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The effect of MgNPs synthesized by using green method on the production and proliferation of Capisum annuum callus culture In Vitro

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Abstract

Nanotechnology, consider as one of the newest important methods towards improve the production of callus using plant tissue culture. The objective of the current work was to create a unique callus induction methodology. and proliferation from Capisum annum in vitro. Therefor different concentrations of Mg nanoparticles were used which synthesized using green method. For callus inducement, young foliage, and stem from the sterile germinated seedlings using Sodium hypochlorite (NaOCL) 6% for 10 min were used as explants. They were cultivated in MS media supported by several combinations of kinetin and 2, 4- Dichlorophenoxyacetic. The maximum percentage responsive reached 100% from leaf explants at 2, 4-D 1.5 mg/L and Kin 1.0 mg/L. While 2, 4-D 1.5 mg/L and Kin 0.5 mg/L achieved the highest fresh weight for callus proliferation. The best MgNPs concentration for callus inducement and proliferation of capsicum leaves and stem explants was MS media supported with 1.0 and 0.3 ml/L respectively. While the highest proliferation mean value for callus was recorded at the concentration of 6.0 ml/L MgNPs in MS medium reached 6.1 gm. AFM photographs prove that MgNPs were in nanometer size and had the spheres and the rods shape. The average grain size of the MgNPs was 23.8 to 38.24 nm.

Keywords: Capisum annuum, MgNPs, green method, callus culture, In Vitro

Introduction

Nowadays, the using of nanotechnology applications has acquired a wide resonance in all fields, from materials science to biotechnology [1,2]. The study of Nano-sciences has received increasing interest from scientists and a researcher, especially metal nanoparticles, because of their specific features compared with bulk metals. As well, its applications have increased in the field of plant biotechnology and agricultural because its environmentally friendly .[3,4,1] Nanotechnology has many applications in agricultural sector like usage of micronutrients, pesticide distribution, plant nutrition and protection, Nano fertilizers, provide protection against pathogen and pests. Also, reduce

the environmental pollution and the agriculture waste, this allows improving agricultural performance.[5,6]

Due to their expensive cost and propensity to pose risks to the environment and health, chemical, and physical methods for creating nanoparticles are gradually being superseded for many applications [7]. The reason is due to the use of some starting reagents and secondary product that being involved in nanoparticles synthesis, and they have a toxic nature [8]. So, the use of plant extracts in metal NPs synthesis has received much attention as an alternative method.[4] Metal oxide nanoparticles in particular have shown great potential for biosynthesis through plant-mediated mechanisms [9]. Plant leaves extract serve as dependable biosynthesis factors to metal oxide NPs formation, where plant extracts include biomolecules that work as reduction and capping factors to generate steady NPs. Metal NPs manufactured biologically or green using a plant route offer the benefits of being simplicity, nontoxic, and ecofriendly [10]. The interactivity between the NPs and plants causes different morphological and physiological changes in plants, the NPs characteristics determine the type of changes in plant tissue.[11] Nanoparticle treatment has successfully removed microbiological pollutants from the explants. Additionally, show how NPs positively affect the development of calluses, somatic embryogenesis, somaclonal variety, organogenesis, transform of genes, and secondary metabolites creation.[12] In plant biotechnology, NPs have the potential to be employed as efficient elicitors, and several investigations proposed they may have a role in boosting the genes expression linked to the synthesis of secondary metabolites [2]. The impacts of nanoparticles onto the various traits of many plants are rely onto the kind of NPs, plants species, the period of exposure, NPs dose. According to several researches, ZnO NPs may be helpful in germination of seeds or various sides of plant development [3]. The fabricated chlorophyll route, which regulating the essential photosynthetic enzymes activity in the chloroplast, is dependent on magnesium as a key component. Also, Mg is crucial to growth and physiology of plants due as it is responsible for the synthesis of adenosine triphosphate and nucleic acids and has multiple regulatory and structural functions within the plant cell [13,14]. Magnesium oxide has physical and chemical properties, which enabled its entry into plant cells and stimulate their growth, callus formation and development. It also has antiviral, antifungal and antibacterial effects against pests.[15]

Magnesium oxide NPs are unusually shaped crystals with extraordinarily large surface areas that have high ionic strength Nano particulate metal oxides [16]. Due to its distinctive architectures, MgO NPs has special optical, electrical, magnetic, thermal, mechanical, and chemical characteristic, make it suitable for use in many application. MgO is a crucial functional metal oxide that has found extensive use in a number of industries, including catalysis, reflective and anti-reflective coatings, refractory materials, catalyze supporter, poison waste recycling, adsorbents, substances added in heavy fuels oils, dyes, substrates like superconducting and ferroelectric thin films, and lithium-ion batteries [17,18]. Several methods for synthesis nano sized MgO particles have been described in the literature, including the sol-gel method, combustion aerosol synthesis, laser vaporization, hydrothermal synthesis, and chemical gas phase deposition

[16]. Currently, the synthesis of MgO NPs by biological methods has become more common using plant extracts. In this paper, a biological technique was used for creating Mg nanoparticles using an extract of Eucalyptus camaldulensis leaves. Capsicum annuum fruits have been used as vegetative, flavour components, natural pigments, and conventional drugs. A broad variety of normal and cayenne pepper is consuming around the world at the present time [19]. In addition, pepper includes remarkable amounts of dyes such as chlorophyll, anthocyanin, and lutein, and also has fabulous chemical components important for health supporting, like different vitamins, minerals, flavonoids, carotenoids, and capsaicinoids [20]. The study aimed to synthesis MgO NPs by green method for induction and maintenance of callus from leaves and stems of pepper using different concentrations. Besides uses various combinations of plant growth regulators to induce callus cells formation from pepper seedlings.

Materials and Methods

The present study was executed in the plant tissue culture laboratory at the biology department in the college of sciences, University of Baghdad, in March 2022 .

Seeds germination and seedlings culture

The seeds were disinfectant by using sodium hypochlorite NaOCL (Clorox) 6 % for 10 minutes. After rinsing the seeds three times with sterilized distilled water, they were put in Petri dishes including filtrate paper saturated by MS medium free from agar, sugar, and hormone. Each Petri dish contained 7-8 seeds, and then incubated at 25 ± 2C° until the seeds germinated. The hypocotyls and cotyledons of pepper seedlings 10 days old (4–5 cm in length) were cutoff into small parts and planted in MS medium with various combinations of growth regulatores (2,4-D and Kinetin) and MgNPs separately[21]

Prepare of the hot aquatic extract of Eucalyptus camaldulensis

Eucalyptus camaldulensis leaves were gathered from the University of Baghdad gardens, then washed well and dried in the oven (Memmert, Germany) at 40 °C, then ground using an electrical grinder (newal, China) for 7-10 minutes [22]. 100 mL of boiling distilled water was mixup with 8–10 g of leaves powder, and then the mixing was stirred by a magnetic stirrer for 2 hours and left overnight. Whitman paper No. 1 was used to filter the mixture, and the extract was kept at 4 °C[23] .

Prepare of MgNPs by using a magnetic stirrer device

MgNPs was manufactured by addition of 45 mL of 1*10-3 M of MgSO4 (Sigma-Aldrich) solution in a flask put on the magnetic stirrer apparatus. When the temperature settled at 70 °C, add on 5 mL of E. camaldulensis leaves hot aquatic extract with continual stirring for 30 min. The latest solution was stored at 4 C ° [24] .

Callus induction of pepper by using plant growth regulators

The MS medium (Hi media, India)[25] which contains all nutrients, was the culture medium employed for this study. MS medium was prepared by dissolving 5 gm in 1 L of distilled water then add 30 g sugar and 7.5 g agar to 1 L of the medium. Different concentrations of 2, 4-D (0.5, 1.0, 1.5 and 2.0) mg/L and Kinetin (0.5 and 1.0) mg/L were prepared, to be used for callus induction; pH was adjusted to 5.8, then autoclaved for 15 min at 121 °C. The explants culture was incubated at 25 ± 2C° and 16/8 hours (light/ dark) photoperiod with the intensity of light up to 1000 lux. After four weeks of incubation the percentage (%) of callus induction was calculated as stated by this equation [26,27] .

Callus induction (%) = [No. of explants showing callus / No. of explants inoculated] x 100

Callus maintenance using plant growth regulators

After 21-28 days of callus initiation, the callus attained a suitable size. A healthy light–yellow callus were cut into pieces weighted 100 mg before being cultured in MS media including 30 gm/L sugar with different concentrations of 2, 4-D (0.5, 1.0, 1.5 and 2.0) mg/L and Kinetin (0.5 and 1.0) mg/L. The callus culture was incubated at 25 ± 2C° and 16/8 hours (light/ dark) photoperiod with the intensity of light up to 1000 lux. Callus fresh weight was estimated under sterile conditions after six weeks of the subculture in the maintenance medium[28]

Callus induction using different concentrations of MgNPs

The explants of leaves and stems from capsicum plant were cultured in MS medium including 30 gm/L sugar supported with diverse doses of MgNPs (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 20.0 mL/L). All the explants cultures were incubated at 25 ± 2C° and 16/8 hours (light/ dark) photoperiod with the intensity of light up to 1000 lux[29] .

Callus maintenance using different concentrations of MgNPs

The callus was separated into pieces weighted 100 mg before being cultured in MS media containing 30 mg/L sugar supported with different concentrations of MgNPs (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 20.0 mL/L). The callus culture was incubated at 25 ± 2C° and 16/8 hours (light/ dark) photoperiod with the intensity of light up to 1000 lux [30] .

The description of MgNPs

The measurement of UV spectrophotometer

Preparatory description of the MgNPs was achieved using UV-vis spectroscopy. The UV-Spectrophotometer-Shimadzu UV-1800 was used to measure the UV-vis absorption spectra of the MgNPs in a quartz cuvette with a 1 cm optical path at room temperature. The wavelength range examined was 190 to 1100 nm[31] .

Measurement with Atomic Force Microscopy (AFM)

AFM (Angstrom AA2000, contact mode, atmospheric circumstances, USA) pictures were used, which clarify topological photographs at high magnification of the surface morphology, the surface morphology of MgNPs was examined. The MgNPs sample was centrifuged in an eppendrof tube with an aliquot of 0.5 mL for five minutes at 10,000 rpm. AFM was used to describe a few drops from the sample after air dried from the slide[32] .

The statistical analysis and experiments design

All the experiments were designed using Completely Randomized Design (CRD) with the existence of ten replicates. The Gen Stat software was utilized for statistical analysis with the employment of the two - way analysis of variance (ANOVA), the various between the means values were specified, and the least significant various were comparison at $p \leq 0.05$ [33] .

Results and Discussion

MgNPs visible observation

The green preparation method for the nanoparticles had a significant impact on the changes that occurred on the MgO NPs, such as a change in color, and determining the size and shape of the nanoparticles. Primitively, the reaction solution color between Eucalyptus oblique leaves extract as a reducing agent, and MgSO4 solution was yellow, but over time with heating, the solution changed to light brown. The colored change in the solution signalizes the forming of MgO NPs, as shown in Figure 1 and this result agree with the result [34] .

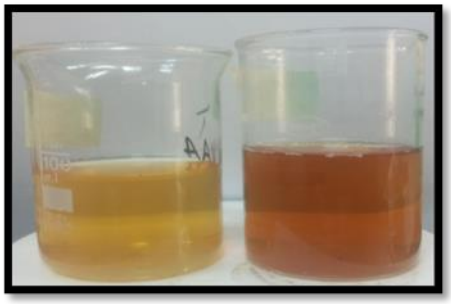


Figure (1): The alteration in color of MgNPs solution with time

A color change in MgO NPs solution after the heating process was observed. This indicates that the secondary metabolites compounds in the leaf extract like alkaloids, phenols, and flavonoids, degrade to works as a reducing and stabilizing factor in the constituencing of MgO nanoparticles [35,23]. In addition, the existence of phytochemicals compounds inside the plant extracts provides the electronic capacity of reduction of MgNPs[36] .

The UV-Vis absorbance to identify MgNPs shaping

The UV-Vis absorbance was analyzed for the sample to determine the manufacture of MgO nanoparticles. The light absorbance in Mie theory is proportional directly to the volume of the metal nanoparticles. But this theory did not apply to the metal oxide

nanoparticles because it is a semiconductor and a gap is present between the conduction band and valence [37]. For this reason, the absorbance of the metal oxide nanoparticles occurs in the UV region between (100-400) nm [9]. So the absorbance of MgO NPs was in the region of UV-A between (350-400) nm, as shown in figure 2 left.

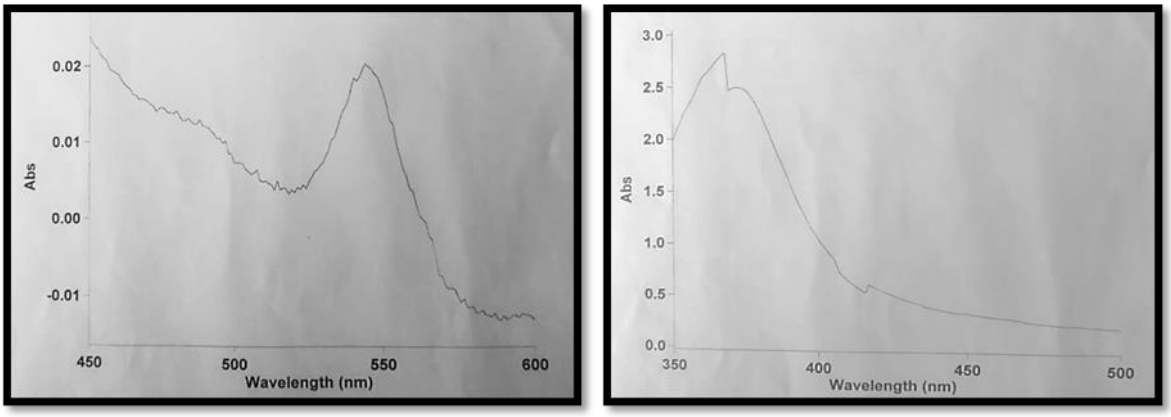


Figure (2): Absorption spectrum of MgO NPs. Right: the region of UV-A between (350-400) nm. Left: The absorption spectrum between (500-600) nm .

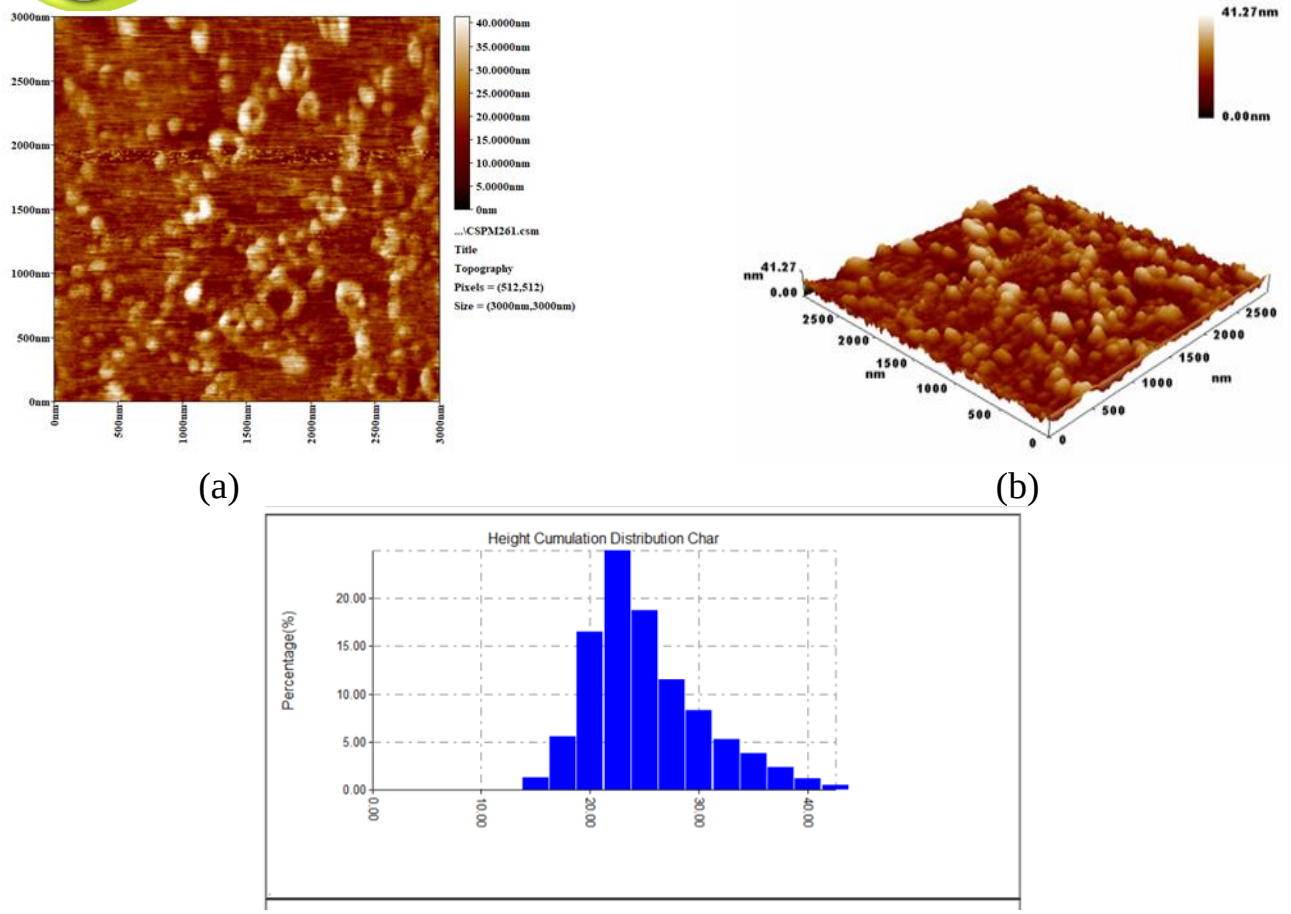
The distinct absorbance peak of MgO nanorod was achieved in the UV spectrum region (365 nm), as shown in Figure (2) and there are other peaks at both 380 and 550 nm. Which maybe refer to the light-reflecting of metal oxide molecules or due to the light-absorbing of the plants secondary compounds as mentioned by [9,4]. The peak at 365 nm detected the subsistence of metal oxide nanoparticle. Whereas the peak at 550 nm may also confirm the presence of a rod-shaped particle, so these outcomes are co-ordinate with the results of [9,38], when the shape of the nanoparticles alters from sphere to rod form as revealed by [39]. The visible spectra of the UV rays are divided into two bands. The reason is due to that shorter and longer wavelength of light is absorbed by the nanorods, where the long wavelength performs the absorption of light along the long axis of the nanorod. Whilst the shorter wavelength represents the absorption of light along the shorter wavelength of the nanorod[40] .

The analysis of Atomic Force Microscopy (AFM)

AFM analysis allows recognizing of the plot topographies, performing the surface altitude and surface fabric of nanoparticles molecules[36,41] .

Furthermore, many shapes of NPs take shape after manufacture by employing the green method, like spheres, rods, plates, and needles. AFM photographs proof that MgO NPs were in nanometer volume with spheres and rod shapes. Figure 3 b interprets the two-dimensional AFM photograph for the manufacture MgO NPs by the green method .

The MgO NPs grain size was ranged from23.8 to 38.24 nm, as shown, and it can be seen that the nanoparticles in the same Figure 3 C that a percentage % of high distribution chart occurs between 20 and 40.%



(a) (b)

(C)

Figure (3): AFM topography for MgNPs (a) 2D & (b) 3D AFM images of MgO NPs, and (c) Partitions of MgO NPs granules which manufactured under heat reaction at 70ºC for 30 min.

Callus inducement from foliate explants of capsicum using PGR

The influence for various doses of 2,4-D and Kin on the responsive to callus inducement on foliate explants of capsicum is display in (Table 1). The leaf response was significant, as the dose 1.0 mg/L of Kin registered 45.3%. Moreover, diminution in Kin concentration significantly influenced the induction of callus. But there was no response for callus inducement from the pepper stems. The implication of the culture medium with 2,4-D doses (0.5, 1.0, and 1.5) mg/L causes a significant increment in the mean responsive value of callus inducement comparison with leaf cultured in medium free from PGR. The highest response was obtained at 1.0 mg/L 2,4-D, which recorded 62 %. Whilst, 2 mg/L 2,4-D was not influencing callus induction for all the concentrations of Kin, which achieved the lowest mean 0.0%

Table (1): The influence of 2,4-D and Kin on mean proportion of callus inducement from foliate explants after culturing on MS medium for 4 weeks, n=4.

2,4-D (mg/l) / Kin (mg/l)	0.0	0.5	1.0	1.5	2.0	Mean
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	73.3	0.0	93.3	20.0	0.0	37.3
1.0	6.7	26.7	93.3	100.0	0.0	45.3
Mean	26.7	8.9	62.2	40.0	0.0	
LSD 0.05	2,4-D= 8.18; Kin= 6.34; Interaction= 14.17					

The means of callus formation at 0.0, 0.5, 1.0, 1.5, and 2.0 mg/L 2,4-D, reached 26.7, 8.9, 62.2, 40.0, and 0.0%. Significant differences were recorded between the means of all the doses of 2,4-D. The means of callus inducement at 0.0, 0.5, and 1.0 mg/L of Kin gets to 0.0, 37.3, and 45.3%. Significant differences were recorded between the means of doses 0.0, 0.5, and 1.0 mg/L of Kin. The overlap between 2,4-D and Kin (figure 4) detected the highest brittle yellowish white callus induction is 100.0% was registered in the treatment 1.5 mg/L 2,4-D + 1.0 mg/L Kin. While the treatments 1.0 mg/L 2,4-D with (1.0 or 0.5) mg/L Kin registered the same value of 93.3% for both, the treatment 0.5 mg/L Kin achieved 73.3% for callus induction .

[42],is in agreement with these results, and expressed that the induction of callus in many species of plant is preferable to higher auxins than cytokinins. [43], are also agreed with these results and recorded that leaf explants cultured on MS medium supplemented with 1.0 mg/L of 2, 4-D was shown a higher value % of callus induction and maintenance. [44], are in agreement with these results by obtaining a higher value % of callus induction and maintenance in leaf explants of dwarf pomegranate in various kinds of cytokinins and 2,4-D[45], mentioned that the diminution or increase in callus induction values % maybe due to cells sensitivity to various plant growth regulators [46], reported that equilibrium between auxin and cytokinin is needed in the development and division of the plant cells [47], explained that the lack of callus inducement in growing explants on MS medium with free hormones maybe because of hormones internal disability, which may reduce stimulation of callus induction.

Maintenance of callus cultures using PGR

Table 2 refers to the existence of a significant increment happened in the mean value % of the callus maintenance in all the doses of Kin contrasted with the mean value at 0.0 mg/L Kin which was 0.465%. The highest rate was obtained using 0.5 mg/L Kin, which listed 2.531%, who significantly various from the mean responsive percentage at 1.5 mg/L Kin reach to 1.972.% .

Table (2): The callus fresh weight (gm) growing in MS medium supported by various concentrations of 2,4-D and Kin, after six weeks, the initial calli fresh weight was 0.1gm, (n=3)

2,4-D / Kin (mg/l) (mg/l)	0.0	0.5	1.0	1.5	2.0	Mean
0.0	0.366	0.485	0.545	0.417	0.511	0.465
0.5	0.0	2.966	3.189	3.465	3.037	2.531
1.0	0.0	2.914	2.554	2.289	2.103	1.972
Mean	0.122	2.122	2.096	2.057	1.883	
LSD 0.05	2,4-D= 0.1178; Kin= 0.0912; Interaction= 0.204					

The highest value % of mean in callus weight was noted at 0.5 mg/L 2,4-D reaching 2.122 %. In a general sense, callus subculture in MS medium supported by 0.5 mg / L Kin and 1.5 mg/L 2,4-D reached 3.465 gm, and displayed better callus reproduction which has yellowish-white color, and is friable, as shown in figure 4.

Figure (4): Callus induction from Capsicum leaf explants culturing in MS medium supported by 2,4-D 1.5 mg/L and Kin 1.0 mg/L after duration of 4 weeks (Left); Callus maintenance in MS medium supported with 0.5 mg/L Kin and 1.5 mg/L 2,4-D after duration of 6 weeks (Middle); The maintenance of callus in MS medium supported with 6.0 mL/L MgNPs after duration of 6 weeks (Right).

The outcomes recorded a significant various in the mean value % in dose 0.5 with 0.0, and 2.0 mg/L 2,4-D, also between the mean value % for callus fresh weight for the concentration 1.0, 0.0, and 2.0 mg/L 2,4-D. The mean value % for the concentration 1.5 mg/L 2,4-D achieved a significant difference with the mean value of the concentration 0.0 and 2.0 mg/L 2,4-D. But no significant various between the concentrations 0.5, 1.5, and 1.0 mg/L 2,4-D. The interaction between 2,4-D and Kin detected that the highest friable yellowish-white callus proliferation (3.465 gm) was achieved in the concentrations 1.5 mg/L 2,4-D + 0.5 mg/L Kin followed by the doses 0.5 mg/L Kin + 1.0 mg/L 2,4-D, in which the callus fresh weight was 3.189 gm.

The outcomes in table 2 also showed existence of a significant various in the influence of 2,4-D levels compared to the control, and the dose of 1.0 mg/L was significantly eminent to the rest of the treatments, then it began to decrease by increase the dose of 2,4- D. The reason for the decrease of callus weight may be due to reaching of growth regulators to the concentration levels which inhibit of cell division and expansion [48]. The results demonstrate the significance of (Kin) in the nutrient media for the formation of callus due to its role in cell division, and because it activates the building of DNA and then the formation of RNA, proteins, and enzymes, and thus the division of the nucleus and cells [49]. The reason for increasing the rates of callus formation in a medium including Kin and 2,4-D compared to the control treatment may be due to their effect on stimulating cells to divide and dilate [50,51]. The appropriate combinations that excel in callus induction may have led to an internal equilibrium between growth regulators and that varies with different cultivation conditions [52,53]. The combination treatments between the auxin and cytokinin are very importance in order to get the high amounts of callus for using in other experiments, and auxins directly affect cell expansion by increasing some enzymes activities, in response to wall flexibility and increasing permeability [54]. Due to their ability to trigger the manufacture of some important proteins and the creation of RNA, cytokinins are crucial for cell division [55]. Results displayed that callus induction demands the existence of 2,4-D as auxin used to fundamental inducement. The auxin will work like an inducement signal for excite the reproductive action of cells. The existence of both Kin and 2,4-D in the medium are substantial to most appropriate calli inducement [56]. Increment in protein synthesis, nucleic acid synthesis, amino acid metabolism, respiratory activities, and changes in the activation of various membrane components and membrane permeability have all been associated with other enhanced metabolic activities in response to auxin treatment[57] .

Callus inducement from leaf and stem Capsicum using MgNPs

The outcomes displayed in figure 5 indicates that a response showed up in the callus induction at the concentrations (0.1, 0.3, 1.0, 2.0, and 6.0 mL/L MgNPs) reached (69.2, 71.8, 86.7, 71.5, and 71.6%) compared with callus induction in MS medium without Mg nanoparticles. While the concentrations of Mg nanoparticles at (0.5, 4.0, 8.0, 10.0, 15.0, and 20.0) mL/L MgNPs in MS medium didn’t have any effect on callus induction.

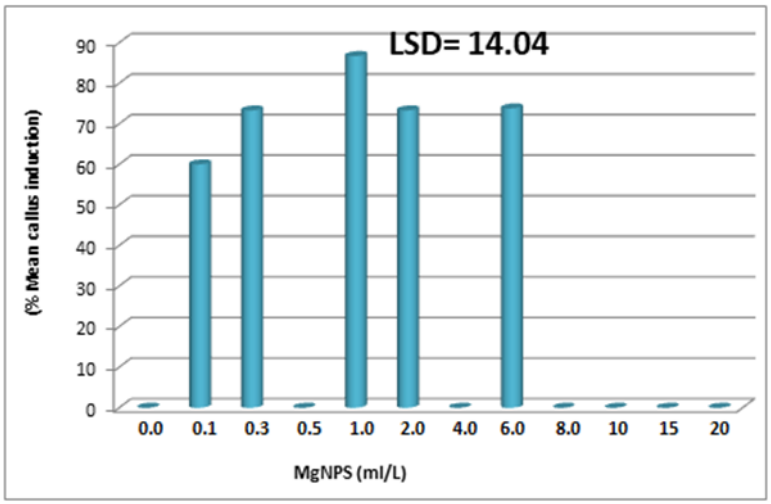


Figure (5): Effect of MgNPS (mL/L) on mean percentage of callus inducement from leaf tissue growing in MS medium after 4 weeks, n= 3

The highest mean value was achieved at the dose 1.0 mL/L MgNPs for callus inducement from leaf explants reaching 86.7 %, which was significantly various from the mean responsive percentage at the concentration of (0.1, 0.3, 2.0, and 6.0 mL/L MgNPs). But no significant various between the mean values for the concentrations (0.3, 2.0, and 6.0) mL/L MgNPs.

The results in figure 6 showed the induction of callus in the concentrations (0.3, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 mL/L MgNPs) reached (79.8, 46.2, 72.5, 71.3, 44.7, 31.9 and 26.3 %) compared with callus induction in MS medium without Mg nanoparticles. While the concentrations of Mg nanoparticles at 0.1, 10.0, 15.0, and 20.0 mL/L MgNPs in MS medium didn't have any effect on callus induction.

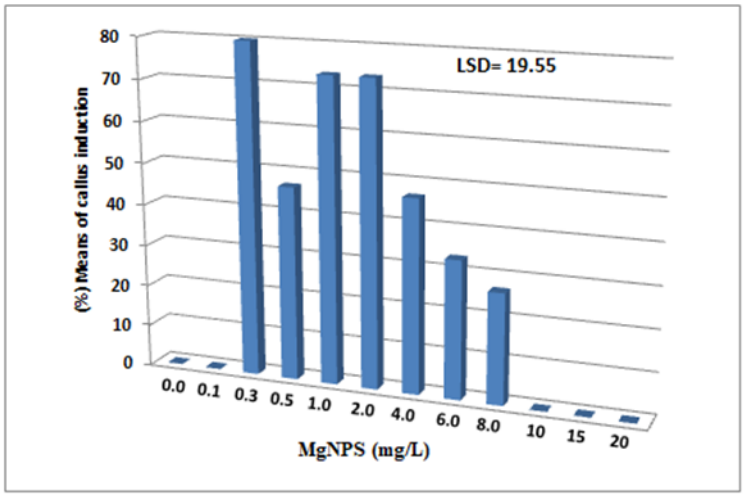


Figure (6): Effect of MgNPS (mL/L) on mean percentage of callus inducement from stem tissue growing in MS medium after 4 weeks, n= 3

The highest mean value was achieved at dose 0.3 mL/L MgNPs for callus induction from stem explants reaching 79.8 %, which was significantly various from the mean responsive percentage at the concentration of (0.3, 8.0, 6.0, 4.0, and 0.5 mL/L MgNPs).

But no significant various between the mean values of the doses (0.3, 1.0, and 2.0) mL/L MgNPs.

The increase in the weight of the callus induction to 79.8 and 86.7 gm from the stem and leaf explants became after treatment in MS medium supported with the concentrations of 0.3 and 1.0 mL/L of MgO NPs. This led to the suggestion that these concentrations represented the optimum concentration limit for Capsicum callus growth. While a decrease in the weight after this dose may have the harmful impact of the MgO nanoparticles. This result is in agreement with the result of [11], which got the highest weight of Artemisia annua callus at maximum dose 0.05 mg/L of CoO NPs. [58], say that NPs possess the capacity to promote the growth and building of chemicals in cells and act as nutrients, which can influence the physiological growth of the plant.

The magnitude of nanoparticles is one of their most significant attribute. The small magnitude of NPs in plant cells prevents them from being recognized as exterior particles in addition to allowing them to enter via the pores in the membrane. [59]. It's been noticed that various concentrations of MgO-NPs implementations significantly increase callus formation from foliage and stem. This outcome corresponds to the result of [15] which used various doses of MgO-NPs to induce callus from cowpea. The best concentration for induction was 550 mg/L. Also, our result is in the same line with the result of [60] who used different concentrations of Al2O3 NPs for callus induction from Ocimum basilicum, 75 mg/L achieved a high proportion of leaf explants that forming callus cells [61], mentioned that nanoparticles (NPs) behave as nutrients and play the main role in many physiological processes including chlorophyll biosynthesis, respiration, and redox reaction. NPs are the most crucial nutrients for a plant's development and metabolism.

Maintenance of callus using MgNPs

The results showed a response in callus proliferation at the concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0 and 15.0) mL/L MgNPs, reached (3.7, 3.6, 3.5, 2.9, 4.6, 4.5, 4.3, 4.5, 4.8, 4.9, 5.1, 5.3, 5.7, 6.1, 5.3, 4.5 and 3.8 gm). Results displayed in Figure 7 reveal significant increases in the mean % fresh weight of callus maintained on MS medium supported by all the doses of MgNPs compared with callus maintained on MS medium with no adding of nanoparticles. Except for the dose of 20.0 mL/L MgNPs, which is not achieved any significant increases in callus fresh weight compare with the callus control treatment, moreover, there were no discernible increases in callus fresh weight sustained in the concentrations (0.1, 0.2, and 0.3 mL/L) of MgNPs, and also between (0.5, 0.6, 0.7, and 0.8 mL/L) of MgNPs .

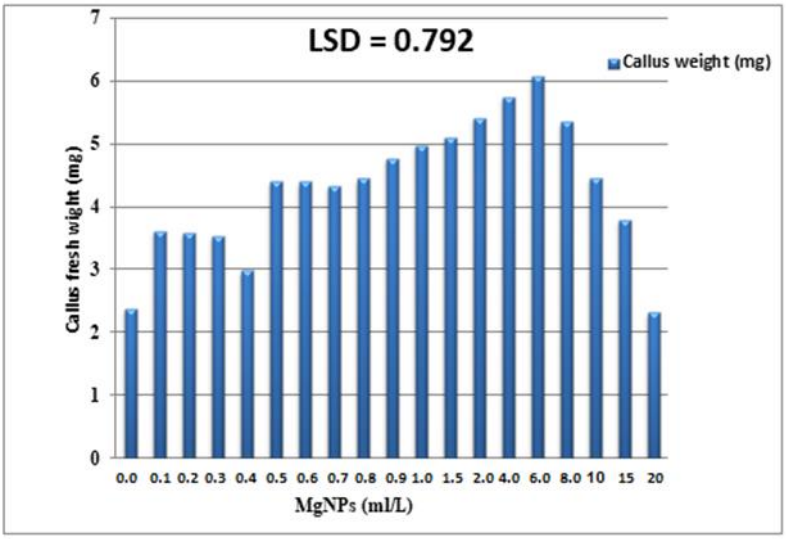


Figure (7): The fresh weight of callus cells (mg) planted in MS medium supported by several doses of MgNPs (ml/L), after 6 weeks, the initial fresh weight is 100 mg, (n=3)

The callus highest proliferation mean value was recorded at the concentration of 6.0 mL/L MgNPs reached (6.1 gm) in the MS medium as shown in (figure 4 right), which is significantly various from the mean values of all callus that proliferate at another MgNPs doses. In addition, the callus produced from 6.0 mL/L was brittle in morphology and pale yellow in color. Depending on their unique characteristics, like the smaller size, the ratio of height surface to size, the capacity to engineer electron substitution, and height level surface reacting abilities, NPs can simply get in and react with varied components of plant cells and tissues [62]. The small size of MgO NPs 23.8 - 38.24 nm facilitated their absorption process by callus cells, noting that all the concentrations used produce a clear excess in the growth of calli cells compared to callus cells not exposed to nanoparticles. Exception for the callus that was exposed to a high concentration of MgNPs of 20 mL/L, which led to a clear inhibition of callus growth. This result is consistent with [63] who say that metal NPs significantly contribute to plant growth and development at the condition of low dose concentration. High levels of metals can have harmful consequences on plants, which include reduced growth and aberrant cell division. The NPs' toxic effects can be based on the size and doses of the nanoparticles, also the precursors that are used in preparation. Various authors achieved that the major mechanism that causes the toxicity of NPs in plant cells is the creation of ROS, whose higher in plants later than exposition to nanoparticles, and leads to oxidative stress in plant cells [64,65,66]. The feature of the small size of NPs facilitates their spreading in cells, whose specified by their transmission and distribute through the plant. Additionally, the cell size influences cellular absorption and release strategies in addition to the distribution inside the cell[59] .

The difference between callus induction and maintenance using MgNPS and PGR

The capsicum callus cells induction from leaf explants achieved a high rate reached 100% by using MS medium supported with 1.0 mg/L Kin + 1.5 mg/L 2,4-D, but there was no response to callus stimulate from stem using hormones as show in figure 8.

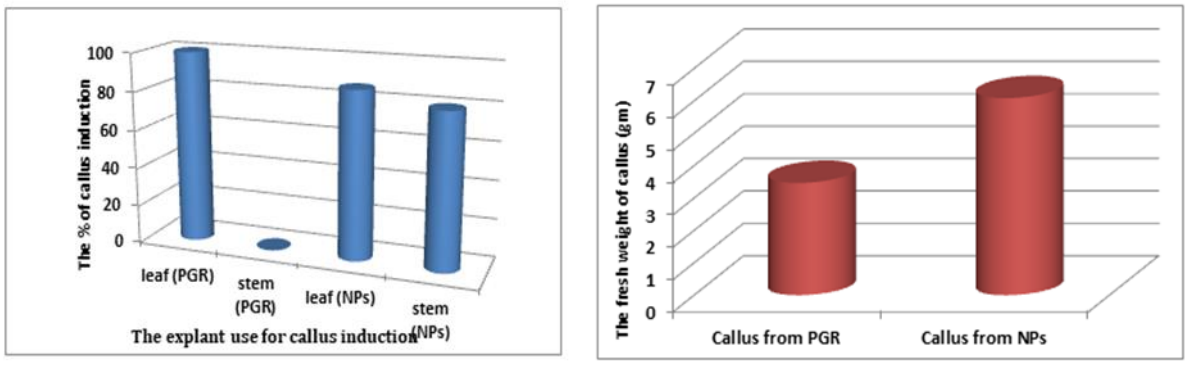


Figure (8): Callus fresh weight (mg) grown in MS medium supported with (1.0 and 0.3) mL/L of MgNPs for inducement callus using leaves and stem explants, and 1.0 mg/L Kin + 1.5 mg/L 2,4-D for induction the leaf callus cells, later than 6 weeks (Left); Callus fresh weight (gm) outgrown in MS medium supported with 1.0 mg/L Kin + 1.5 mg/L 2,4-D and 6 mL/L MgO NPs for callus maintenance, after 6 weeks (Right).

While the induction of callus cells using Mg nanoparticles from the leaves explants achieved a high rate reached 86.7% using a concentration of 1mL/L, while the induction of callus from the stem explants achieved a high rate reached 79.8% using 0.3 mL/L from MgNPs. This indicates the efficiency of Mg nanoparticles in callus induction from various parts of the pepper plants, especially the stem explants which cannot achieved by using hormones. The callus induction from the stems of pepper seedlings using MgNPs is a great advantage represented in benefiting from the production of callus from all parts of the plant seedling. Also, MS medium supported by 1.0 mg/L Kin + 1.5 mg/L 2,4-D achieved weight gain of calli proliferation reached to 3.465 gm as shown in figure 8. While the MgNPs supported medium achieved an increase in the weight of the callus, which amounted to twice the weight of the callus cultured in the hormone-supported medium. Callus weight reached 6.1 gm using 6 mL/L from MgNPs supplemented with MS medium. The use of Mg nanoparticles to maintain and multiply callus will save effort and time and achieve an increase in the amounts of callus cells. The use of nanoparticles in the production of callus is important from an economic point of view, as it saves time, and effort in testing many hormonal combinations and knowing their suitability for the production of callus for each part of the plant .

Nanoparticles enhanced callus cell induction and growth from Capsicum explants Magnesium nanoparticles proved their ability to induce callus from leaves and stems seedlings and were better than PGR to stimulate the stem callus cell. The optimal dose of MgNPs promoted the outgrowth of callus cells with increased the fresh weighing of the callus better than the use of PGR in the MS medium. High dose of NPs suppress the outgrowth of callus due to increased toxicity of nanoparticles in plants.

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