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INDUCTION CALLUS OF MORINGA PLANT (MORINGA OLEIFERA) IN VITRO

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Abstract: This study aims to find an easy and efficient way to induce callus tissue of (Moringa oleifera) in vitro. The seeds were used as explant for cultivation in vitro, these parts were sterilized with sodium hypochlorite (Naocl) at concentrations (0.0, 0.5, 1.0, 2.0%) for a period of time (5,10 ,15, 20 minutes) and mercury chloride solution 0.06,0.07,0.08 0.1 mg/L) and for a period of time (2,3,4,5 minutes). Explant were planted in the medium (Murashige and Skoog (MS) in addition to different concentrations of plant growth regulators (BA, NAA), either individually or in combination. The results showed that the use of sodium hypochlorite solution (NaocL) with a concentration of 2% and a sterilization period of 20 minutes gave the highest sterilization rate of 100% without causing damage to the plant part. Also, the seeds produced callus after 48 days of culture on Ms medium containing the best combination of plant growth regulators (BA 4.0, NAA 0.5 mg/L)

Keywords: -

Introduction

Medicinal plants occupy a large place in agricultural production and industrial and received great care in many of the producing countries as it is a "natural" source of the active substances that go into its preparation Medicine. Plants produce complex organic compounds that have no function Directly in growth are called secondary metabolites or compounds(Shoji et al., 2002)Phytochemical Compound Among these plants is the Moringa tree or the miracle tree, or Moringa oleifera belongs to the Moringa family Moringaceae, cultivated as ornamental trees for the beauty of its flowers and shade bumper, or to make a vegetable fence or as a windbreak (Duke, J. A. 1983; Fahey, J. W. 2005; Poteet, M.D. 2006). In addition, almost all parts of the tree are of interest Nutritional and medicinal, the plant constitutes an integrated food Regions of Africa and Asia, where the leaves are used as a food supplement For those with immunodeficiency, as it contains large amounts of Vitamins, carbohydrates, amino acids, and beta Carotene, iron, potassium, phosphorus, and calcium And the elements zinc, semenium, and antioxidants (Farooq. 2012; Yadav, et.al. 2016)

. The plant is also rich in some compounds such as: Zeatin It is a plant hormone belonging to the group of cytokinins Compound Aging – Anti Helps in cell division, as it contains a number of compounds Others of medical importance are β-sitosterol and L- caffeoylquinic acid and the compounds quercetin and the kaempferol and the other two compounds belong to the group Phenols are important for plants and humans alike Works to reduce the risk of cancer and some diseases Heart and circulatory system as well as their effectiveness as antioxidants And antibiotics against pathogenic fungi and bacteria (Abdulkarim ,et al 2005; Makonnen et al,1997). (The researchers were able to use tissue culture technology in Increasing the production of medicinal plants of effective compounds Medicinal uses, compared to the amount produced by the mother plant. This technology provided many opportunities for the production of metabolites Secondary school continuously and throughout
the year (.Park et .al,2008). The possibility of controlling the environmental conditions and the components of the medium the nutrients needed by the cultivated plant part .The Current study aimed to in duction of callus tissue for M.olferia. using different concentrations of growth regulators (Benzyladenine (BA) and naphthylacetic acid (NAA) combination.

Methods

The study was conducted in the Plant Tissue Culture Laboratory of the Department of the College of Science / University of Babylon for a period of 12-3-2022 until 4-20-2023 for the purpose of induction callus tissue, as well as a sterilization experiment for the Moringa.oleifera plant using tissue culture technology.

Seed collection and sterilization

Moringa seeds were collected. From the local market of the city of Hilla, it was diagnosed in the herbarium of the College of Science / University of Babylon. An appropriate number of seeds is placed in a vial that is washed with distilled water three times to get rid of suspended dust and impurities, and then transferred to the cabinet - airflow, packaging and sterilization. Sterilization experiments will be carried out on the seed.

Figure (3.1) Seed collection and sterilization

Culture media preparation

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) powder from Himedia - India was used as fenugreek seed culture medium. The culture medium was prepared by Dissolving 4.9 g of MS medium powder in a liter of distilled water, then added sugar at a concentration of 30 g/L

. Then it was placed on the shaker for 5 minutes to make sure the sugar and medium ingredients melt. After that, Adjust the pH of the medium to 5.8 by adding IN sodium hydroxide (NaOH) or IN hydrochloric acid (HCI), then 7 g/L of agar was added and the medium put on the hot stirrer until boiled and the agar was dissolved. When the solution appeared clear, 10 ml of the medium was placed on each of the sterilized culture tubes (size 20 ml), then placed in the autoclave at a temperature of 121°C and a pressure of 15-inch lb. sq' for 15 minutes. After sterilization, the medium was left to cool and ready for cultivation.

Sterilization of tools used in agriculture

All tools, including forceps, tubes, petri dishes and glass bottles, were sterilized in an oven at a temperature of (100°C) for four hours. As for the distilled water used in washing the explant, it was sterilized by (Autoclave) at a temperature of 121°C and a pressure of 1.04 kg / cm for 20 minutes. In addition to using alcohol at a concentration of 95% to sterilize blades and forceps during work, but when sterilizing hands, ethyl alcohol was used at a concentration of 70%.

Experiments

Surface sterilization experiment for the plant part

After selecting the explant(seeds) of the Moringa plant, the seeds were placed in a glass beaker with a capacity of (1 liter), then placed under running water for more than an hour and washed with water and liquid soap for (1h) to remove dust and some surface contaminants, after that they were transferred to the laminar air flow table. (Laminar air flow cabinet) to be ready for the subsequent sterilization experiments, two types of sterile solutions were used with different concentrations and a different period of time in order to be approved in the subsequent experiments, and from these solutions mercury chloride with concentrations (0.1, 0.2, 0.3, 0.4, 0.5) and time periods (2, 3,4,5 minutes) and a solution of NAOCL, i.e. commercial minor, at a concentration of 0.06,0.07,0.08,0.1%, for a period of time (5,10,15,20 minutes with continuous shaking to reduce the surface tension of the seed and penetration of the sterile material with continuous shaking to reduce the surface tension of the seeds, after that the sterilization of the seeds was carried out in all Concentrations and time periods specified for the experiment, the seeds were washed thoroughly with distilled water more than once to remove the residual effect of the sterilized material, then the seeds were culture in the nutrient media prepared for cultivation

by ten replications for each treatment, then the percentage of contamination was calculated after two weeks of cultured.

Pollution percentage = Number of Contaminated Plant parts/Total Number of Plant parts * 100

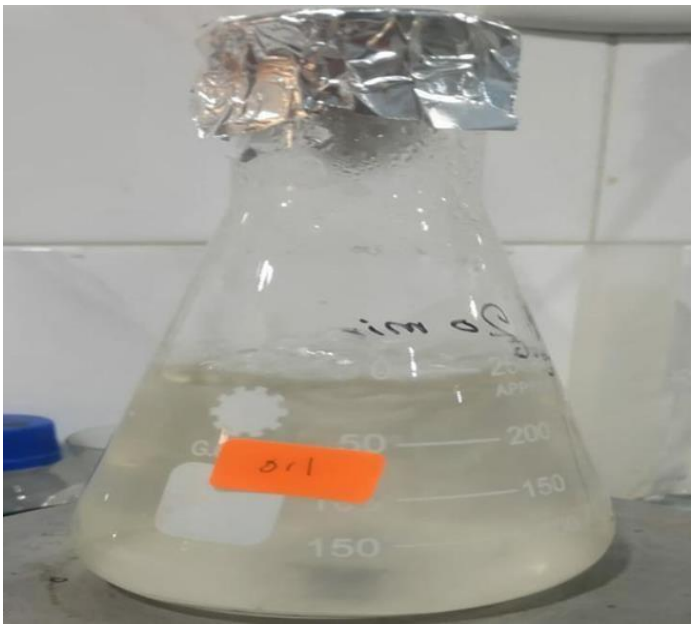

Figure (3.2) preparation of MS media

culturing of explants (seeds)

After preparing MS medium and sterilizing the seeds, the seeds were taken and culture in all culture tubes containing 10mL of MS medium free plant growth regulators, then incubated under lighting conditions for 16 hours every day.

Figure (3.3) culturing of explants on MS medium inside (Air laminar flow cabinet device)

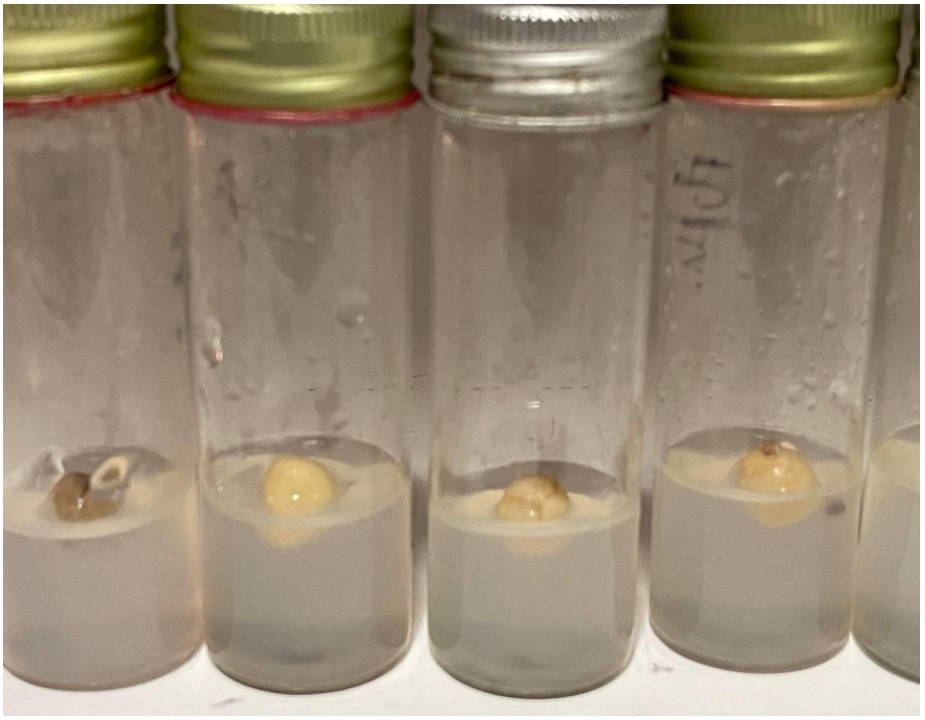

Figure (3.4) Cultured of seeds explants on MS medium

Callus induction

After sterilization Moringa seeds were cultured on MS medium supplemented with different concentrations of auxins NAA(0.0,0.05, 0.5,1.0,2.0) mg/L and different concentrations of BA (0.0, 3.0,4.0, 5.0, 6.0 mg/L) for callus induction from Moringa seeds. 48 days After cultured callus were recorded.

Estimation of fresh weight

Callus appeared on the seeds after days of seed cultivation on MS medium, and after 45 days the growth of callus was completed as the fresh weight of callus induced from seeds of all (BA and NAA) concentrations used in the experiment was calculated, as the callus was extracted from glass tubes and the fresh weight was measured Using a sensitive electric balance after removing the remnants of the medium suspended on the callus by washing with distilled water.

Design and analysis of experiments

A factorial experiment with a complete random design (CRD) was applied to study the effect of the studied factors on different traits, and the significant differences between the averages were compared with the least significant difference (LSD) at a probability level of 0.05. The statistical program SAS was used in the results.

Result and Discussion

sterilization in the mercury chloride solution of the explant (the seed)

The results show in Figure (1) that the percentage of contamination of the cultivated plant part (the seed) decreases as the concentration of the sterilization solution increases. The contamination rate is 100%. In spite of the fact that the rest of the treatments had less contamination than the comparison treatment, the contamination percentages were high, reaching 77.50, 66.00, and 47.50% for the treatments 0.05,0.07,0.08 mg HgCl2 per 100 ml of water, respectively. The results shown in Figure (1) also showed that there is an effect of the sterilization period on the percentage of contamination, as it is noted that the percentage of contamination decreases with the longer the sterilization periods. The lowest percentage of contamination was at the sterilization period of 20 minutes, compared to the percentage of contamination at five minutes, which amounted to 78%. It is also clear from the results that there is an effect of interaction between the concentrations of the sterilization solution and the sterilization period in the percentage of contamination: the results indicated that the lowest contamination percentage was 0.0% when using the concentration of 0.1 mg HgCl2 / 100 ml water for the periods of 4 and 5 minutes. As for the highest contamination percentage, it was when the concentration of 0.0% of the sterilization solution mixed with all sterilization periods, although the interference of 0.1 mg HgCl2 / 100 ml for 20 minutes gave the lowest contamination percentage, but it caused burning and death of the explant (seed), and therefore it is preferable to use the treatment 0.1mg HgCl2 / 100 ml water for 20 minutes, which did not cause any damage to the explant.

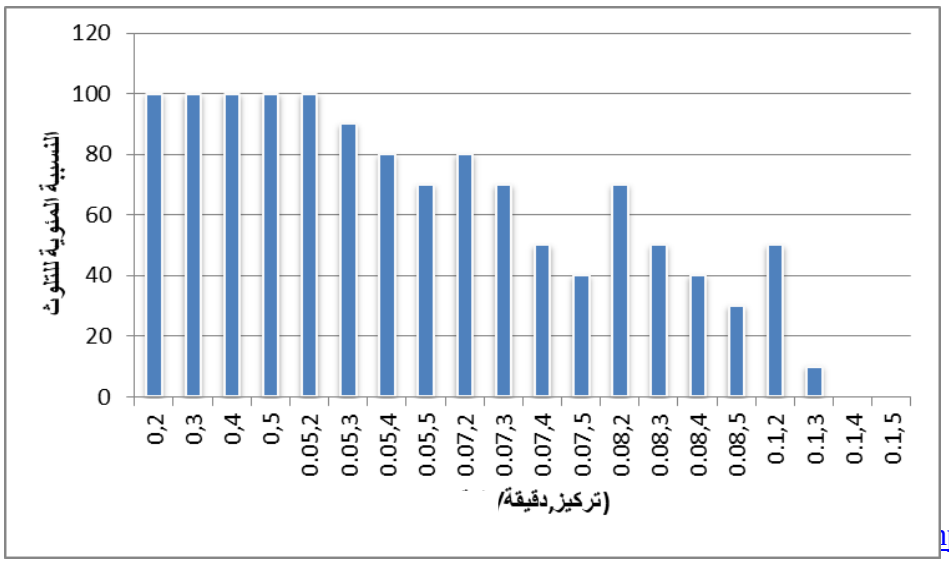

Figure (4-1) The effect of sterilization with mercuric chloride solution.

With regard to sterilization with mercury chloride solution of the explant(seeds), the results showed that the percentage of contamination of the cultivated part decreases as the concentration of the sterilization solution increases, as the lowest percentage of contamination reached 20% when the concentration treatment was 0.1 mg HgCl2 / 100 ml water compared to the comparison treatment, which amounted to the percentage of contamination is 100%, as shown in Figure (1). In spite of the fact that the rest of the treatments had less contamination compared to the comparison treatment, the percentages of contamination were high, reaching 60, 68 and 48% for the treatments 0.07, 0.05 and 10.08 mg HgCL2

/ 100 ml water, respectively. The results shown in Figure (1) also indicated that there is an effect of the sterilization period on the percentage of contamination, as it is noted that the percentage of contamination decreases with the longer the sterilization period. The lowest percentage of contamination was 54% when sterilizing for 5 minutes, compared to the sterilization period .2 minutes, which was 84%, while the contamination rate reached 76% when the sterilization period was 3 minutes. The chart generated the results that there is an effect of overlap between the

concentrations of the sterilization solution and the sterilization period in the percentage of contamination, as the lowest contamination rate reached 0.0% when using the concentration of 0.1 mg HgCL2 for 5 minutes. As for the highest percentage of contamination, it was when the concentration overlapped 0.0 with all sterilizations. Therefore, it is preferable to use the treatment 0.1 mg 2 / 100 ml water for 5 days. The effect of this sterile substance is attributed to the role of the mercury ion (Hg +2), which eliminates contaminated microorganisms through its high ability to bind Sulfhydryl groups, thus affecting the vital processes of the fungal or bacterial cell.

The use of mercury chloride solution is common in the sterilization of plant parts and has benefits and harms, but the time period for sterilization varies according to the type and explant used. It was noted that low concentrations of HgcL2 mercury chloride solution were not effective in getting rid of pollutants, while high concentrations of it led to the death and burning of explant, and this is due to the toxic effect of this substance on plant tissues and the difficulty of removing it, and this is consistent with what was reached by the mechanism, which indicated that The use of mercury chloride solution at a concentration of 0.1 mg HgCL2 / 100 ml of water for a period of 3-4 minutes preceded by the use of 70% ethyl alcohol for 30 seconds to sterilize the seeds of the Moringa plant was effective in obtaining a good sterilization rate.

Sterilization with sodium hypochlorite solution of the shoot (seed) of the Moringa plant

The results showed, as shown in Figure (3), that the percentage of contamination of the cultivated explant (the seed) decreases as the concentration of the NaOCT sterilization solution increases, and the results indicate that the percentage of contamination has decreased to the lowest possible, reaching 6% at a concentration of 2% of the active substance.

Compared to the comparison treatment, in which the contamination rate reached 100%, however, the high concentration of the sterilization solution led to the death and burning of the plant parts, while the contamination rate reached 16% when the concentration was 2% of the sterilization material, with no burning damage and death of the plant part. the cultivated seed) when using this concentration for sterilization. The results also indicated that there is an effect of the duration of sterilization on the percentage of contamination. Noting that the contamination rate decreases with the longer the sterilization period, as the contamination rate reached 36% when

the sterilization period was 20 minutes, compared to 62% when the sterilization period was minutes. As for the overlap between the concentrations of the sterilization solution and the sterilization period, the results showed that the treatment of 1.5% of the sterilization solution with a period of 20 minutes and the treatment of 2% of the sterilization solution with the duration of 15 and 20 minutes had an effect on reducing the percentage of contamination to as low as 0.0% for each. As for the highest percentage of contamination, it was when using all concentrations of the sterilization material for a period of 5 minutes. Although the interference at a concentration of 2% of the sterilization solution for a period of 20 minutes gave the lowest contamination rate of 0.0%, but it caused damage to the plant parts (burning or death), so it is preferable to use the interaction treatment 2% of the sterilization solution for a period of 20 minutes or Interferon 2% of the active substance for 15 minutes.

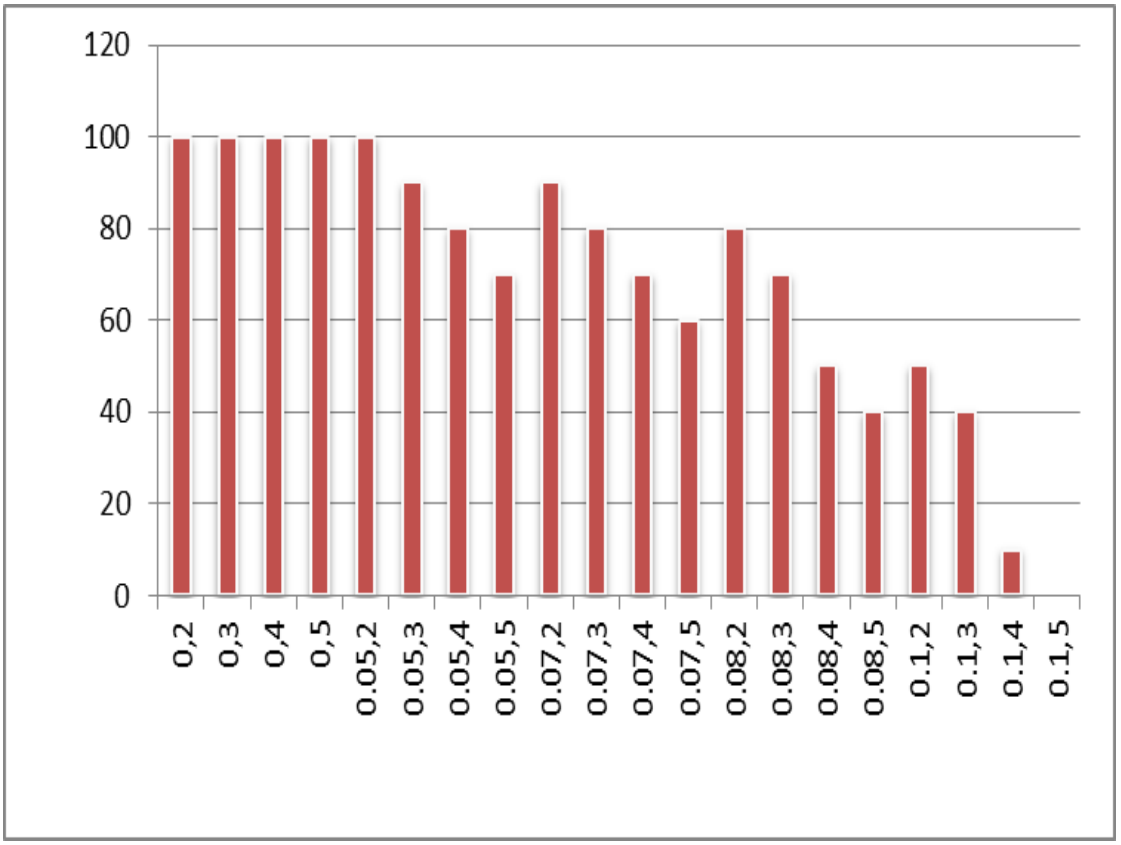

Figure (4-2) The effect of sterilization with sodium hypochlorite solution after 14 days .

Figure (4_3) the best concentration of sodium hypochlorite solution (NaOcL) 2% for sterilization .

Figure (4-4) Bacterial and fungal contamination of the explant

Callus formation experiments

Effect of NAA and BA (mg/L) and the interaction between them on the average fresh weight (mg) of callus tissue after 48day of cultivating the plant part (seed)

The data in Table (1) show that the concentration of NAA had a significant effect on the average fresh weight of callus. If it increased gradually with increasing the concentration up to 2 mg / liter, which gave the highest rate of 1090 mg. It is also noted from the data that the NAA-free medium did not have any stimulation of the cultivated explant, which indicates the importance of oxygen in the shoot medium because of its role in stimulating division, as well. It is noted that the callus emerging from the seed and growing on the media containing oxygen only differentiates to be roots, as the callus emerging when oxygen is added to the nutrient media containing the vegetable part of the seed of the Moringa plant differentiates to be roots

Figure (4-5) Callus induction .

Figure (4.6) seeds explant on MS media with control treatment.

The results in Table (1) show that the concentration of BA had a significant effect on the average callus fresh weight. The concentration of 5 mg significantly outweighed the effect with the highest average fresh weight of 264 mg. In Maine, the concentration of 6 mg BA / L gave the lowest average fresh weight of 10 mg, which did not differ significantly from the control treatment. The data in Table ((1) also shows that there is a significant effect of the interaction between BA and NAA on the average fresh weight of callus; the treatment of 0.5 mg / L NAA with 4 mg / L BA was significantly superior by giving the highest average fresh weight of callus amounted to 1239 mg as in the figure (5) The results showed in Table (1) that the lowest average of fresh callus weight was 10 mg for the overlap between high concentrations of MBA 6 mg/L and NAA at concentration 2 mg/L.

Table (1) The effect of BA and NAA (mg / L) and the interaction between them on the average fresh weight (mg) of callus tissue after 48 day of cultivating the vegetative part (seed) of Moringa plant in MS medium

The reason for the increase in callus fresh weight at a concentration of 0.5 mg/L NAA and 4 mg/L BA may be due to a balance between auxins and cytokinins added with plant endogenous hormones that work together to promote cell division and cell elongation, while the decrease in callus fresh weight may be due at high concentrations of 6 mg / L and NAA 2 mg / L to an imbalance between auxins and cytokines [97]. It was also noted that the callus produced from the vegetative part (the seed) does not have the ability to differentiate and form vegetative branches,

so it began to deteriorate and die after a period of 8 weeks of cultivation, as in, and for this reason it was excluded from the study, and this It does not agree with both [55] [6] and

[66] who obtained, when propagating the Moringa plant outside the living body, the adventitious buds from the leaf, and this may be d genetic

variations in the studied cultivars or the different components of the nutrient media.

BA Rate (mg)	2	1	0.5	0.05	0	NAA
358	1090	450	240	10	0.00	0
527	391	452	926	736	132	3
635	523	901	1239	287	229	4
327	204	294	540	334	264	5
10	10	10	10	10	10	6
-	443	421	591	275	127	NAA Rate (mg)
34=BA *NAA			16=BA	103=NAA		L.S.D0.05

Table (1) Effect of interaction between the NAA and BA concentration in the fresh weight (mg) of Moringa callus

Conclusion

It became clear through this study as follows:

1- The use of seeds as an explant in the process of inducing callus in vitro.

2- Sodium hypochlorite solution at a concentration of 0.2 for 20 minutes the best treatment to sterilize the seeds explant.

3- The mercury chloride solution at concentration of 0.07 for 3 minutes the best treatment to sterilize the seed explants.

4- The best concentration of MS was found with the combination of 4 mg/L of BA with 0.5 of NAA to obtain the best results in inducing callus from seeds in vitro.

Recommendations

1- Use other plant parts (such as leaves, roots, stems).

2- Carrying out genetic studies on callus-producing plants, the necessity of diagnosing genetic variations, and the possibility of obtaining new varieties.

3- The use of tissue culture technology in the production of secondary metabolize substances such as (active compounds, enzymatic and non- enzymatic antioxidants) in callus of Moringa plant

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