Effect of Silver Nanoparticles in Stimulating Some Active Compounds in Garlic Callus Under Salt Stress, in Vitro

Ali Abdulkhudur Ghalib Al-Taie, Mansoor Abed Aboohanah and Falah Hasan Issa

1-2 Faculty of Agriculture, University of Kufa, Iraq.
3 College of Agriculture, Al-Muthanna University, Iraq.

Email: alia.altaee@student.uokufa.edu.iq
Email: falah70-hasan@mu.edu.iq

Abstract

This study was conducted in tissue culture laboratory, Faculty of agriculture, University of Kufa in 2020. The experiment included study the effect of two factors: First, different concentrations of Silver nanoparticles (0, 1, and 2 mg.L⁻¹), and second factor : different concentrations of NaCl (0, 25 and 50 mmol.L⁻¹) in Allicin, Di-allylsulfide and Vinyldithiin content in callus garlic. In vitro. A Completely Randomized Design (C.R.D). Silver nanoparticles showed a significant increase in the active compounds. The concentration (2 mg.L⁻¹) gave highest rate in Allicin, Di-allylsulfide and Vinyldithiin content (261.36, 529.23 and 309.41µg.g⁻¹ F.W.) respectively. Also, three are significant increased between concentrations of NaCl. The concentration (50 mmol.L⁻¹) gave highest rate in Allicin, Di-allylsulfide and Vinyldithiin content (250.17, 521.57 and 279.15µg.g⁻¹ F.W.) respectively. While, the interaction treatment (2 mg.L⁻¹ of Silver nanoparticles and 50 mmol.L⁻¹ NaCl) recorded significant increase in Allicin, Di-allylsulfide and Vinyldithiin content (316.18, 619.06 and 379.39µg.g⁻¹ F.W) respectively.

Keywords: Silver, Garlic Callus, Stress, Nanoparticles.

1. Introduction

Garlic plants Allium sativum L. belong to the order Liliales, and this order includes eight families, including the Liliaceae family, to which garlic belongs [1]. Cultivation of the crop is spread all over the world mostly with temperate climates. Garlic produces bulbs and has a stalk (scape). The inflorescences rarely give seeds but often set bulbs on the top of the inflorescences [2]. has a medicinal value that may exceed the nutritional value, which makes it a plant that can be used in the treatment or resistance of many diseases, benefits patients with diabetes and high blood pressure through its effect in lowering blood sugar, reducing high blood pressure and preventing blood clotting, reduces the incidence of cancerous diseases because it contains antioxidants, a circulatory stimulant and is useful in Reducing harmful low-density lipoproteins It has benefits for treating intestinal and stomach problems because it differentiates between beneficial and harmful bacteria in the intestine and has an antibacterial effect such as stomach germs [3, 4].

Nanotechnology is one of the most active areas of research at the present time and has an important impact on the global economy and industry positively. It is a scientific field concerned with the study of materials at a scale of less than 100 nanometers, as the particles of matter at this scale show physical and chemical properties that differ from them when they are in their traditional dimensions. Then, materials can be manufactured and shaped for the purpose of obtaining better properties than their properties at their natural scale [5]. Silver nanoparticles are used in plant tissue culture to reduce bacterial infections, callus induction, formation of organs, tissues and somatic cells, genetic engineering and production of various metabolites. When added to MS growth medium, including total phenols, antioxidant compounds and lipid peroxides. When different concentrations of silver nanoparticles were applied as a catalyst for secondary metabolites of Didonia callus, it caused a significant increase in the production of Quercetin and Luteolin. They also found an increase in the active compounds as a result of adding silver nitrate nanoparticles to the callus of the Calendula officinalis plant [6, 7, 8, 9, 10]. The addition of NaCl salt to the media affects the physical and chemical properties of callus, which are affected by different degrees based on the salt concentration, the duration of exposure to it, the type and variety of the plant from which the callus is derived, and also affects the growth reduction in most tissue cells due to the effect of the salts mentioned previously. Under stress conditions, the plant tries to protect itself through various protective and defensive mechanisms, including morphological or structural, in addition to possessing enzymatic and non-enzymatic systems to protect its cells from the...
negative effects of stress, and sometimes the formation of secondary compounds that have roles that are no less important than the main reactions and the accumulation of many compounds, most of them with Medicinal value such as alkaloids, tannins, phenols and flavonoids [11-14]. This study was conducted to determine effect of different concentrations of silver nanoparticles and sodium chloride (NaCl) in some of active substances in garlic callus, under in vitro conditions.

2. Material and Methods

The experiment was conducted in tissue culture laboratory, Faculty of Agriculture, University of Kufa in 2020. This experiment included study the effect of two factors: First, different concentrations of Silver nanoparticles (T1: 0, T2: 1, and T3: 2 mg.L⁻¹), and second factor: different concentrations of NaCl (L1: 0, L2: 25 and L3: 50 mmol.L⁻¹), in Allicin, Di-allylsulfide and Vinyldithiin content in callus garlic, In vitro. The Local garlic used were obtained from local markets, and the cloves of garlic were sterilized 20 minutes with 15%, v/v of NaOCl (6% active ingredient) then washed by sterile distilled water three times. After sterilization the garlic cloves was placed in a test tube contain MS media (table 1) volume 10 ml. The explants were incubated to the growth room 25 ± 2 ° C. After 40 days of callus intiation, callus maintenance on the same callus media Provided with experiment factors, concentrations of silver nanoparticles and NaCl. All explants were incubated in growth room 25 ± 2 ° C. after four wee ks of cultivation, Allicin, Di-allylsulfide and Vinyldithiin content in callus Weight Fresh (F.W.) were recorded using HPLC technique Type Column: phenomenex C-18, 3µm particle size (50x2.0mm I.D). Study was performed using the Completely Randomized Design (C.R.D) were used with three replicates and means were compared according to L.S.D. test at the level of 0.05.

| Components | Concentration (mg.L⁻¹) |
|------------|-----------------------|
| MS salts   | Full strength         |
| Sucreose   | 30000                 |
| Myo inositol | 100                  |
| Agar       | 7000                  |
| BA         | 0.5                   |
| IAA        | 3                     |
| 2.4.D      | 1                     |

3. Result

3.1 Allicin content

The results presented in Table 2 showed that silver nanoparticles when (T3) treatment recorded the highest rate of callus content of Allicin amounting to (261.36 µg.g⁻¹ F.W.) as compared with control treatment (T1) which recorded the lowest rate (113.66 µg.g⁻¹ F.W.), while the NaCl salt recorded the highest rate of Allicin when (L3) treatment amounted to (250.17 µg.g⁻¹ F.W.) as compared with (L1) treatment which recorded the lowest rate (164.93 µg.g⁻¹ F.W.). The interaction between silver nanoparticles and NaCl recorded significant increase in Allicin content. The treatment (T3, L3) achieved the highest rate (316.18 µg.g⁻¹ F.W.) as compared with treatment (T1, L1) which recorded (79.10 µg.g⁻¹ F.W.)

| Table 2. Effect of silver nanoparticles and NaCl on Allicin content (µg.g⁻¹ F.W.) in garlic callus. |
|---------------------------------------------------------------|
| Silver nanoparticles (mg.L⁻¹) | NaCl salt mmol.L⁻¹ (L) | Mean of Silver nanoparticle |
| (T)                           | 0      | 25 | 50 | L1 | L2 | L3 |          |
| 0 T1                          | 79.10  | 79.21 | 182.66  | 113.66 |
| 1 T2                          | 217.56 | 172.70 | 251.67 | 213.97 |
| 2 T3                          | 198.13 | 269.75 | 316.18 | 261.36 |
| Mean of NaCl                  | 164.93 | 173.89 | 250.17 |          |
| L.S.D. 0.05                   | 0.2109 L= 0.2109 TL= 0.3652 |

3.2 Di-allyldisulfide content

The results presented in (Table 3) shows a significant effect of silver nanoparticles in Di-allyldisulfide content. The concentration (T3) recorded the highest rate (529.23 µg.g⁻¹ F.W.) as compared with concentration (T1) which recorded the
lowest rate (291.81 µg.g⁻¹ F.W.). Also, there are a significantly affected of NaCl salt in Di-allyldisulfide content. The concentration (L3) recorded the highest rate (521.57 µg.g⁻¹ F.W.) as compared with treatment (L1) which recorded (338.86 µg.g⁻¹ F.W.). The interaction between silver nanoparticles and NaCl salt a significant effect in Di-allyldisulfide content. The treatment (T3, L3) recorded the highest rate (619.06 µg.g⁻¹ F.W.) as compared with (T1, L2) which recorded (182.45 µg.g⁻¹ F.W.).

### Table 3. Effect of silver nanoparticles and NaCl on Di-allyldisulfide content (µg.g⁻¹ F.W.) in garlic callus.

| Silver nanoparticles (mg.L⁻¹) | NaCl salt mmol.L⁻¹ (L) | Mean of Silver nanoparticle |
|-----------------------------|------------------------|-----------------------------|
|                            | 0         | 25       | 50       |               |
|                            | L1        | L2        | L3        |               |
| 0 T1                       | 229.76    | 182.45   | 463.22   | 291.81        |
| 1 T2                       | 377.52    | 467.44   | 482.44   | 442.47        |
| 2 T3                       | 409.30    | 559.32   | 619.06   | 529.23        |
| Mean of NaCl               | 338.86    | 403.07   | 521.57   |               |
| L.S.D. 0.05                |           |          | T=0.4285 | L= 0.4285 TL= 0.7421 |

### 3.3 Vinyldithiin content

The obtained results Table No. (4) showed a significant differences between concentrations of silver nanoparticles in Vinyldithiin content of garlic callus. The concentration (T3) was recorded (309.61 µg.g⁻¹ F.W.) as compared with concentration (T1) which recorded lowest rate (102.80 µg.g⁻¹ F.W.). Also, it was the NaCl salt had an influence on Vinyldithiin content of garlic callus. The (L3) concentration achieved the highest rate (279.15 µg.g⁻¹ F.W.) compared with the (L1) concentration which recorded lowest rate (165.85 µg.g⁻¹ F.W.). The interaction between silver nanoparticles and NaCl salt were also a significant effect on in Vinyldithiin content. The treatment (T3, L3) showed the highest rate (379.59 µg.g⁻¹ F.W.) compared with the lowest treatment (T1, L2), which recorded (16.08 µg.g⁻¹ F.W.).

### Table 4. Effect of silver nanoparticles and NaCl on Vinyldithiin content (µg.g⁻¹ F.W.) in garlic callus.

| Silver nanoparticles (mg.L⁻¹) | NaCl salt mmol.L⁻¹ (L) | Mean of Silver nanoparticle |
|-----------------------------|------------------------|-----------------------------|
|                            | 0         | 25       | 50       |               |
|                            | L1        | L2        | L3        |               |
| 0 T1                       | 112.64    | 16.08    | 179.69   | 102.80        |
| 1 T2                       | 145.54    | 203.19   | 278.38   | 209.04        |
| 2 T3                       | 239.37    | 309.48   | 379.39   | 309.41        |
| Mean of NaCl               | 165.85    | 176.25   | 279.15   |               |
| L.S.D. 0.05                |           |          | T=0.4160 | L= 0.4160 TL= 0.7206 |

### 4. Discussion

According to our results, there are increased of the secondary metabolites significantly (Allicin, Di-allyldisulfide and Vinyldithiin content in callus) and the highest values were obtained from the at interaction between silver nanoparticles and NaCl. Silver nanoparticles can act as a catalyst for the production of more copies of the genes of the biosynthesis pathway for many active compounds. The increase in growth is attributed to the ability of silver nanoparticles to cause an enzymatic change inside the cell that reduced ethylene production, which led to this kind of increase, and the addition of silver nanoparticles nanostimulants with different concentrations are determined according to the type of plant and the type and size of nanoparticles. Increasing the concentration of silver nanoparticles leads to an increase in stresses on the cell as well as an increase in the production of antioxidants in order to reduce free radicals (reactive oxygen species) ROS inside the cell as much as possible, and the addition of silver nanoparticles affected DNA and gene expression in a number of higher plants and the addition led to a change in both lipids, proteins and vitamins, as well as the level of phytohormones auxin and cytokinin. [15- 20].

The plant works to increase the effectiveness of the defensive enzyme system, which are the important enzymes in osmotic and ionic regulation. The negative effects of salinity [21]. The reason for increasing plant production of secondary metabolites when using NaCl as a type of abiotic stimuli may be due to the plant’s role in modifying cell osmosis by increasing the negative water potential of the plant cell exposed to osmotic stress [22].
Conclusion

As a result of this study, the highest production of Allicin, Di-allyldisulfide and Vinyldithiin content in garlic callus, was found in the 2 mg.L⁻¹ of silver nanoparticles with 50 mmol.L⁻¹ of sodium chloride (NaCl), thus, silver nanoparticles and NaCl may be a promising compounds for use in production of secondary metabolite.

References

[1] Al Katib, Youssef Mansour. (2000). Classification of Seed Plants, Dar Al-Kutub Press for Printing and Publishing, University of Mosul, Iraq.
[2] Lallemand, J., Messiaen, G.M., Briand, F. and Etoh, T. (1997) Delimitation of varietal groups in garlic (Allium sativum L.) by morphological, physiological and biochemical characters. Acta Horticulturae 433, 123-132.
[3] Bouras, M., Abu Turabi, B. and Al-Basit. I. (2006). Vegetable crop production. university of Damascus. Syrian Arab Republic.
[4] Healthyme.(2006).Garlic.http://www.ahealthyme.com/topic/topic13835.
[5] Al-Iskandarani, Muhammad Sharif. (2010). Nanotechnology for a better tomorrow. The National Council for Culture, Letters and Arts. Knowledge World Series. Kuwait.
[6] Al-Obaidi, Hashem Kazem Muhammad. (2016). Increasing of some medicinal flavonoids compounds of Dodonaea viscosa L. using nanoparticles of silver in vitro. Iraqi Journal of Science. 57 (1): 338-343.
[7] Bhat, P. and A. Bhat. (2016). Silver nanoparticles for the enhancement of accumulation of capsaicin in suspension culture of Capsicum sp. Journal of Experimental Sciences. 7(2), 1-6.
[8] Jimenez-Medina, E.; Garcia-Lora, A.; Paco, L.; Algarra, I.; Collado, A. and Garrido, F. (2006). A new extract of the plant Calendula officinalis produces a dual in vitro effect cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer, 6, pp:119.
[9] Santoscoy, R.; J. L. Castillo; J. Sato; J. Bello. (2017). Antimicrobial and hormetic effects of silver nanoparticles on In vitro regeneration of vanilla (Vanilla planifolia Jacks. ex Andrews) using a temporary immersion system. Plant Cell Tiss Organ Cult, 1240-1269.
[10] Sarmast, M. K. and H. Salehi. (2019). Silver Nanoparticles: An Influential Element in Plant Nano biotechnology. Biotechnology.publishedonline Molecular, www.researchgate.net/publication/301907423.
[11] Mohammed, M.A., Abdulridha, W.M., Abd, A.N., (2018), Thickness effect on some physical properties of the Ag thin films prepared by thermal evaporation technique, Journal of Global Pharma Technology, 10(3), pp. 613–619.
[12] Hadi, S.M. (2015). Effect of Abiotic Stress on The accumulation of some metabolitesin Ruta graveolens (InVitro). Ph.D Dissertation Biotechnology. College of Science. Al-Nahrain University.
[13] Hussein, E. A. and Aqlan. E. M. (2011). Effect of mannitol and sodium chloride on some total secondary metabolites of fenugreek calli cultured In vitro. Plant Tissue Cult. & Biotech. 21(1): 35–43.
[14] Madlomo. A.F. (2016). Effect of salt stress and some Abiotic Elicitors on active ingredient in Trigonella foenum graecum cultured In Vitro. Ph.D Dissertation Biology- Biotechnology. College of Science. Kufa University.
[15] Aghdaei, M., H. Salehi and M.K. Sarmast. (2012). Effects of silver nanoparticles on Tecomella undulata (Roxb.) Seem. Micro propagation. Advances in Horticultural Science. 10, 13128-12748.
[16] Dimkpa, C.; J. Mclean ; N. Martineau ; D. Britt ; R. Haverkamp and A. Anderson.( 2013). Silver Nanoparticles Disrupt Wheat (Triticum aestivum L.) Growth in a Sand Matrix. Environ. Sci. Technol, 47, 1082–1090.
[17] Homaei, M.; A. Ehsanpour.( 2016). Silver nanoparticles and silver ions: Oxidative stress responses and toxicity in potato (Solanum tuberosum L.) Grown in vitro. Hortic Environ Biotechnol,(57) : 544–553.
[18] Saha, N. And S. D. Gupta.( 2018). Promotion of shoot regeneration of Sverttiya chirata by biosynthesized silver nanoparticles and their involvement in ethylene interceptions and activation of antioxidant activity. Plant Cell, Tissue and Organ Culture. https://doi.org/10.1007/s11240-018-1423-8.
[19] Mohammed, M.A., Salman, S.R., Abdulridha, W.M., (2020), Structural, optical, electrical and gas sensor properties of zro2 thin films prepared by sol-gel technique. NeuroQuantology, 18(3), pp. 22–27.
[20] Zuverza-Mena, N.; R. Armendariz ; J. Peralta-Videa and J.Gardea-Torresday. (2016). Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. Frontiers in Plant Science, vol. 7: 1-11.
[21] Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotech. Advan. 27, 84-93.
[22] Evans, W. C. (2002). Trease and Evans Pharmacognosy 15th ed. W. B. Saunders Company Ltd. London. UK.