus cells.

Table 3.Capsaicin Alkaloid (µg/g) in the leaves and callus of *C. annuum*

Alkaloid compound	Leaves of Intact plant µg/ 2 gram	Callus without Fe_3O_4NPs-NPs µg / 0.15 gram	Fold of increase	L.S.D
Capsaicin	2.5	3.8	1.5	0.87 *
* (P≤0.05)				

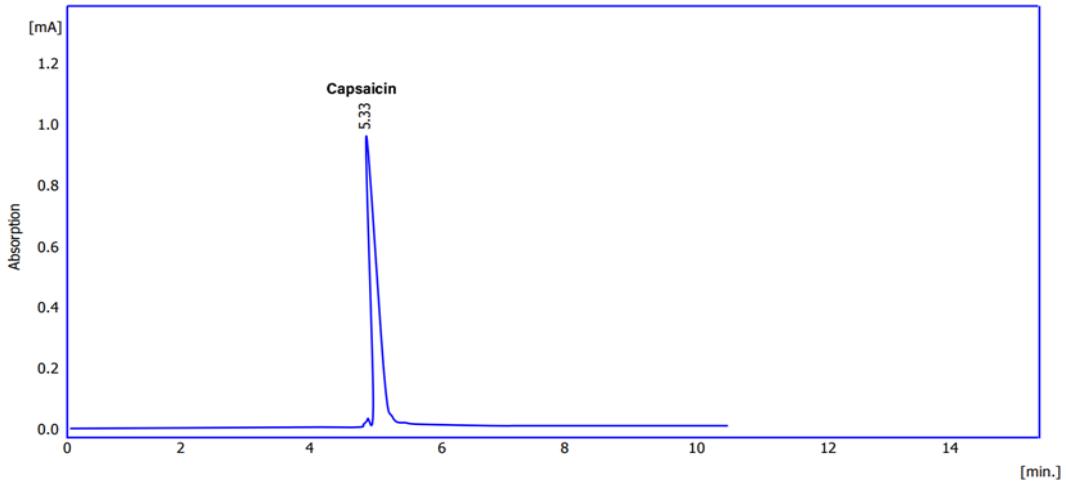

Fig. 2. HPLC curve for Capsaicin standard

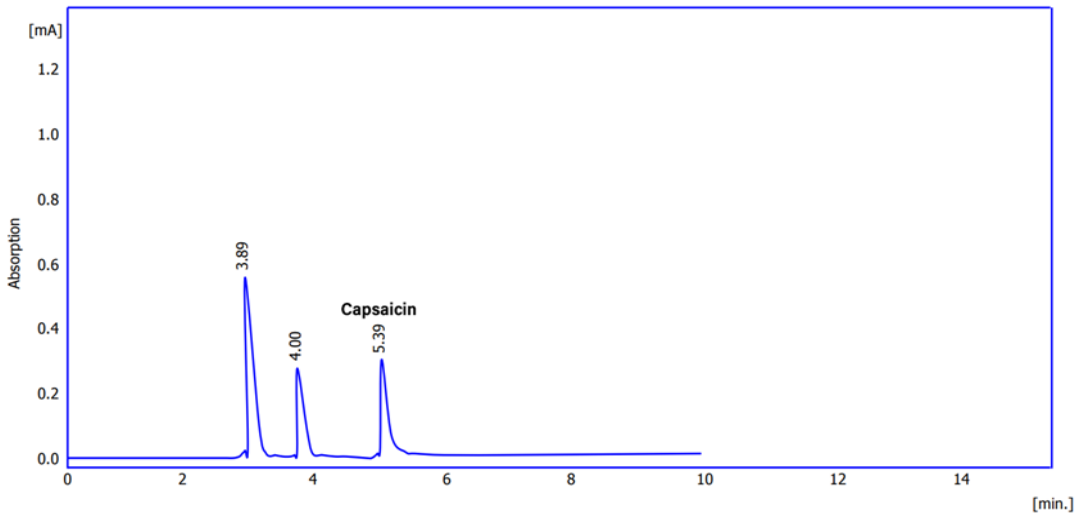

Fig. 3. HPLC curve for Capsaicin in Leaves of plant

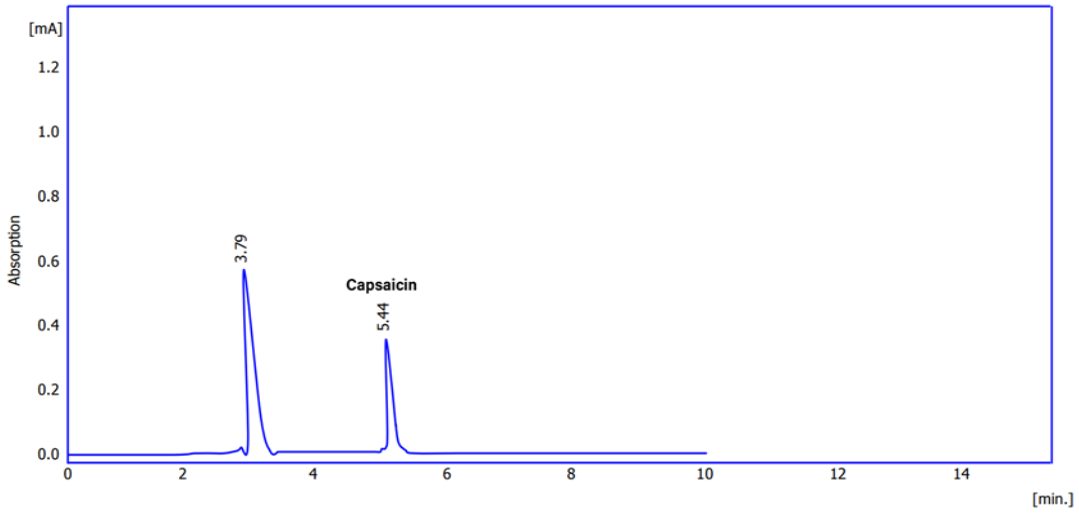

Fig. 4 . HPLC curve for Capsaicin in callus 0 mg/L Fe_3O_4NPs-NPs

Action of iron oxide Nanoparticles on Callus weighing: When Fe_3O_4NPs were added to the callus at varying concentrations, it was shown that adding 2 mg/L to the callus gave slightly higher but insignificant fresh and dry weights of (402.13, 32.20) mg compared to 0 mg/L, which were (397.20, 31.73) mg respectively Table (4). These findings are in line with those of Kokina *et al.* (24), who looked into how different iron oxide NP concentrations affected *Linum usitatissimum* callus cultures. The results showed that the

largest callus was seen in MS medium that had 1.5 mg/L of Fe_3O_4NPs added to it. This concentration most intensively stimulated callus size, as did Irum *et al.* (18),who increased callogenesis in *Cicer arietinum* callus formation at an optimum dose of Iron oxide NPs. This is most likely due to iron's nutritive properties and its significance in a number of physiological functions, such as respiration, redox reactions, and chlorophyll synthesis (36). Iron is the essential nutrient for plant metabolism and growth (33), and its deficiency is a common nutritional disorder that leads to reduced yield and productivity. Both weights decreased significantly with the gradual increase in iron oxide nanoparticles concentration to reach the lowest weights (29.16,365.13)mg respectively observed at 10 mg/L, which differed considerably from other treatments. Due to the increased concentration of nanoparticle catalysts, the cells were made under stress, and hence, the weights decreased. This was in agreement with Kohi and Lahoti (25) when zinc oxide (NPs) were included at high concentration in the culture medium of *Brassica napus* callus, and other researchers have also reached similar results during their studies (1) disclosed similar remarks during their research.

Table 4. Fresh and dry weight of callus cells (mg) with Iron Oxide NPs

Iron Oxide NPs (mg/L)	Fresh. W	Fresh. W
0	397.20	31.73
2 mg	402.13	32.20
4 mg	388.33	31.03
6 mg	380.33	30.40
8 mg	374.43	29.93
10 mg	365.13	29.16
LSD: 0.05	8.05*	0.63 *

Effect of iron oxide NPs on the Capsaicin in callus extract of *C. annuum*.

Embed media with Fe_3O_4 nanoparticles at different concentrations led to an increase of Capsaicin, consistent with the rise in the nanomaterial, reaching the highest value of 6.2 µg/g at 6 mg/L, which is significantly different from other treatments. In addition, the amount of Capsaicin is 1.6 times higher than the amount of Capsaicin 3.8 µg/g achieved in callus grown on media-free nano table (5) (Figures 5,6,7,8 and 9).

Table 5. Effect of varying Fe_3O_4NPs concentrations on the amount of capsaicin in callus extract.

Active Alkaloid µg/g	Fe_3O_4-NPs concentration (mg/L)						L.S.D
	0	2	4	6	8	10	
Capsaicin	3.8	4.1	5.2	6.2	5.5	5.4	1.08 *

* (P≤0.05).

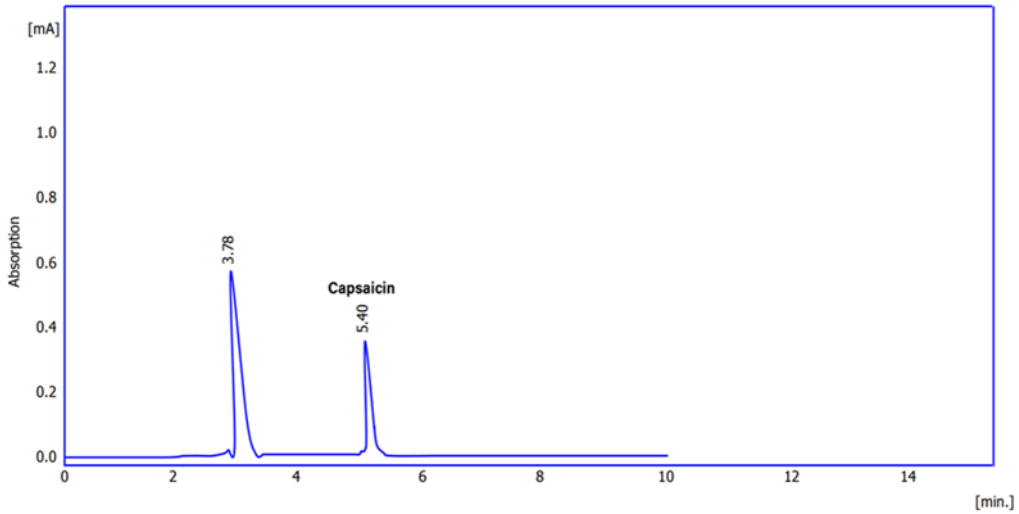

Fig. 5. HPLC curve for Capsaicin in callus 2mg/L Fe_3O_4-NPs

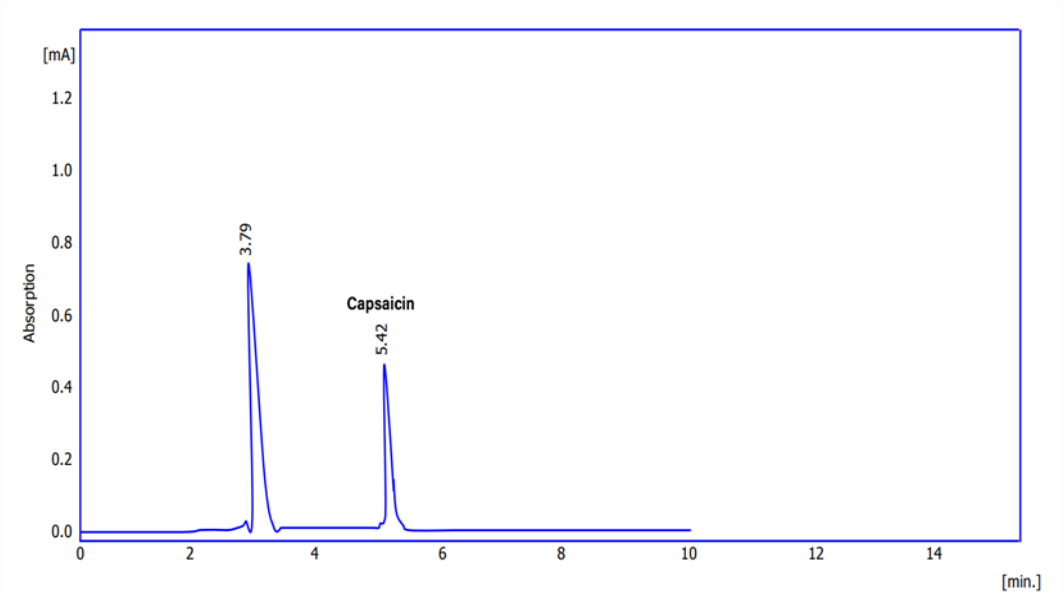

Fig. 6. HPLC curve for Capsaicin in callus 4 mg/L Fe_3O_4-NPs

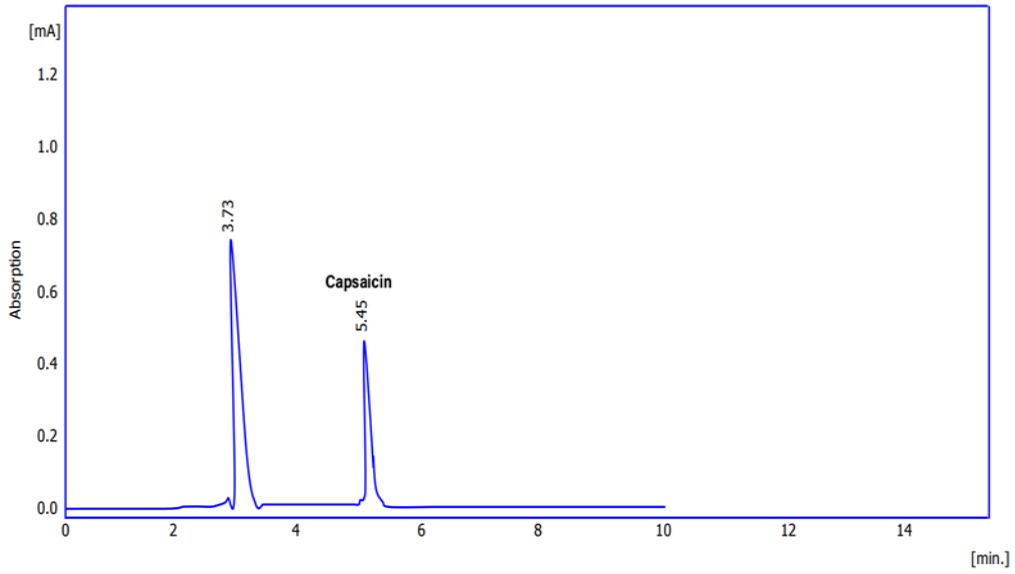

Fig. 7. HPLC curve for Capsaicin in callus 6 mg/L Fe_3O_4-NPs

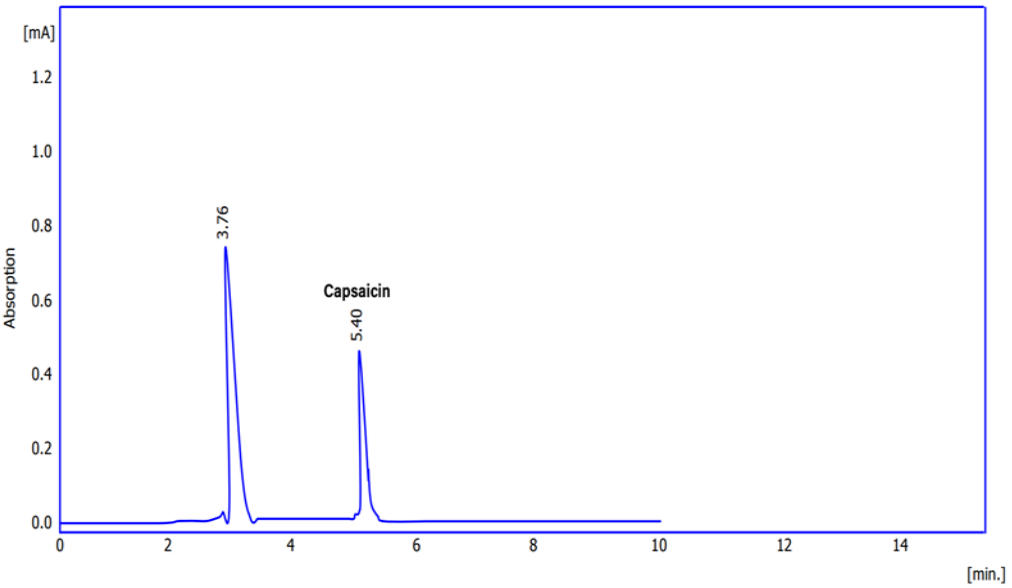

Fig. 8. HPLC curve for Capsaicin in callus 8 mg/L Fe_3O_4-NPs

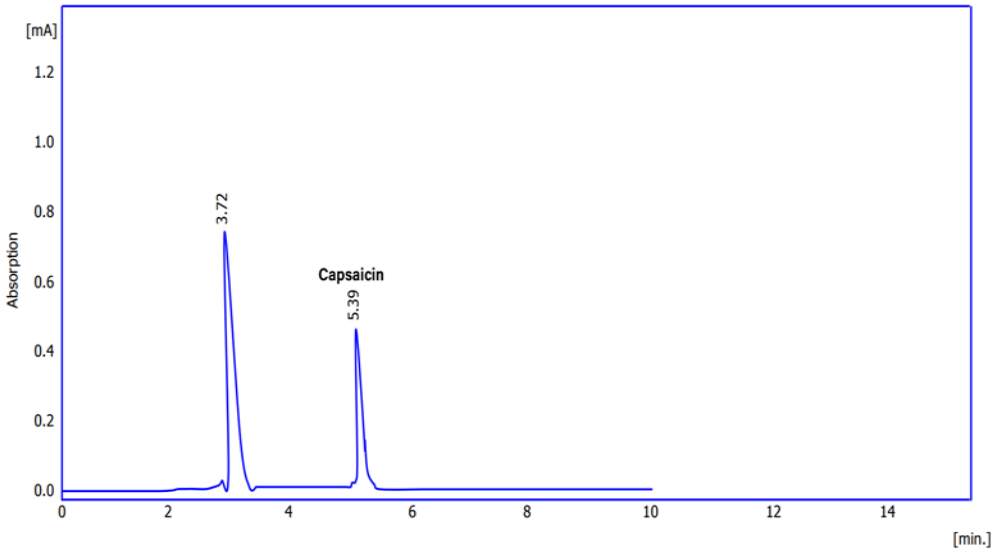

Fig. 9. HPLC curve for Capsaicin in callus 10 mg/L Fe_3O_4-NPs

It is believed that the accumulation of pharmaceutical compounds *in vitro* by Fe_3O_4 nanoparticles accelerates the enzyme activity. Thus, the enzyme defense system will be stimulated by reactive oxygen species (ROS), with the high expression of target genes since *Artemisia annua* contains a lot of artemisinin (5). Other researchers have also demonstrated increased amounts in callus using the same nanoparticles on curcuminoids in *Curcuma longa* (17) and trans-cinnamic acid content in *Narcissus tazetta* (7). When the nano level rose above 6 mg/L, which is the best result, it led to an insignificant decrease (Figure10) .This may come back to the use of high levels of Fe_3O_4 nanoparticles, causing stress on the plant cells, which further affects their growth and development and thus reflects on secondary metabolism (19).

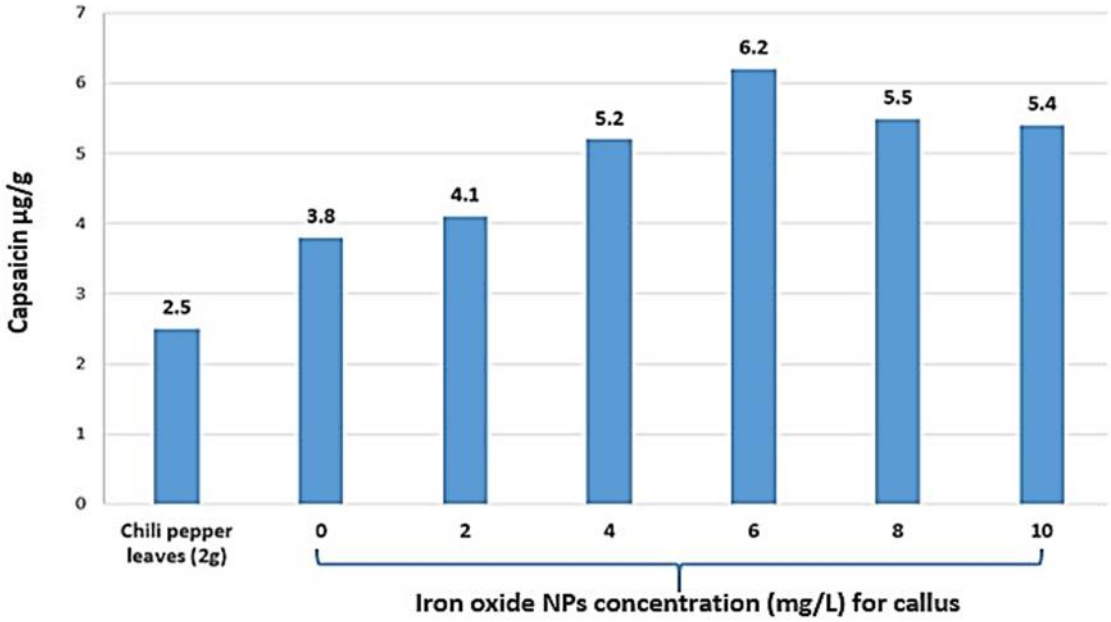

Fig. 10. Capsaicin levels in plant leaves and nano-stimulated callus

REFERENCES

1. Abed, A. S. and E. H. Jassim. 2024. Enhancement of the production of tropane alkaloids in the *Hyoscyamus niger* L. callus using different biotic elicitors. Iraqi Journal of Science,65(9): 4993-5004. https://doi.org/10.24996/ijs.2024.65.9.16

2. Al-Shimmary, S. M. , Z. H.Shehab, E and . H. Jassim. 2025. Exploring the antibacterial, antifungal and anticancer effects of a novel bacteriocin- cerium oxide nanoconjugate. Inorganic Chemistry Communications, 174,113925. https://doi.org/10.1016/j.inoche.2025.113925

3. Alwash ,B.M.J., Z. O. Salman and S. F.Hamad. 2020. Qualitative and quantitative evaluation of active constituents in callus of *Lavandula angustifolia* plant *in vitro* Baghdad Science Journal, 17(2)(SI):591-598. . https://doi.org/10.21123/bsj.2020.17.2 (SI).0591

4. Antonelli A., C. Fry , R. Smith , M. Simmonds , P. Kersey, H. Pritchard,et al.2020. State of the World's Plants and Fungi. Royal Botanic Gardens: Kew. org. pp:96 https://doi.org/10.34885/172

5. Ayoobi, A., A.Saboora , E. Asgarani, and T. Efferth. 2024. Iron oxide nanoparticles (Fe3O4-NPs) elicited *Artemisia annua* L. *in vitro*, toward enhancing artemisinin production through overexpression of key genes in the terpenoids biosynthetic pathway and induction of oxidative stress. Plant Cell, Tissue and Organ Culture (PCTOC), 156(3): 85. https://doi.org/10.1007/s11240-024-02705-9

6.Ban,M.A.and O. M. S. Ibrahim.2024. Effect of *Syzygium aromaticum* L. essential oil extract against pathogenic *Candida albicans in-vitro.* Iraqi Journal of Agricultural Sciences ,55(6):2177-2185. https://doi.org/10.36103/4984kw67

7.Bayat, L., and A. Saboora .2023. Improvement of production of alkaloid and flavonoid compounds in *Narcissus tazetta* L. callus under iron oxide nanoparticles (Fe3O4-NPs) and trans-cinnamic acid and L-phenylalanine treatments. Journal of Plant Biological Sciences,15(1),:85-110. DOI: https://www.sid.ir/paper/655659/en

8. Behbehani, J. M., M.Irshad , S. Shreaz, and M. Karched .2023. Anticandidal activity of capsaicin and Its effect on ergosterol biosynthesis and membrane integrity of *Candida albicans*. International Journal of Molecular Sciences, 24(2):1046. https://doi.org/10.3390/ijms24021046

9. Chanthai, S., J. Juangsamoot, C. Ruangviriyachai and S. Techawongstien . 2012. Determination of capsaicin and dihydrocapsaicin in some chilli varieties using accelerated solvent extraction associated with solid-phase extraction methods and RP-HPLC-fluorescence. Journal of Chemistry, 9 (3), 1550-1561. https://doi.org/10.1155/2012/380574

10.Dudwal, M. 2021. A review study on medicinal uses and health advantage of chili pepper. International Journal of Innovative Research in Computer Science and Technology, 9(5), 83-86.2021 https://doi.org/10.55524/ijircst.2021.9.5.17

11. Ebrahimzadeh, H., A. Ataei-Azimi and M. Noori-Daloi.1996. The distribution of indole alkaloids in different organs of *Catharanthus roseus* L. G. Don. (*Vinca rosea* L.) Daru. J. Sch. Pharm., 6(1):11-24. https://api.semanticscholar.org/CorpusID:89807655

12. Eshraq, J. k. , S. A. AL sahib, and B. M. AL-Timimi.2024. Evaluate the effect of cinnamon zeylanicum oil extract in inhibition of bacteria in laboratory biscuit. Iraqi Journal of Agricultural Sciences , 55(3), 1048-1063. https://doi.org/10.36103/h2cgqb53

13. George, E.F., M.A. Hall and D. G.J. Klerk. 2008. Plant Propagation by Tissue Culture. 3rd ed., Springer, Dordrecht, Netherlands, pp: 501.

14. Großkinsky, D. K. and J.Petrášek. 2019. Auxins and cytokinins the dynamic duo of growth regulating phytohormones heading for new shores. New Phytol., 221(3):1187–1190. https://doi.org/10.1111/nph.15556

15. Ibeh, A. G. and K. C. Egbucha. 2023. Appraisal of some medicinal and other uses of chilli pepper (*Capsicum frutescens* Linn.). World Journal of Pharmaceutical Research,12(4),119-133, https://doi.org/10.20959/wjpr20234-27281

16. Ibrahem ,N. M., E. J. Khadum and S. H. Mutlag. 2022. Isolation of catchin and epigallocatchin From Iraqi rhus coriaria by Preparative high-Performance Liquid chromatography (PHPLC). Iraqi Journal of Pharmaceutical Sciences,31(2), 271-282.. https://doi.org/10.31351/vol31iss2pp271-282

17. Iqbal, M., Z. E. Aftab, H. Anjum, T., Rizwana, H., Akram, W., A. Aftab and G. Li.2024. Nano-Integrated plant tissue culture to increase the rate of callus induction, growth, and curcuminoid production in *Curcuma longa*. Plants, 13(13):1819. doi:10.3390/plants13131819

18. Irum, S., N. Jabeen, K. S. Ahmad, Shafique, S.; Khan, T.F. and H. Gul. 2020. Biogenic iron oxide nanoparticles enhance callogenesis and regeneration pattern of recalcitrant *Cicer arietinum* L. PLoS ONE

15(11): e0242829
https://doi.org/10.1371/journal.pone.0242829
19. Izad, A. A., R. Nulit , C. A. C. Abdullah ,T. H. Fang and M. H. Ibrahim.2019.Growth , leaf gas exchange and biochemical changes of oil palm (*Elaeis guineensis* Jacq.) seedlings as affected by iron oxide nanoparticles.AIMS Materials Science,6(6):960-984.
doi:10.3934/matersci.2019.6.960
20. Jasim, H.Y. and H. M. Habeeb . 2024. Effect of biotic and abiotic elicitors on *Salvadora persica* callus *in vitro* . Baghdad Science Journal, 21, (9):2829.
https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/9439
21. Joshi, N., K.Bhattarai , S.Sinha , B. Rawat, , N. Rai , Anand, J., and J. M. Rawat .2024. Production of secondary metabolites from medicinal plants through tissue culture. In Secondary Metabolites and Biotherapeutics (pp.63-77).AcademicPress.
https://doi.org/10.1016/B978-0-443-16158-2.00007-0
22. Kdhim S.J., E.H. Jassim and H.H. Salih.2020. Effect of salicylic acid on increasing of some phenolic acid and flavonoids in *Coriandrum sativum* callus. Plant Archives, 20 (1):3007-3014.
https://www.plantarchives.org/SPECIAL%20ISSUE%2020-1/3007-3014%20(231).pdf
23. Kim, S., J. Chen ,T. Cheng , A. Gindulyte , J. He ,S. He , Q. Li , B. A. Shoemaker, P. A. Thiessen , B. E.Yu , L. Zaslavsky, J. Zhang and E. Bolton .2023. Pub Chem 2023 update. Nucleic Acids Research, 51(D1), D1373–D1380.
https://doi.org/10.1093/nar/gkac956
24. Kokina,I.,I.Mickeviča,I.Jahundoviča, A. Ogurcovs, M. Krasovska, Jermaļonoka, M. Mihailova,E.Tamanis and V.Gerbreders.2017. Plant explants grown on medium supplemented with Fe3O4 nanoparticles have a significant increase in embryogenesis. Journal of Nanomaterials,(1),4587147.
https://doi.org/10.1155/2017/4587147
25.Kouhi,S.M.M.andM.Lahouti.2018.Application of ZnO nanoparticles for inducing callus in tissue culture of Rapeseed.International Journal of Nanoscience and Nanotechnology, 14(2):133-141.
doi:https://www.ijnnonline.net/article_31221_81efffe6ced02f12a43080a6cc55331d.pdf
26. Kumar, S., M. Sarpras, F. Mushtaq, S. Singh, A. Thattantavide, and A. Kumar.2024. Capsaicin-Producing Plants: Species Diversity, Distribution and Botanical Details. In Capsaicinoids: From Natural Sources to Biosynthesis and their Clinical Applications, pp. 1-24. Singapore: Springer Nature Singapore.
doi:10.1007/978-981-99-7779-6_1
27. Lee, Y. , D.E.Lee, H.S. Lee , S.K. Kim ,W.S. Lee , S.H. Kim and M.W. Kim .2011. Influence of auxins, cytokinins, and nitrogen on production of rutin from callus and adventitious roots of the white mulberry tree (*Morus alba* L.). Plant Cell, Tissue and Organ Culture,105(1):9-19.
https://doi.10.1007/s11240-010-9832-3
28. Mutasher, H.H. and H.J. Attiya .2019. Induced callus from seedlings of *Peganum harmala* L. and studying harmine compound concentration and by GC analysis. *in vitro in vivo* . Iraqi Journal of Science,60(7):1442-1451.
https://doi.org/10.24996/ijs.2019.60.7.4
29. Muthuswamy, R. , S. Asish and M. Nison . 2021 . Review on capsicum frutescens , a tribal herbal food used as medicine. Research Journal of Pharmacognosy and Phytochemistry , 13(4), 191-194.
doi:10.52711/0975-4385.2021.00033
30.Normasari,R.,E. L. Arumingtyas ,R. Retnowati, and W. Widoretno.2023. The Combination Effect of Auxin and Cytokinin on Callus Induction of Patchouli (Pogostemon Cablin Benth.) from Leaf Explants , In 3rd International Conference on Biology, Science and Education (IcoBioSE 2021) ,pp.551-557.
doi:10.2991/978-94-6463-166-1_66
31. Oubaid ,E. N., A. R. Abu-Raghif and I. M. Al-Sudani. 2023. Phytochemical screening and antioxidant activity of uncaria to mentosa extract: In Vitro and InVivo Studies. Medical Journal of Babylon, 2023;20(1):136-42.
https://doi.org/10.4103/MJBL.MJBL_310_22
32. Ramawat, K. G. 2008. Plant Biotechnology. Schand Company LTD, Ram Nagar, New Delhi. India ,pp :24-40.
33. Samaranayake, P., B. D. Peiris and S.Dssanayake. 2012. Effect of excessive ferrous (Fe2+) on growth and iron content in

rice (*Oryza sativa*).International Journal of Agriculture and Biology 14(2):296–298. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20123099887

34. SAS. , Statistical Analysis System, User's Guide. Statistical, Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA. 2018.

35. Shaghati , H. A , E. H. Jassim, and L. A. K. AL-Zubaidi. 2023. Evaluation of the inhibitory activity of *Syzygium aromaticum*

36. extract- chitosan nanoparticles against biofilm formation of *Klebsiella pneumonia*.

37. Bionatura,8 (1) :1-14 https://ouci.dntb.gov.ua/en/works/9GwKwvJ9/

36. Zargar, S. M., G. K. Agrawal, R.Rakwal, and Y. Fukao. 2015. Quantitative proteomics reveals role of sugar in decreasing photosynthetic activity due to Fe deficiency. Front PlantSci.,6:592. https://doi.org/10.3389/fpls.2015.00592