Effect of silver nanoparticles and NaCl salt on the growth and content of some active compounds of *Allium sativum* L. callus ex vivo

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Abstract

The experiment was carried out in the palm propagation unit at the Faculty of Agriculture / University of Kufa, in 2020-2021. The study involved the effect of two factors: First, different concentrations of AgNPs (A1= 0, A2= 1, andA3= 2 mg L^{-1}), and second factor: different levels of sodium chloride salt (B1= 0, B2= 25 and B3= 50 mmol L^{-1}) On the Biomass weight and growth rate of the callus as well as their effect on the content of the compounds S-allyl cysteine, Allyl methyl trisulfide and Diallyltrisulfide In the callus cells of garlic plants, ex vivo. A completely randomized design factorial experiment (C., R., D.). AgNPs showed superiority in the Biomass weight and growth rate. The focus (1mg L^{-1}) Record the highest rate of Biomass weight and growth rate reached (468.4 mg and 13.16 $mgday^{-1}$) sequentially. As well, ther are Positive increase between different levels of sodium chloride salt. Focus (25 $mmolL^{-1}$) was the highest in the Biomass weight and growth rate reached (462.8 mg and 12.96 $mg.day^{-1}$) sequentially. AgNPs Showed superiority in the secondary metabolites. The focus (2mg L^{-1}) Record the highest rate in S-allylcysteine, Allyl methyl trisulfide and Diallyltrisulfide content (168.28, 225.41and 533.14µg ml^{-1}) sequentially. As well, theer are a Positive increase between concentrations of sodium chloride salt. The focus (50 mmol L^{-1}) Record the highest rate in S-allylcysteine, Allyl methyl trisulfide and Diallyltrisulfide content (98.26, 201.85 and 510.23 µg ml^{-1}) sequentially. Whereas, the Overlap treatment (2 mg L^{-1} of AgNPs and 50 mmol L^{-1} of NaCl) recorded a Positive increase in S-allylcysteine, Allyl methyl trisulfide and Diallyltrisulfide content (233.84, 285.31 and 608.44 µg ml^{-1}) sequentially.

Keywords: Silver Nanoparticles, sodium chloride salt, Garlic Callus.

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Introduction

The scientific name of the garlic plant, *Allium sativum* L. is one of the plants Belongs to Narcissus family, and It is considered an important crop, as it is cultivated for its nutritional and medicinal benefits (2). Garlic is used fresh, dried, or squeezed, and its juice is used in cooking and seasoning meat and fish because of its desirable flavor due to the presence of volatile oils. Diabetes, through its effect in lowering blood sugar, reducing high blood pressure, and preventing clots, is considered one of the plants that strengthen the body's immunity and reduce the incidence of cancerous diseases because it contains antioxidants with its ability to help toxins the body get rid of especially heavy elements and earns it activity and vitality. It is also used to relieve objective pain (14 and 6 B). The cultivated area in Iraq amounted to (2534 dunums) with average productivity of the garlic-producing governorates (11 governorates) amounting to (1650.4 kg. dunums^{-1}) and the total produced crop amounted to (4182 tons) (9).

The plant tissue culture technique and its various applications have provided a wide range for the possibility of obtaining important compounds from an economic point of view, including pharmaceutical compounds whose preparation in the laboratory is difficult with the high cost of manufacturing (3). The importance of nanotechnology affects the Nowadays, the term nanoparticles and nanotechnology has become very popular and widely used. But it still needs a clear definition. Nanoparticles are solid particles with a size in the range of 10-1000 nanometers (12). While nanotechnology refers to any technology that is implemented at the Nanoscale where the material is restructured at the atomic and molecular levels with a size in the range of 1-100 nanometers (8). AgNPs are used in the agricultural field and in plant tissue culture technology by preventing contamination Antimicrobial and induction of callus, genetic engineering, and in increasing the production of secondary metabolites. It also stimulates some of the active compounds that are used in the pharmaceutical industry when added to a growth medium, such as flavonoids, total phenols, and antioxidant compounds (17). As for abiotic traits, they include physical traits such as exposing crops to low or high temperatures, ultraviolet rays, osmotic pressure, type of illumination, or intensity of illumination. The manifestations may be abiotic to include chemical agents such as salts of heavy metals. The use of sodium chloride salt in many tissue cultures as a cheap abiotic agent has the ability to increase the production of secondary compounds such as alkaloids and phenols through its effect on the cell membranes of plants and also affects the osmotic pressure It causes a stress on the cell, which leads to it stimulating the production of these compounds as a means of defense and creating a state of balanced osmotic pressure. (4 and 5A).

The current study aimed to use the effect of silver nanoparticles as well as sodium chloride salt on the growth and callus content of garlic plants in some active compounds.

Materials and Methods

The experiment was carried out in the palm propagation unit at the Faculty of Agriculture, University of Kufa, in 2020-2021. Used local garlic was obtained from local markets, garlic cloves were sterilized for 20 minutes at 15% vol.vol^{-1} of sodium hypochlorite (6% active component) and thereafter rinse with sterile distilled water three times. Then garlic cloves are planted in a bottle containing 10 ml of growth medium (Table 1). Plants were incubated in a growth chamber 25°C. After 6 weeks for the purpose of callus formation. Then the induced callus was taken and applied to the same components of the induction medium to obtain the required amount to conduct the experiment of stimulating the active compounds. 100 mg of callus was taken and placed on the same callus-perpetuating medium with the addition of silver nanoparticles with different concentrations (A1: 0, A2: 1, and A3: 2 mg.L^{-1}) with NaCl salt of different concentrations (B1: 0, B2: 25 and B3: 50 mmol.L^{-1}) to stimulate callus growth and production of active compounds Ten replicates were recorded for each treatment. Then the callus was brood in the growth chamber at 25°C after 28 days of agriculture. We calculated the weight of the Biomass with the growth rate of the callus as well as the concentration of the active compounds in the callus tissue S-allylcysteine, Allyl methyl trisulfide, Diallyltrisulfide, using the HPLC technique. (colum): (50x2.0mm ID), particle size: phenomenex C-18, 3μm Experiment design is factorial, completely randomized (CRD). The data were separated at the 0.05 level.

Table (1) Contents of the media used to stimulate the callus

Compounds	focus
Murashige and Skoog salts	gm L^{-1}4.43
Sucrose	30 gm L^{-1}
Myo- inositol	100 mg L^{-1}
Agar Agar	gm L^{-1}7
Benzyl adenine	mg L^{-1}0.5
Indol- 3- acetic acid	mg L^{-1}3
2,4-Dichlorophenoxy acetic acid	1 mg L^{-1}

Results

Effect of silver nanoparticles and sodium chloride salt on the Biomass weight (mg) of garlic callus in Vitro

It is evident from Table (2) that the rate of effect of silver nanoparticles at a concentration (1 mg.L^{-1}) significantly affected the Biomass weight of callus cells, as it recorded the highest weight of (468 mg) compared to the two comparison treatments and the highest concentration of silver nanoparticles (2 mg.L^{-1}) who recorded the lowest weights amounted to

(378.9, 326.4 mg) sequentially, and from the same table, we note that the effect of sodium chloride salt was significant and when treatment B2 recorded the highest average weight of Biomass amounted to (462.8 mg) compared to treatments B1 and B3 were recorded (369.4, 341.6 mg) sequentially. As for the interaction between the two factors, silver nanoparticles, and sodium chloride salt significantly affected the Biomass of garlic callus, as the interaction A2B2 treatment gave the highest weight (660.7 mg) compared to the lowest weight recorded when the interaction A3B3 was (314.3 mg).

Table (2): Effect of different concentration of AgNPs, and NaCl salt and their interplay on the weight of the Biomass (mg) in the callus of garlic plants in Vitro.

concentration AgNPs ($mg.l^{-1}$)	concentration sodium chloride salt $mmol.l^{-1}$			Mean of Silver nanoparticle
	0 B1	25 B2	50 B3	
A1 (0)	406.7	392	338	378.9
(1)A2	372.3	660.7	372.3	468.4
A3 (2)	329.3	335.7	314.3	326.4
Mean of NaCl salt	369.4	462.8	341.6	

L.S.D. $_{0.05}$ A= 26.63 B= 26.63 AB= 46.13

Effect of silver nanoparticles and NaCl salt in the growth rate of ($mg\ day^{-1}$) in garlic plant callus in Vitro. mg day

From Table (3), we see that silver nanoparticles significantly affected the growth rate of garlic plant callus, recording the highest rate when treatment A2 reached (13.16 $mg.day^{-1}$) compared to treatments A1 and A3, which recorded the lowest rates, respectively, (9.96, 8.09 $mg.day^{-1}$), and from the same table, we find that sodium chloride salt significantly affected the growth rate of callus, as it recorded the highest rate (12.96 $mg.day^{-1}$) for treatment B2 compared to treatments B1, B2, which recorded the lowest rates of (9.62, 8.63 $mg.day^{-1}$) sequentially.

With regard to the interaction between the two experimental factors, we find that there is a significant effect on the growth rate of the callus, as the highest growth rate was recorded (20.02 $mg.day^{-1}$) for the A2B2 interaction treatment, compared with the lowest growth rate was when the A3B3 interaction was (7.65 $mg\ day^{-1}$).

Table (3) Effect of different concentrations of AgNPs, and NaCl salt and their interaction on the growth rate of callus mg.day^{-1} in callus of garlic plants in Vitro

concentration AgNPs (mg.l^{-1})	concentration sodium chloride salt mmol.l^{-1}			Mean of Silver nanoparticle
	0 B1	25 B2	50 B3	
A1 (0)	10.95	10.43	8.50	9.96
(1)A2	9.73	20.02	9.73	13.16
A3 (2)	8.19	8.42	7.65	8.09
Mean of NaCl salt	9.62	12.96	8.63	
L.S.D. $_{0.05}$	A=0.953		B= 0.953	AB=1.650

Effect of AgNPs, and NaCl salt on S-allylcysteine complex stimulation in garlic plant callus in Vitro.

Table (4) shows that silver nanoparticles significantly affected the callus content of S-allylcysteine, as the highest content of S-allylcysteine was recorded when treatment A3 was (168.28 µg ml^{-1}) compared to the lowest content of (24.65µg ml^{-1}) at The comparison treatment, from the same table, we find that the effect of sodium chloride salt was significant, giving the highest content of the compound when treatment B3 reached (98.26 µg ml^{-1}) compared to the control treatment that gave the lowest content of (63.57 µg ml^{-1}). The interaction, we note that it significantly affected the content of the compound, recording the highest reading of (233.84 µg ml^{-1}) when the interaction A3B3 was treated compared to the lowest reading which was recorded when the interaction A1B2 was (14.46 µg ml^{-1}).

Table (4): Effect of different focus of AgNPs, and NaCl salt and their interaction on the content of S-allylcysteine (µg ml^{-1}) in garlic plant callus in Vitro.

concentration AgNPs (mg L^{-1})	concentration sodium chloride salt mmol L^{-1}			Mean of Silver nanoparticle
	0 B1	25 B2	50 B3	
A1 (0)	32.12	14.46	27.38	24.65
(1)A2	49.88	105.71	33.55	63.04
A3 (2)	108.71	162.28	233.84	168.28
Mean of NaCl salt	63.57	94.15	98.26	
L.S.D. $_{0.05}$	A= 1.560		B= 1.560	AB= 2.701

Effect of AgNPs, and NaCl salt on the stimulation of Allyl methyl trisulfide in garlic plant callus in Vitro.

It is noted from Table (5) the Positive increase of AgNPs on the callus content of the compound Allyl methyl trisulfide, as treatment A3 excelled by giving it the highest rate of (225.41 µg.ml^{-1}) compared to the control treatment A1, which recorded the lowest rate of (56.60 µg.ml^{-1}), We also note from the same table the significant effect of sodium chloride salt on the content of the same compound, as treatment B3 achieved the highest content (201.85 µg.ml^{-1}) compared to the lowest content of (83.62 µg.ml^{-1}) when the comparison treatment.

The results showed that the interplay between AgNPs and NaCl salt had a the Positive impact on the content of the callus tissue of the compound Allyl methyl trisulfide, as the interaction A3B3 treatment excelled by giving it the highest content of the compound amounted to (285.31 µg.ml^{-1}) compared to the lowest content when treatment A1B2 It reached (18.39 µg.ml^{-1}).

Table (5) Effect of different focus of AgNPs, and NaCl salt and their interaction on the content of Allyl methyl trisulfide (µg.ml^{-1}) in garlic callus in Vitro.

concentration AgNPs (mg.l^{-1})	concentration sodium chloride salt mmol.l^{-1}			Mean of Silver nanoparticle
	0 B1	25 B2	50 B3	
A1 (0)	23.48	18.39	127.94	56.60
(1)A2	64.28	141.84	192.30	132.81
A3 (2)	163.10	227.81	285.31	225.41
Mean of NaCl salt	83.62	129.35	201.85	
L.S.D. $_{0.05}$	A= 0.861	B= 0.861		AB= 1.490

Effect of AgNPs, and NaCl salt in the stimulation of Diallyltrisulfide complex in garlic plant callus in Vitro.

It is noted from Table (6) the significant effect of silver nanoparticles on the content of callus tissue cells of the compound Diallyltrisulfide, as the A3 treatment excelled by giving it the highest rate of the compound content amounted to (533.14 µg.ml^{-1}) compared to the control treatment A1, which recorded the lowest rate of the compound content. It reached (293.59 µg.ml^{-1}), and it is noted from the table the significant effect of sodium chloride salt on the concentration content of the same compound, as the B3 treatment achieved the highest rate of (510.23 µg.ml^{-1}) compared to the control treatment that achieved the lowest rate of the compound concentration content. Diallyltrisulfide (358.24 µg.mg^{-1}).The results showed that the interplay between AgNPs, sodium chloride salt had a Positive effect on the callus tissue content of the compound, as the interaction A3B3 treatment excelled by giving it the highest content of (608.44 µg.ml^{-1}) compared to the control treatment that achieved the lowest content of Diallyltrisulfide compound which reached (237.74 µg.ml^{-1}).

Table (6) Effect of different focus of AgNPs, and NaCl salt and their interaction on the content of Diallyltrisulfide (µg ml^{-1}) in garlic plant callus in Vitro

concentration AgNPs (mg.l^{-1})	concentration sodium chloride salt mmol.l^{-1}			Mean of Silver nanoparticle
	0 B1	25 B2	50 B3	
A1 (0)	237.74	238.41	404.62	293.59
(1)A2	422.00	439.53	517.63	459.72
A3 (2)	414.98	576.01	608.44	533.14
Mean of NaCl salt	358.24	417.98	510.23	
L.S.D. $_{0.05}$	A= 1.063	B= 1.063		AB= 1.842

Discussion

The reason for this increase, as in Tables (2,3) may be due to the fact that when silver nanoparticles were added to the growth medium, it was their ability It led to a decrease in the production of ethylene inside the cell as a result of an enzymatic change that led to this increase, and the addition of a nano-material as a catalyst with different concentrations according to the type of plant. According to the type and size of the nanomaterial used in the stimulation, as the cell does not resist it or attack it and isolate it because it does not pollute the cell. Thus, it works effectively in stimulating positive physiological and metabolic processes and reducing the production of ethylene. (1) and (18), The addition of the nano-material altered the level of endogenous growth regulators in the plant, such as auxin and cytokinin, and also changed the cell contents of proteins, fats and vitamins. (21) and (20). We also find from the same two tables that there is an increase in the weight of the Biomass, as well as the growth rate of the callus at the concentration (25 mmol.L^{-1}) of sodium chloride salt. The reason for this increase may be due to the importance of salts in plant growth in general and their impact on many processes The physiological and metabolic actions of the plant also affect the appearance of the plant and because it supplies the plant with ions and nutrients, including the sodium ion, which increases the permeability of the membranes, which helps to increase the absorption of water and other nutrients and everything contained in the nutritional medium from a source of carbohydrates, sucrose, vitamins and growth hormones such as auxins and cytokines Which increases cell division and helps to form proteins and amino acids necessary for the growth of the plant part (16). In the same regard, we find that the plant part did not reach the stage of salt stress, as there is a difference between salt stress and ion stress, as the first term is used when the salt concentration is so high that the water stress of the growth medium decreases to a significant level (0.05 - 0.1 MPa), but if the decrease in the water potential is slight and imperceptible, this means that the effect here is in the form of ion stress (10). It is clear from the results of Tables (4, 5, 6) that adding AgNPs to the callus growth medium doubled the production of the compounds under study. One of the active compounds (19) and by growing the concentricity of AgNPs give rise to increased stress on the cell as well as increased The addition of silver nanoparticles as a catalyst for the production of larger quantities of secondary metabolic compounds outside the vivo, which are of multiple pharmaceutical importance, and the reason for this increase may be due to the stress they cause to the plant. of cells and tissue death in high concentrations, and the most important proposed mechanism is using silver nanoparticles as a catalyst by inducing stress in plants, which leads to an excessive increase in the production of free radicals ROS and oxidizing factors that attack organelles and compounds of large molecular weight such as proteins, carbohydrates, cellulose and others, which ultimately results Subjecting cells to stress and increasing the production of secondary metabolic compounds, or that silver nanoparticles act as centers that send signals in the cell for some genes and increase their gene expression. These genes increase the production of certain proteins excessively, which makes the cell under stress and increases the production of various secondary metabolites. (15). The plant has developed a complex defense mechanism such as enzymatic and non-enzymatic antioxidants, to adapt to ionic, osmotic and oxidative stresses caused by NaCl salt stress (13). As a result, secondary metabolites such as phenols, terpenes and alkaloids accumulate as non-enzymatic antioxidants under different environmental stresses and act as membrane stabilizers during abiotic stress (18). As the sodium chloride salt in the food medium increases the amount of hydrogen peroxide in the callus tissue, and in the presence of free oxygen, the hydroxyl radical is formed in their interaction, which causes oxidative damage to cells. For plant survival, lower levels of H_2O_2 and O_2 are required (11) and (7).

Conclusions

The best results were obtained in this study of S-allylcysteine, Allyl methyl trisulfide and Diallyltrisulfide For garlic callus content, at a concentration of 2 mg L^{-1} of AgNPs, as well as at a concentration of 50 mmol L^{-1} of NaCl salt and when treating the interaction between them in addition to their effect on the weight of the Biomass and the growth rate of callus, but at a lower concentration of 1mg.L^{-1} of AgNPs and 25 mmol .L^{-1} of NaCl, AgNPs It may be a good substance to use to increase the active compounds of many plants metabolites as well as the sodium chloride salt as an inexpensive catalyst.

References:

1- Aghdaei, M.; H. Salehi and Sarmast, M. K. 2012. Effects of silver nanoparticles on *Tecomella undulata* (Roxb.) Seem. Micro propagation. Advances in Horticultural Science, 26 (1):21-24. https://www.jstor.org/stable/42882856

2- Al-Safadi, B. A.; N. M. Orabi and Al-Din, M. I. 1998. Improving garlic resistance to white rot, its yield and storage capacity by using gamma rays. Final report on scientific research, Department of Agriculture, Atomic Energy Commission. Damascus.

3- Al-Sowaidi, W. M. M. and H. K. M. Al-Oubaidi. 2015. Increasing (*glycosides compounds*) of Olea europaea L. From shoot tips using agno3 particle in vitro. International

Journal of Psychopharmacology, 6(1): 31-35.

4- Al-Sumaidai, K. M. I. 2017. Applications of Plant Biotechnology. AL-Nahrain University. Ministry of Higher Education and Scientific Research. The Republic of Iraq.

5- Al-Taie, A. A. G.; M. A. Aboohanah and Issa, F. H. 2021 A. Effect of silver nanoparticles in stimulating some active compounds in Garlic callus under salt stress, *in Vitro*. IOP. Conf. Ser.: Earth Environ. Sci. 923.

6- Al-Taie, A. A.G.; M. A. Aboohanah and Issa, F. H. 2021 B. Effect of Cysteine in Stimulating Some Active Compounds in Garlic Callus Under Different Light Spectra *in Vitro*. IOP. Conf. Ser.: Earth Environ. Sci. 910.

7- Arif, Y.; P. Singh; H. Siddiqui; A. Bajguz and Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: Anomic approach towards salt stress tolerance. Plant Physiology and Biochemistry, 156: 64–77.

8- Bhushan, B. 2017. Introduction to Nanotechnology. Handbook of Nanotechnology. Springer, Berlin, Heidelberg. Germany. pp. 1-19.

9- Central Bureau and Statistics, Directorate of Agricultural Statistics. 2020.

10- Mahajan, S. and N. Tuteja. 2005. Salinity and drought stress an overview. Archives of Biochemistry and Biophysics, 444:139-158.

11- Mallik, S.; M. Nayak; B. B. Sahu; A. K. Panigrahi and Shaw, B. P. 2011. Response of antioxidant enzymes to high NaCl concentration in different salt-tolerant plants. Biol. Plant, 55:191-195.

12- Mohanraj, V. J. and Y. Chen. 2006. Nanoparticles. Tropical Journal of Pharmaceutical Research, 5(1): 561-573.

13- Naik, P. M. and J. M. Al–Khayri. 2016. Abiotic and biotic elicitors–role in secondary metabolites production through *in vitro* culture of medicinal plants: A review, Journal of Advanced Research in Biotechnology, 1(2): 1-7.

- Rana, S. V.; R. Pal; K. Vaiphei; K. S. Sharma and Ola, R. P. 2011. Garlic in health and disease. Nutrition Research Reviews, 24: 60-71. DOI:10.1017/S0954422410000338

14- Saha, N. and S. Gupta. 2017. Low-dose toxicity of biogenic silver nanoparticles fabricated by Swertia chirata on root tips and flower buds of *Allium cepa*. Journals of Hazardous Materials, 330: 18–28. Doi: 10.1016/j.jhazmat.2017.01.021.

15- Saqr, M. T. 2006. Fundamentals of Plant Biochemistry and Physiology. Faculty of Agriculture, Mansoura University. Egypt. pp. 230.

16- Sarmast, M. K. and H. Salehi. 2019. Silver Nanoparticles: An Influential Element in Plant Nanobiotechnology. Molecular Biotechnology, 58(7):441-449. DOI: 10.1007/s12033-016-9943-0

17- Taiz, L. and E. Zeiger. 2006. Plant physiology, 4th edn. Sinauer Associates Inc. Publishers, Massachusetts. USA.

18- Tymoszuk, A. and D. Kulus. 2020. Silver nanoparticles induce genetic, biochemical, and phenotype variation in Chrysanthemum. Plant Cell, Tissue and Organ Culture, 143: 331–344.

19- Yin, L.; B. Gill; B. Wright and Bernhardt, E. 2012. Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. Plos One, 7: 1-7.

20- Zuverza-Mena, N.; R. Armendariz, J. Peralta-Videa and J.Gardea-Torresdey. 2016. Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. Frontiers in Plant Science, 7: 1-11. Doi: 10.3389/fpls.2016.00090