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STIMULATING THE PRODUCTION OF BIOACTIVE COMPOUND QUERCETIN FROM *MORINGA OLEIFERA* L. CALLUS BY USING CHITOSAN AND SILVER NANOPARTICLES

Bassam Meftin Ewhayid[1], Eman Mohammed Abdulzahra[2] and Majid Abdulhameed Ibrahim[3]*

[1, 2] Department of Biology, College of Science, University of Basrah, Basrah Iraq

[3] Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Basrah Iraq

*Correspondence author: e-mail: majid.abdulhameedl@uobasrah.edu.iq

Abstract

A study was conducted in the Colleges of Science and Pharmacy Laboratories, University of Basrah, and Private Fadak Tissue Culture Laboratory, Basrah, Iraq. The explants of the nodule stem segments of Moringa were cultured by tissue culture technique to induce callus and produce active substances by adding different concentrations of 0, 10, 20, and 30 mgL^{-1} silver nanoparticles (NPs), and 5, and 10 mgL^{-1} chitosan to culture media. The content of quercetin in leaves of moringa trees was reached 3.637 moles L^{-1}. The MS medium supplemented with 20 mgL^{-1} silver NPs recorded the highest content of quercetin reaching 17.761 moles L^{-1}. This treatment was a significant difference in callus content of quercetin compared to the other treatments. The MS medium supplemented 10 mgL^{-1} chitosan recorded the second rank that reached 15.245 moles L^{-1} quercetin in moringa callus. While the control treatment recorded the lowest content of quercetin in callus reached 2.932 moles L^{-1}. The other treatments 10, 30 mgL^{-1} silver NPs, and 5 mgL^{-1} chitosan were recorded 4.484, 7.243 and 1.529 moles L^{-1} quercetin, respectively.

Keywords: Bioactive substance, callus induction, explant, MS salts, nodule segment, quercetin

1. Introduction

Medicinal plants are among the most important plants from which bioactive substances are extracted to use in the pharmaceutical industry, drugs and traditional medical treatment (Al-Jabir et al., 2020; 2021). The moringa tree, *M. oleifera* L., is an important plant that humans have been interested in since ancient times, and it belongs to the family Moringaceae, (Poteet and Number, 2006). It is rich in many compounds that are of medical importance, such as quercetin, which reduces the risk of cancerous diseases, as well as some cardiac and vascular system diseases. It is also effective as an antioxidant and antibiotic against bacteria, fungi and viruses, as it turned out that it may have anti-Covid19 properties (Makonnen et al., 1997; Abdulkarim et al., 2005). It also contains a high percentage of minerals, proteins and carbohydrates and contains biological activities such as vitamins, carotenoids, polyphenols, alkaloids, tannins and saponins (Oladeji et al., 2017). In addition to the glycosides, flavonoids (including quercetin), and phytosterols (Yadav et al., 2017).

Quercetin also recognized as 3,3′,4′,5,7-pentahydroxy flavone is a widely spread phytoflavonoid. It is seen in many vegetables, seeds, leaves, and grains. It is associated with the remaining saccharides to take shape quercetin glycosides (Li et al., 2016). Studies show that quercetin

addition may enhance antiviral, antioxidant, antiinflammatory, and immunoprotective effects (Nair et al., 2002; Uchide and Toyoda, 2011). Quercetin has been investigated in different types of viral infection required to its good antiviral effects in frustrating polymerases and reversing transcriptase (Shinozukaet al., 1988; Speddinget al., 1989).

Quercetin could be an effective, safe, and affordable antiviral and immunomodulatory strategy for the treatment of mild and severe cases (Yi et al., 2004). The compound supplies a direct mechanical basis for its practical clinical use as well as for its immunological actions. Remarkably, the few in vivo models inspected demonstrate increased duration of a lethal viral infection when treated with quercetin (Daviset al., 2008). Quercetin targets viral polymerases and disrupts replication by inhibiting reverse transcriptase enzymes. Quercetin also inhibits SARS protease by binding to GLN189 site, which is similarly expressed by SARS-COV-2 (Chen etal., 2006; Zhanget al., 2020).

Chitosan is the most important compound with bioactivities, as it works as an antioxidant, and antimicrobial, and enhances plant growth. Chitosan is a natural carbohydrate polymer (Chibu, 2001). It is a strong reducer that functions an essential role in plant resistance in defense mechanisms (as an antioxidant) and diseases, as well as has a critical and effective position in plant growth (Uthairatanakijet al., 2007; Safana et al., 2022).

Nanoparticles are defined as particles that sizes range from 1 to 100 nanometers. These particles also have unique capabilities and distinctive physical properties that in turn enhance the metabolism in the plant (Giraldoet al., 2014). Galbraith (2007) and Torneyet al. (2007), showed that nanomaterial enter into plant cells, and transfer nucleic acids, chemicals and DNA to plant cells, as this domain of research, presented new options in the specialization of plant biotechnologies. Materials and nanoparticles interact with plant cells and cause many phenotypic and physiological changes. Al-Aubaidi (2016) used different concentrations of silver nanoparticles to produce some active secondary compounds in the hopbush (*Dodonaea viscosa* L.) plant by in vitro culture technique. Whereas, silver nitrate treatment at 2 mg L^{-1} led to a considerable increase in the production of the active secondary compounds of quercein and luetolin in tissue cultures of hopbush plant. The 0.5 mg L^{-1} silver nanoparticles recorded a significant superiority in the concentration of the compound apigenin.

This study aims to stimulate the production of the bioactive compound quercetin in moringa callus induced on the nutrient medium prepared with different concentrations of silver nanoparticles and chitosan and compared it with the natural amount in the leaves of moringa trees.

2. Material and Methods

The study was carried out in Fadak Laboratory for tissue culture in Basra Province, Abu Al-Khaseeb District, and in the laboratories of the Colleges of Pharmacy and Science, University of Basrah, Garmat Ali, Basrah, Iraq for the period from 25/12/2020 to 22/01/2021.

2.1 Explants Sources

In this study, explants for nodule stem of the Moringa (*Moringa oleifera* L.) plant obtained from trees were used. These explants were cultivated on an MS medium having growth regulators to initiate and multiplied callus for the next experiments.

2.2 Sterilization of Explants Surfaces

The nodule stem segments were removed from the mother moringa plant using a sharp scalpel (blade) with a length of 1 cm, washed well. Then a surface sterilization process was carried out by transferring them to airflow pathfinder chamber with 70% ethanol and 1.05%% sodium hypochlorite diluted with distilled water with drops of the diffuser Tween-20 for 15 minutes (Ibrahim and Draaj, 2020). Then they washed with sterilized distill water for several times. The explants were used by 10 replicates per treatment.

2.3 Medium Culture Preparation

The medium was prepared from formula MS salts (Murashige and Skoog, 1962) with a weight of 4.43 gL^{-1} produced by the Indian HIMEDIA company. The 30 g sucrose and different concentrations of growth regulators were added to this MS medium. The pH of the medium was regulated pH to 5.8-5.7 using 0.1N each of NaOH or HCl. Then 8gL^{-1} agar was appended to the medium of culture. After homogenization of the medium, the media were distributed in glass jars. These jars were transferred to an autoclave for sterilization at a temperature of 121°C and 1.04 $kg.cm^2$ pressure for 20 minutes. Then these jars were kept at growth room.

2.4 Callus culture on MS medium provided with different concentrations of chitosan or silver nitrate NPs

The 150 mg of induced callus from nodule stem explant was weighed and cultured on MS medium provided with 4.0 mgL^{-1} benzyl adenine (BA), and 0.5 mgL^{-1} naphthalene acetic acid (NAA) for callus multiplication. Then producing callus were cultured on MS medium containing a different concentrations of chitosan (0, 5 and 10 mgL^{-1}), and silver NPs at 0, 10, 20 and 30 mgL^{-1} concentrations. Each concentration was replicated ten times. The cultures were incubated in the dark, at a temperature of 25±2°C. After five weeks, the quercetin was estimated in the callus of all treatments and leaves of moringa trees.

2.5 Estimation of Quercetin using High Performance Liquid Chromatography (HPLC)

Samples were taken from the callus of treatments of silver NPs and chitosan as well as samples of leaves of moringa trees and entered into the Freeze-Dryer (Labconco type) and connected to the device and the device turned on for 48 hours to get rid of moisture in the samples. After the lyophilization has being completed, they were finely ground to be dissolved in organic solvents in preparation for inclusion in HPLC experiments. 5 mg of pure quercetin and plant samples were weighed and placed in 1.5 mg vials. 400 µl of solvent 70% ethanol with 30% acetonitrile was added and mixed to ensure good mixing and dissolution of the particles as 100% dissolved. The solution was purified with Whatman paper. The 10 microliters of quercetin standard solution were withdrawn and injected into the High-Performance Liquid Chromatography device (Al-Bahadli, 2020). As the retention time was 1.73 minutes, while the peak area of the standard solution was 826453.8. The plant samples were then injected separately and sequentially with a 10 µl Hamilton Syringe at the injection site. The fixed phase of the device (Nuceosil 100-S), the column used (Arcus type) with dimensions (4.6×250 mm), the mobile phase (Water and Acetonitrile) with a ratio of 70:30 (v: v), a flow rate of 1 ml L^{-1} and a wave length: 280 nm. The concentrations of the active substance were quantitatively determined using the comparison between the standard and the sample under the same conditions using the following equation:

Sample concentration (mg/g) = area of sample/area of standard solution x number of dilution times.

2.6 Experimental Design and Statistical Analysis

All experiments included in the study were carried out in a Randomized Completely Design (C.R.D). The results were resolved using the ANOVA table and the statistical program (GenStat). The significance was compared between the mean of treatments using the L.S.D (Least Significant Difference Test) to show the statistical differences between the treatments at the 0.01 probability level (Al-Sahoki and Waheeb, 1990).

3. Results

The results in Figure 1 show that the treatments of silver NPs and chitosan had a significant effect in the quercetin content of the callus tissues of the moringa tree compared to the control treatment within a significant level of 1%. It was noticed that the 20 mgL^{-1} silver NPs was a significant superior in callus content of quercetin over all treatments, which reached 17.761 moles L^{-1}. The treatment of 10 mgL^{-1} chitosan was followed by this treatment and it recorded a 15.245 moles L^{-1}. While the control treatment was recorded the lowest value in quercetin compared to these two treatments, it reached 2.932 moles L^{-1} quercetin. The quercetin content in leaves of moringa tree was reached 3.637 moles L^{-1} quercetin. The quercetin content of callus in the treatments of 10, 30 mgL^{-1} silver NPs, and 5 mgL^{-1} chitosan was recorded 4.838, 7.243 and 1.529 moles L^{-1} quercetin, respectively.

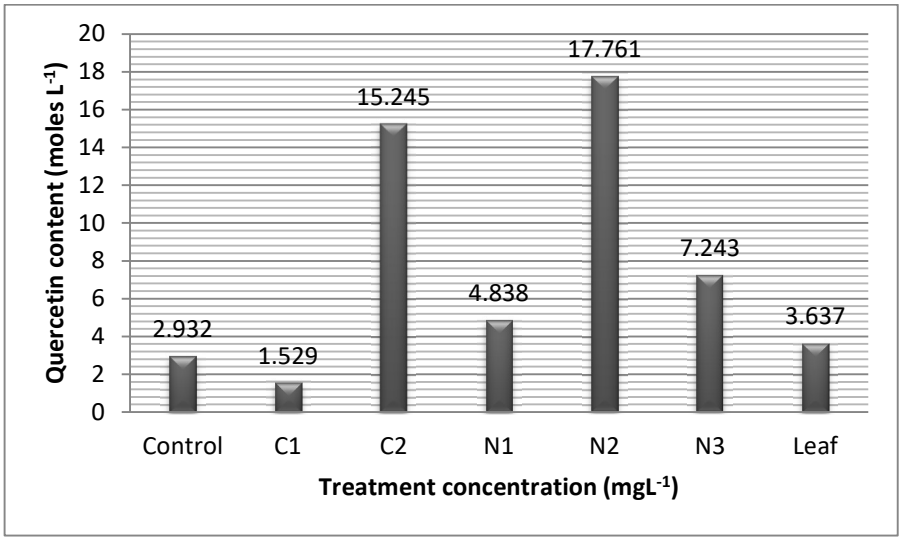

Figure 1: The effect of different concentrations of chitosan or silver NPs on quercetin content in callus and leaf of moringa tree. (Control: without chitosan or silver NPs; C1: 5 mgL^{-1} chitosan; C2: 10 mgL^{-1} chitosan; N1: 10 mgL^{-1} silver NPs; N2: 10 mgL^{-1} silver NPs; N3: 10 mgL^{-1} silver NPs; Leaf: leaf of moringa tree).

The results indicate an increase in the content of quercetin produced from callus tissue when adding chitosan and nanoparticles silver stimulants (Figures 2-8). It is noted that the culture medium prepared by the nanoparticles silver treatment at 20 mgL^{-1} concentration stimulated the production of quercetin.

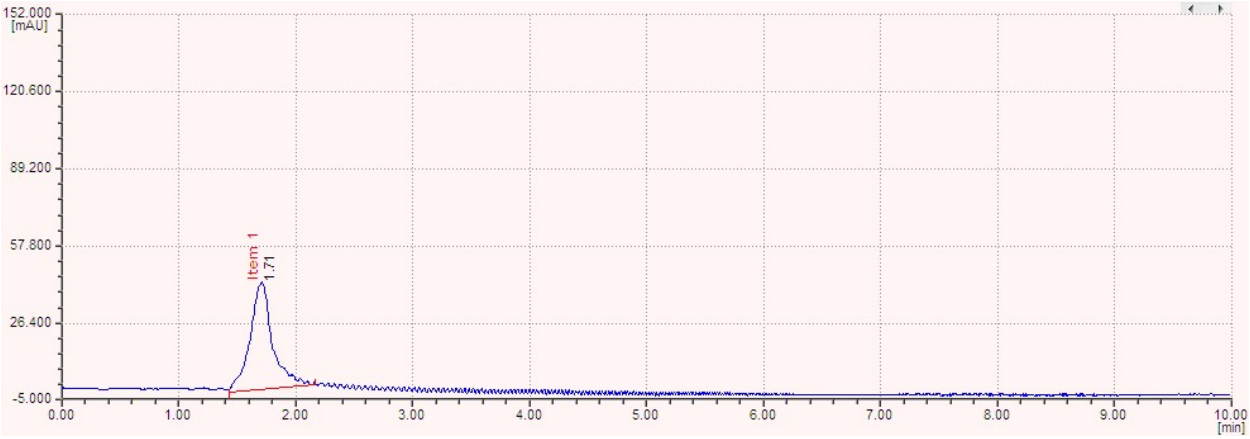

Figure 2: The curve that represents the quercetin content in callus of control treatment.

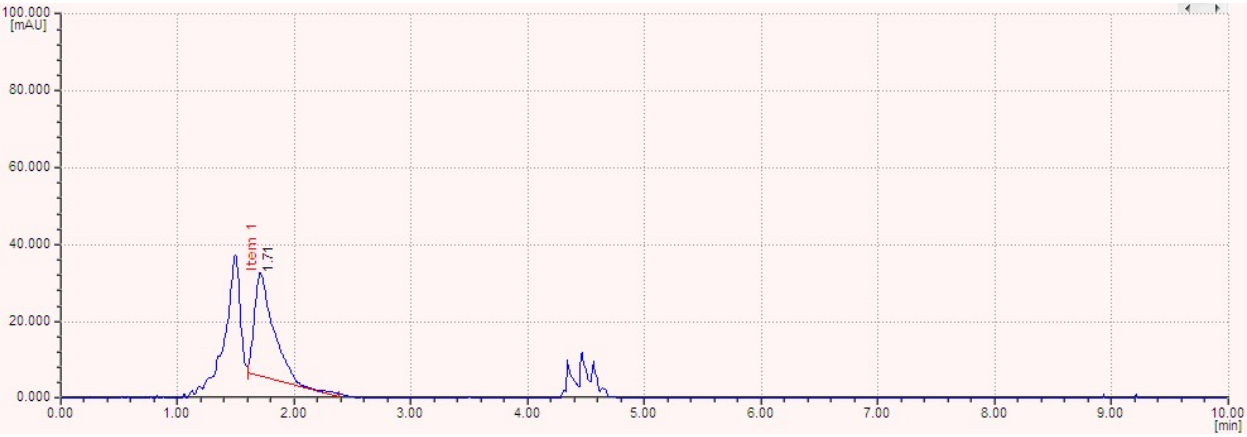

Figure 3: The curve that represents the quercetin content in callus of 5 mgL^{-1} chitosan treatment.

Figure 4: The curve that represents the quercetin content in callus of 10 mgL^{-1} chitosan treatment.

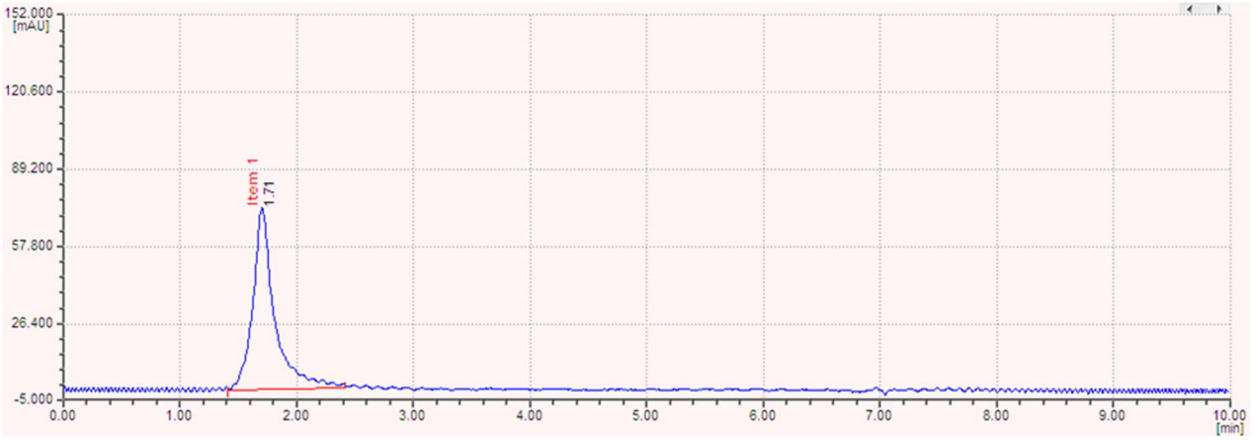

Figure 5: The curve that represents the quercetin content in callus of 10 mgL^{-1} silver NPs treatment.

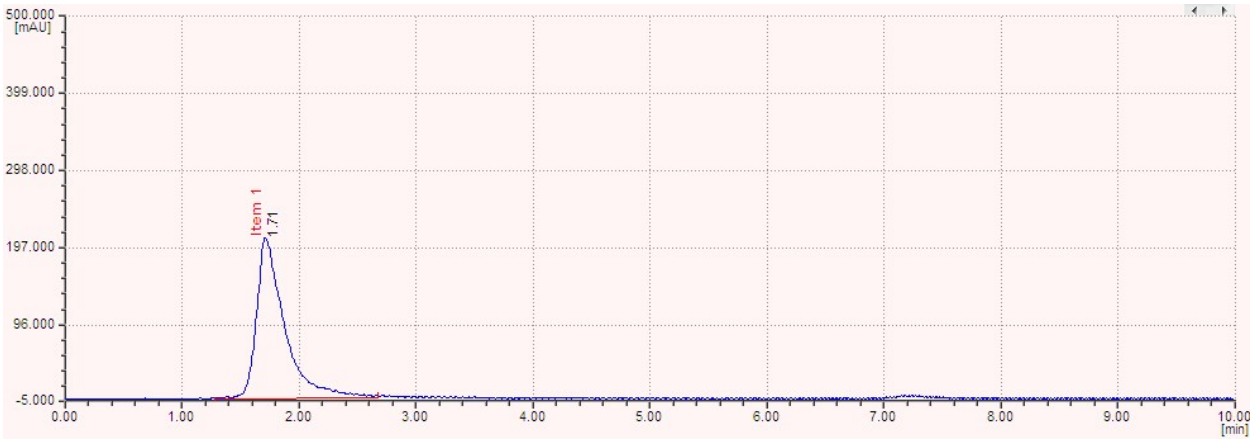

Figure 6: The curve that represents the quercetin content in callus of 20 mgL^{-1} silver NPs treatment.

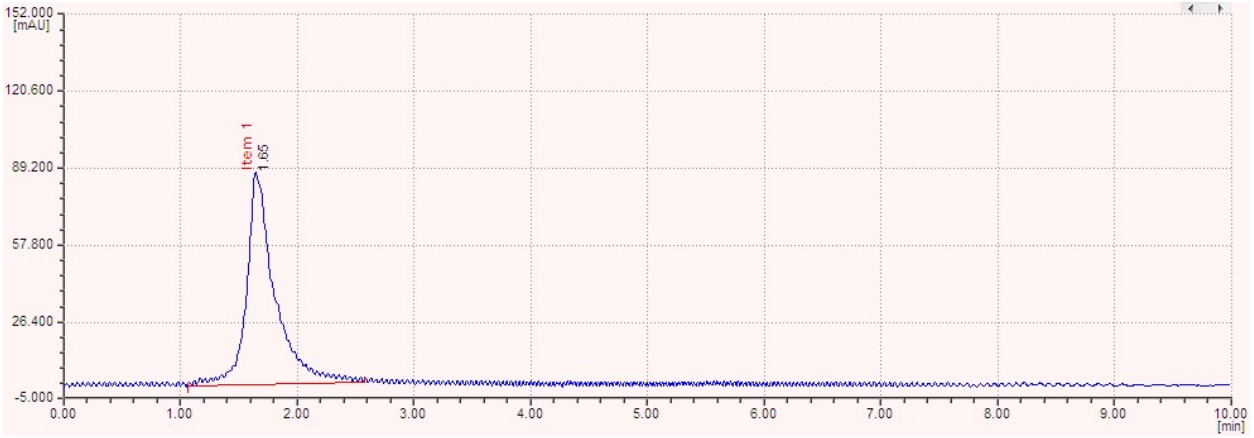

Figure 7: The curve that represents the quercetin content in callus of 30 mgL^{-1} silver NPs treatment.

Figure 8: The curve that represents the quercetin content in leaves of moringa (*Moringa oleifera* L.) tree.

4. Discussion

These results are consistent with the results that found by Al-Aubaidi (2016), and his study was about the hopbush (*Dodonaea viscosa* L) plant. The reason for the decrease in the production of the quercetin when increasing the concentration of nanoparticles silver (30 mgL^{-1} Ag NPs) may be due to the increased stress on the cells in the callus tissue, which reduces the bioactivities of the cells to face stresses. These results were not agreed to the findings of other studies. Which they studied on effect of the addition of Ag NPs and $AgNO_3$ in the increase of secondary compounds in the callus of the olive (*Olea europaea*) tree (Vasudevanet al., 2004; Jiménezet al., 2006).

Through the results, it was also observed that the MS medium provided with 10 mg L^{-1} chitosan had a high response in the production of the quercetin compound. The reason may be because chitosan is a carbohydrate compound and that carbohydrates contribute to the production of bioactive compounds. As they are considered a source of energy and this is consistent with what it was reached by Ibrahim (2017), which showed that increasing the addition of carbohydrates in the culture media helps to increase the production of bioactive compounds resulting from increased energy in cells. The addition of growth regulators represented by benzyl adenine and naphthalene acetic acid had a supportive and important role in the induction and multiplication of callus, which may have been positively reflected in its content of bioactive substances (Ibrahim, 2022).

5. Conclusion

The in vitro culture of the nodule stem segments of the moringa tree as explants on the MS medium supplemented with benzyl adenine and naphthalene acetic acid combination leads to the induction of callus. The optimum addition of silver nanoparticles or chitosan concentration to the MS media increased the callus content of the bioactive compound quercetin compared to the control treatment and moringa leaf content.

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