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Impact of Different Concentrations of Aluminum Chloride on Callus Induction and Organogenesis in Date Palm (*Phoenix dactylifera* L.) cv. "Barhi"

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Abstract

The present study was conducted in the Plant Tissue Culture Laboratory, Date Palm Research Center, University of Basrah, Basrah, Iraq for the years 2023 and 2024. The investigation aims to test the effect of adding different concentrations of aluminum chloride (0, 15, 30, and 45mg L^{-1}) to the MS medium prepared to induce callus and indirect shoot regeneration of date palm (*Phoenix dactylifera* L. cv. Barhi) grown using an *in vitro* culture technique. The results showed that the aluminum chloride treatment at a concentration of 45mg L^{-1} significantly outperformed the 15 mg L^{-1} treatment of aluminum chloride and the control treatment in response to callus induction (%), fresh and dry weight (mg). The control treatment recorded the lowest values in response to callus induction and fresh and dry callus weight. The results indicate that the aluminum chloride treatment at a concentration of 45mg L^{-1} significantly outperformed the 15mg L^{-1} treatment of aluminum chloride and the control treatment in response to shoot regeneration, number of shoots per culture, shoot length, and number of leaves per shoot. The control treatment recorded the lowest values in these characteristics.

Keywords: Indirect shoot, in vitro, induction, callus, organogenesis.

I. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) belongs to the family Arecaceae and the order Palmae (Ibrahim *et al*., 2018). It is a family of monocotyledonous, dioecious flowering vascular plants and is a semi-tropical evergreen fruit tree (Al-Khayri, 2007). Date palm trees are characterized by their ability to grow vegetatively in various climatic conditions. They are also widely cultivated in arid and semi-arid areas and North Africa (Ibrahim, 2019). They are one of the oldest Asian and African plants, grown for their sweet-tasting, edible fruits and their industrial and medicinal benefits (Echegaray *et al*., 2020). Date palms reproduce in two ways: sexually, by seeds, and by offshoots. This is the traditional method of vegetative propagation and is common in palm propagation (Ibrahim *et al*., 2013). The second propagation is genetically similar to the mother plants. However, it is a strenuous and difficult process because it requires a lot of care and its survivor rate is low (Ibrahim *et al*., 2017; Ibrahim *et al*., 2021). In addition, the one date palm production ranges from 1-30 offshoots, especially in high-quality cultivars (Al-Mssallem *et al*., 2024). Barhi cultivar is distinguished by low production of offshoots and difficulty in obtaining them because of the costly prices of offshoots. Therefore, many researchers have resorted to propagating rare, high-quality, and seductive date palm cultivars through tissue culture techniques to overcome the low production of offshoots and their high prices (Ibrahim *et al*., 2013; Hashem *et al*., 2018; Ibrahim *et al*., 2018). Through plant tissue culture technology, a large number of plants can be obtained in a short time. The plants produced in this way are free of pathogens, so they can be exported without resorting to agricultural quarantine. They are also easy to transport and trade. They can produce many offshoots. They are characterized by a high degree of genetic compatibility with the

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mother plant (Abhman *et al*. 2001; Khairallah 2007). Aluminum (Al) is the third most abundant element in the Earth's crust (after oxygen and silicon), accounting for about 7% by mass (Rabinovich, 2013). In soil, aluminum ions can be toxic to plants, but in combination with other minerals (Rahman *et al*., 2018). Aluminum increases plant growth activation and stimulation by enhancing phosphorus availability and activating genes associated with abiotic stress (Muhammad *et al*., 2018; Noor *et al*., 2023). Several studies have found that low aluminum concentrations lead to stimulate cell elongation and development. Some elements, including aluminum, have been identified as essential and beneficial for some plant species (Muhammad *et al*., 2018; Ofoe *et al*., 2023). Adding it to the micropropagation medium has been found to promote the uptake of several nutrients, such as K, P, Mg, Fe, and Ca (Gribble *et al*., 2002; Ezaki, 2004; George *et al*., 2008). Plant cells have highly efficient defense systems to respond to biotic and abiotic stresses. Some elements, including aluminum, have been identified as essential and beneficial for some plant species (Singhal *et al*., 2022). Zayed *et al*. (2019) also observed that treatment with low concentrations of aluminum chloride stimulated and promoted the differentiation of the fragile callus of date palm cv. Siwi. Aluminum chloride enhanced shoot regeneration in date palm (Al-Mayahi, 2019). It increased the vegetative growth of shoots and microtubers production in *Gloriosa superba* L. (Subiramani *et al*., 2019). Therefore, this investigation aims to test the effect of adding different concentrations of aluminum chloride to the MS medium prepared to induce callus and indirect shoot regeneration of date palm (*Phoenix dactylifera* L. cv. Barhi) grown using an in vitro culture technique.

II. MATERIALS AND METHODS

The present study was conducted in the Plant Tissue Culture Laboratory, Date Palm Research Center, University of Basrah, Basrah, Iraq for the years 2023 and 2024. It used six-month-old Barhi date palm callus (Plate 1, A) obtained from immature inflorescences grown in 4.43g L^{-1} MS (Murashige and Skooge,1962) medium, with the addition of 40g L^{-1} Sucrose, 200mg L^{-1} Sodium hydrogen orthophosphates, 50mg L^{-1} Adenine sulfate, 100mg L^{-1} Myo-inositol, 0.5mg L^{-1} Thiamine-HCl, 3mg L^{-1} Glycine, 2mg L^{-1} Riboflavin, 0.2mg L^{-1} -Nephthathene acetic acid (NAA), 0.1mg L^{-1} Benzyl adenine (BA), 100mg L^{-1} Glutamine, 3g L^{-1} Neutralized activated charcoal and 7g L^{-1} Agar-agar. Four concentrations of aluminum chloride (0, 15, 30, and 45 mg L^{-1}) were added to the MS medium. The pH of the medium was adjusted to 5.7 using a Lutron pH Meter, Model 206, by calibrating the culture medium with 0.1 N NaOH and HCl solutions. Distribute the MS medium in 18 x 2.5 cm Pyrex test tubes at a rate of 20 ml per culture tube and 50 ml per 350 ml jar. The MS medium of culture tubes was sterilized by an Autoclave device at a temperature of 121°C and a steam pressure of 1.05 kg cm2 for 20 minutes.

The following characteristics were calculated after eight weeks of in vitro culture:

1. % response to callus induction
2. Fresh and dry weight of callus (mg)
3. % response to shoot regeneration
4. Number of shoots per 50mg callus
5. Shoot length (cm)
6. Number of leaves per shoot

Experimental design and statistical analysis

The experiments were carried out by using the Complete Randomized Design (C.R.D). The significance of the treatment means was tested according to the Revised-Least Significant Difference (R-L.S.D) test at a probability level of 5% described by Hoshmand (2018). The ready-made statistical analysis program was used in the analysis of variance of the data (SPSS (2017) version 22).

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III. RESULTS AND DISCUSSION

Effect of different concentrations of aluminum chloride on the percentage of callus induction:

The results in Figure (1) indicate the effect of different concentrations of aluminum chloride on the percentage of callus induction of Barhi date palms after four weeks of *in vitro* culture. The same figure shows significant differences between the treatments in the percentage of callus induction. It is noted from the figure that the aluminum chloride treatment at a concentration of 45mg L^{-1} significantly outperformed both the 15 mg L^{-1} treatment of aluminum chloride and the control treatment (Plate 1,B). This treatment recorded the highest percentage of callus induction, reaching 100%. There were no significant differences between the 45 and 30mg L^{-1} treatments in the percentage of callus induction. The aluminum chloride treatment at a concentration of 30mg L^{-1} also significantly outperformed the control treatments and the aluminum chloride treatment at a concentration of 15mg L^{-1} in the percentage of callus induction. There were no significant differences between the 15mg L^{-1} aluminum chloride and the control treatment. The control treatment recorded the lowest percentage of response to callus induction, reaching 84%.

Figure (2) shows the effect of different concentrations of aluminum chloride on the fresh weight of Barhi date palm callus induced after four weeks of *in vitro* culture. The data from the same figure indicate significant differences in the fresh weight of the induced callus. The treatment of 45mg L^{-1} of aluminum chloride was significantly superior compared to the rest of the other treatments except for 30 mg L^{-1} of aluminum chloride, which in turn recorded the highest fresh weight of callus reaching 290.41mg. It was followed by the treatment of 30 mg L^{-1} of aluminum chloride, which in turn significantly outperformed the control and 15mg L^{-1} of aluminum chloride treatments in the fresh weight of callus. The 15mg L^{-1} aluminum chloride was significantly superior to the control treatment in the fresh weight of the callus. The lowest fresh weight of the callus reached 174.8mg, which was recorded by the control treatment.

Figure (3) shows the effect of different concentrations of aluminum chloride on the dry weight (mg) of induced callus of Barhi date palm cultivar after four weeks of *in vitro* culture. The data from the same figure indicate significant differences in the dry weight of induced callus, as the treatment of 45mg L^{-1} of aluminum chloride was significantly superior compared to the rest of the treatments, which in turn recorded the highest dry weight of callus, reaching 61.64mg. This was followed by the treatment of 30mg L^{-1} of aluminum chloride, which in turn significantly outperformed the control and 15mg L^{-1} of aluminum chloride treatments in the dry weight of callus. The treatment of 15mg L^{-1} of aluminum chloride was significantly superior to the control treatment in the dry weight of callus. The lowest dry weight of the callus was 29.6mg, which was recorded by the control treatment.

The present study showed that the best growth and response rate to callus induction and fresh and dry weight were treated with aluminum chloride at a concentration of 45mg L^{-1}. These results were consistent with the results found by Al-Mayahi, (2019). This researcher used concentrations between 25 and 50 mg L^{-1} of aluminum chloride in inducing callus via in vitro culture of another date palm cultivar. The results of this study did not agree with the results obtained by Zayed *et al*. (2019). This researcher used low concentrations ranging between 0.03 and 0.06mg L^{-1} aluminum chloride. The reason for this is that aluminum chloride and some heavy metals are used as mineral stimulants for growth, and callus induction, and increase the fresh and dry weight of date palm callus as a result of increasing the rate of cell division and expansion (Muhammad *et al*., 2018; Al-Mayahi, 2019).

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Effect of different concentrations of aluminum chloride on the percentage of response to shoot regeneration:

Figure (4) shows the effect of different concentrations of aluminum chloride on the percentage of shoot regeneration of Barhi date palms after four weeks of *in vitro* culture. This figure shows significant differences between treatments in the percentage of response to shoot regeneration. The results indicate that the aluminum chloride treatment at a concentration of 45mg L^{-1} significantly outperformed both the 15mg L^{-1} treatment of aluminum chloride and the control treatment. This treatment recorded the highest percentage of response to branch generation, reaching 100%. There were no significant differences between the 45 and 30mg L^{-1} treatments in the percentage of response to shoot regeneration. The aluminum chloride treatment at a concentration of 30mg L^{-1} significantly outperformed the control treatments and the aluminum chloride treatment at a concentration of 15mg L^{-1} in the percentage of shoot regeneration. The results from the same figure show that the treatment of aluminum chloride at a concentration of 15mg L^{-1} was significantly superior to the control treatment. The lowest percentage of shoot regeneration was recorded by the control treatment, which amounted to 47%.

Figure (5) shows the effect of different concentrations of aluminum chloride on the number of shoots of the Barhi date palm cultivar induced after four weeks of *in vitro* culture. The data from this figure indicate significant differences in the number of shoots. The 45mg L^{-1} of aluminum chloride was significantly superior compared to the other treatments (Plate 1,C). It recorded the highest number of branches, which amounted to 44 shoots per culture. This was followed by the 30mg L^{-1} treatment of aluminum chloride, which in turn was significantly superior to the control and 15mg L^{-1} of aluminum chloride in the number of shoots. The 15mg L^{-1} treatment of aluminum chloride was significantly superior to the control treatment in the number of branches. The control treatment recorded the lowest number of shoots amounting to 9.4 shoots per culture.

The results in Figure (6) showed the effect of different concentrations of aluminum chloride on the shoot length of Barhi date palm cultivar induced after eight weeks of *in vitro* culture. There are significant differences between the treatments in the number of shoots. The treatment of 45mg L^{-1} of aluminum chloride was significantly superior compared to the other treatments (Plate 1, D). It recorded the highest shoot length of 8.7cm. This was followed by the treatment of 30mg L^{-1} of aluminum chloride. It was significantly superior to the control and 15mg L^{-1} of aluminum chloride treatments in shoot length. The treatment of 15 mg L^{-1} of aluminum chloride was significantly superior to the control treatment in shoot length. The control treatment recorded the lowest shoot length reaching 1.9 cm.

The results of Figure (7) indicate the effect of different concentrations of aluminum chloride on the number of leaves per shoot of Barhi date palm cultivar after eight weeks of in vitro culture. There are significant differences between the treatments in the number of leaves per shoot. It is noted from this figure that the aluminum chloride treatment at a concentration of 45mg L^{-1} significantly outperformed both the 15mg L^{-1} treatment of aluminum chloride and the control treatment (Plate 1, E). This treatment recorded the highest number of leaves, reaching 2.6 leaves per shoot. There were no significant differences between the 45 and 30mg L^{-1} of aluminum chloride treatments. The aluminum chloride treatment at 30 mg L^{-1} also significantly outperformed the control and 15mg L^{-1} aluminum chloride treatments in the number of leaves per shoot. There were no significant differences between the 15 mg L^{-1} of aluminum chloride and the control treatment in leaves per shoot.

The present results showed that the best treatment of aluminum chloride was at a concentration of 45 mg L^{-1}, which gave the best result for shoot regeneration, number of shoots, shoot lengths, and number of leaves per shoot. The results of this study were consistent with the results obtained by the researcher Al-Mayahi (2019). Shoot formation is the first step in the regeneration of explants by in vitro culture. Aluminum chloride worked to regenerate the plant by forming shoots through the differentiation of callus cells and the formation

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of buds that grew, elongated, and formed vegetative shoots (Bidabadi and Jain, 2020). $AlCl_3$ can stimulate increased production of bioactive compounds by modifying the secondary metabolism of the plant. It also improves the plant defense mechanism by increasing the activities of various antioxidant enzymes, i.e. it protects the cell from oxidative damage (Ramirez-Estrada *et al*., 2016). Aluminum chloride also participates in the defense mechanism and enhances stress resistance (Kaur *et al*., 2016). One study showed that low concentrations of aluminum chloride encourage bud induction (Muhammad *et al*., 2018; Ofoe *et al*., 2023). Another study showed that high concentrations of aluminum chloride promote bud growth (Zafar *et al*., 2017). Therefore, we can say that the effective and optimal concentration of aluminum chloride varies according to the plant species.

IV. CONCLUSION

The addition of aluminum chloride to MS medium increased callus induction and shoot regeneration, increased the number of shoots, and improved their vegetative properties in micropropagation of Barhi date palm (*Phoenix dactylifera* L.) via *in vitro* culture technique. A high concentration of aluminum chloride was better in callus induction and organogenesis compared to low concentrations.

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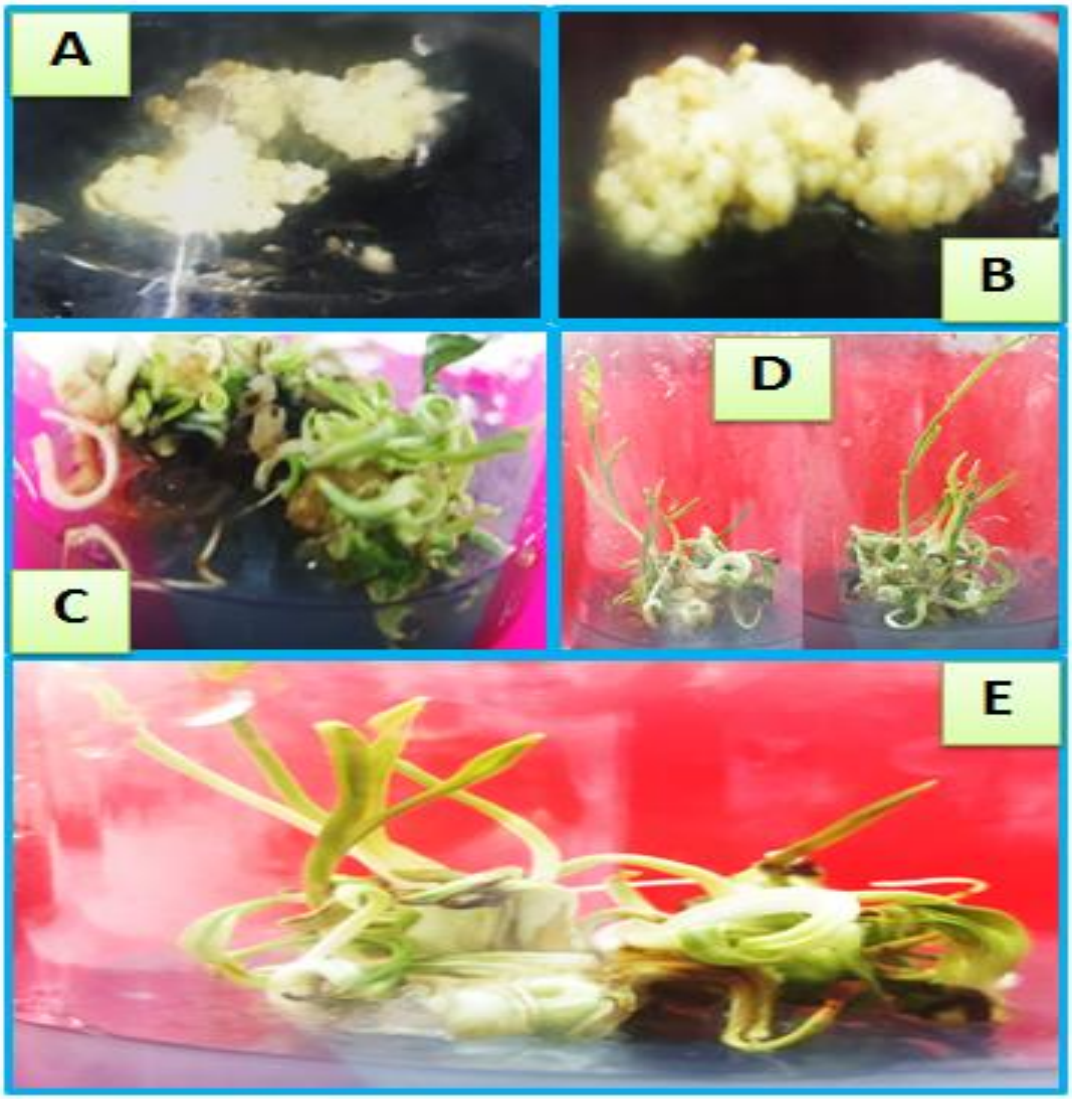

Plate (1): Effect of different concentrations of aluminum chloride on callus induction and shoot regeneration of date palm (*Phoenix dactylifera* L. cv. Barhi) via in vitro culture. A. Primary callus of date palm female inflorescence; B. Callus induction at 45mg L^{-1} of aluminum chloride; C. Shoot regeneration at 45mg L^{-1} of aluminum chloride; D. Shoot length (cm) at 45mg L^{-1} of aluminum chloride; E. Leaves per shoot at 45mg L^{-1} of aluminum chloride.

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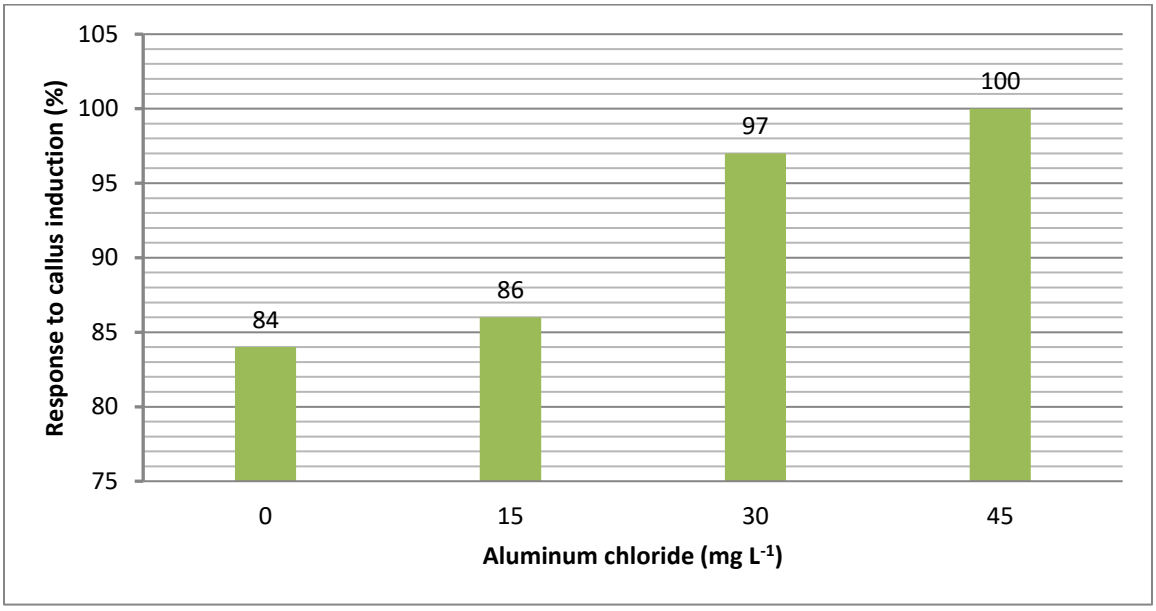

Figure (1): Effect of Aluminum chloride on response to callus induction after four weeks of culture (R-LSD 0.05= 3.83).

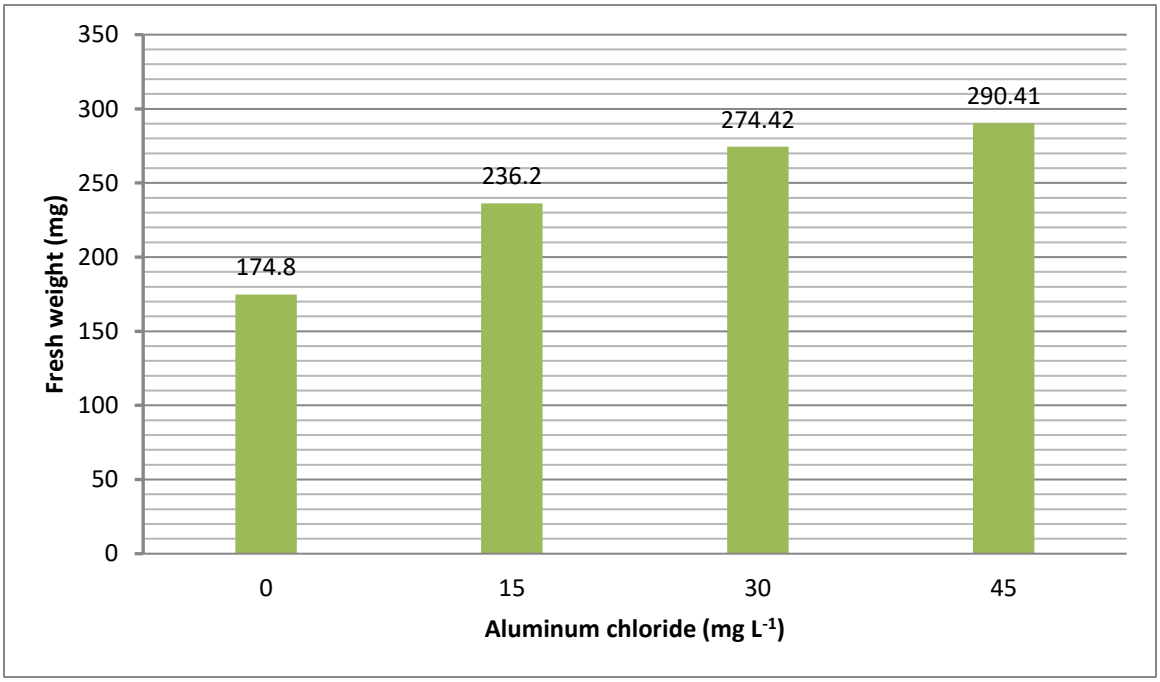

Figure (2): Effect of Aluminum chloride on fresh weight (mg) after four weeks of culture (R-LSD 0.05= 8.25).

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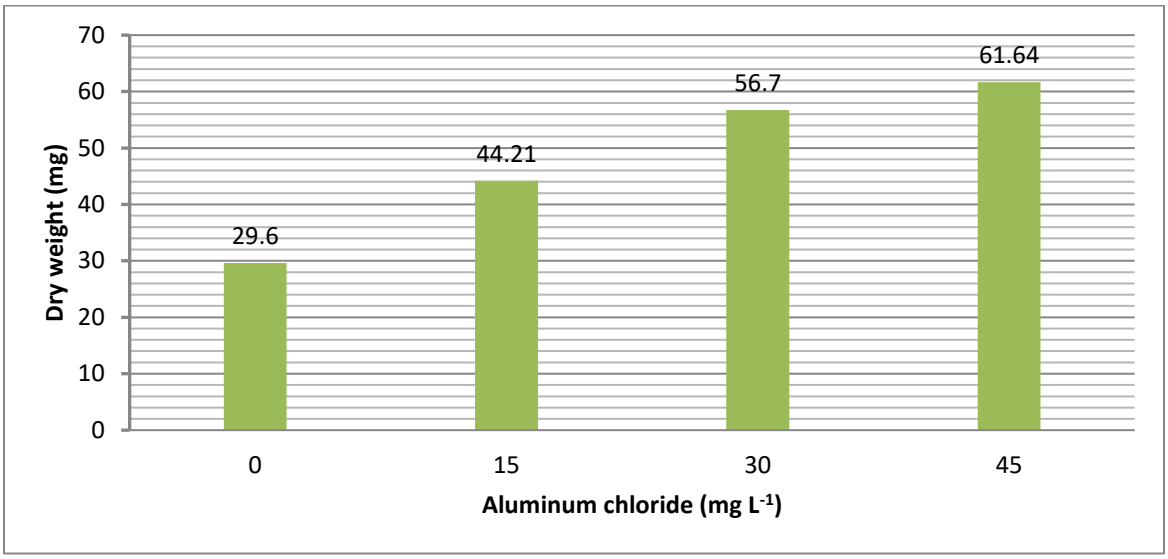

Figure (3): Effect of Aluminum chloride on dry weight (mg) after four weeks of culture (R-LSD 0.05= 2.50).

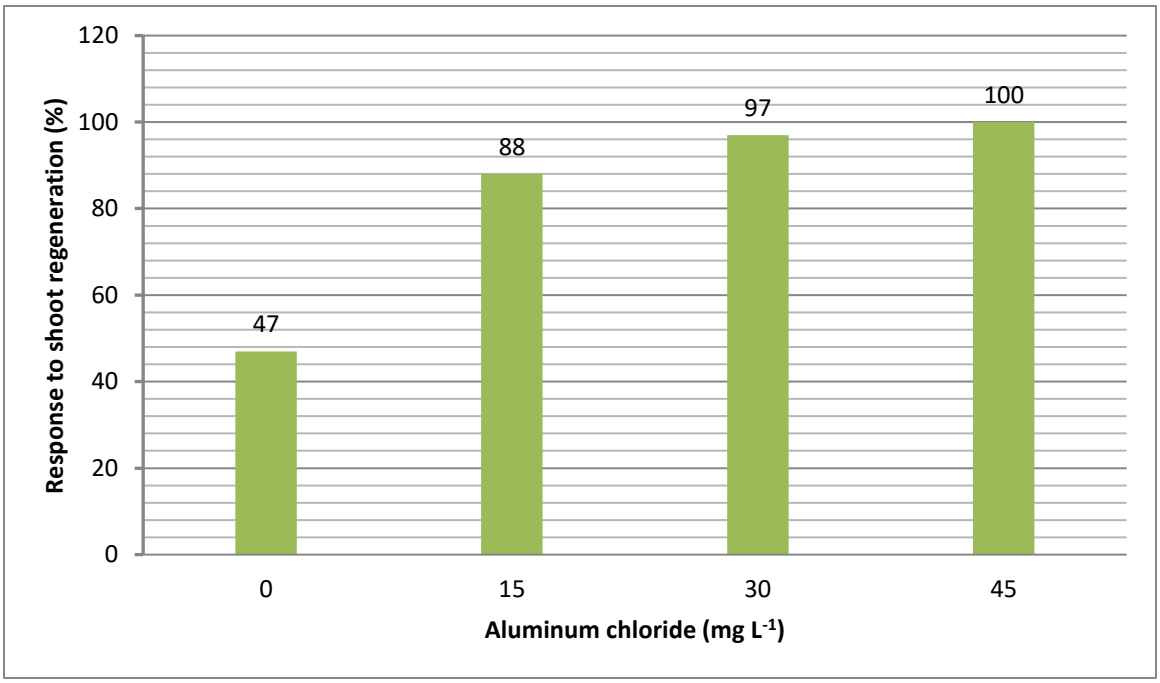

Figure (4): Effect of Aluminum chloride on response to shoot regeneration after four weeks of culture (R-LSD 0.05= 3.99).

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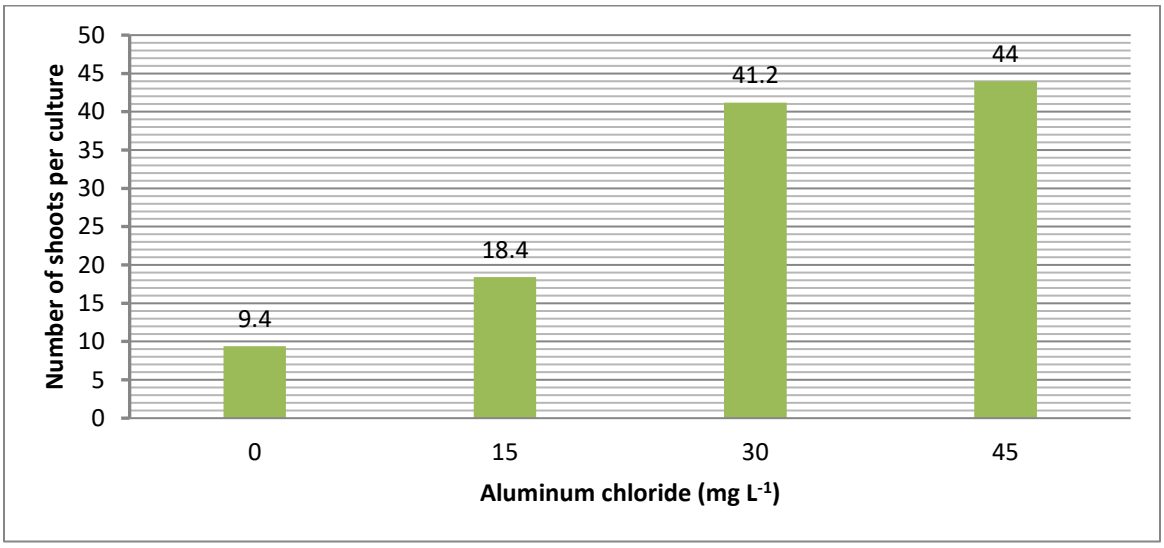

Figure (5): Effect of Aluminum chloride on number of shoots per culture after four weeks of culture (R-LSD 0.05= 2.07).

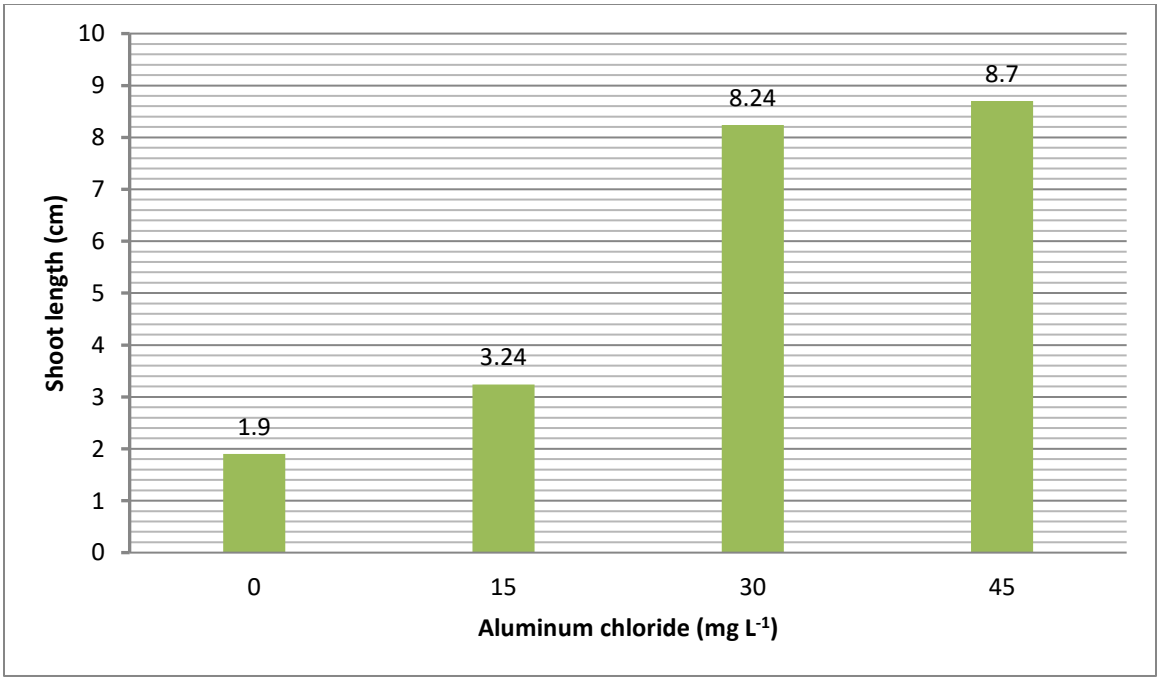

Figure (6): Effect of Aluminum chloride on shoot length (cm) after eight weeks of culture (R-LSD 0.05= 0.30).

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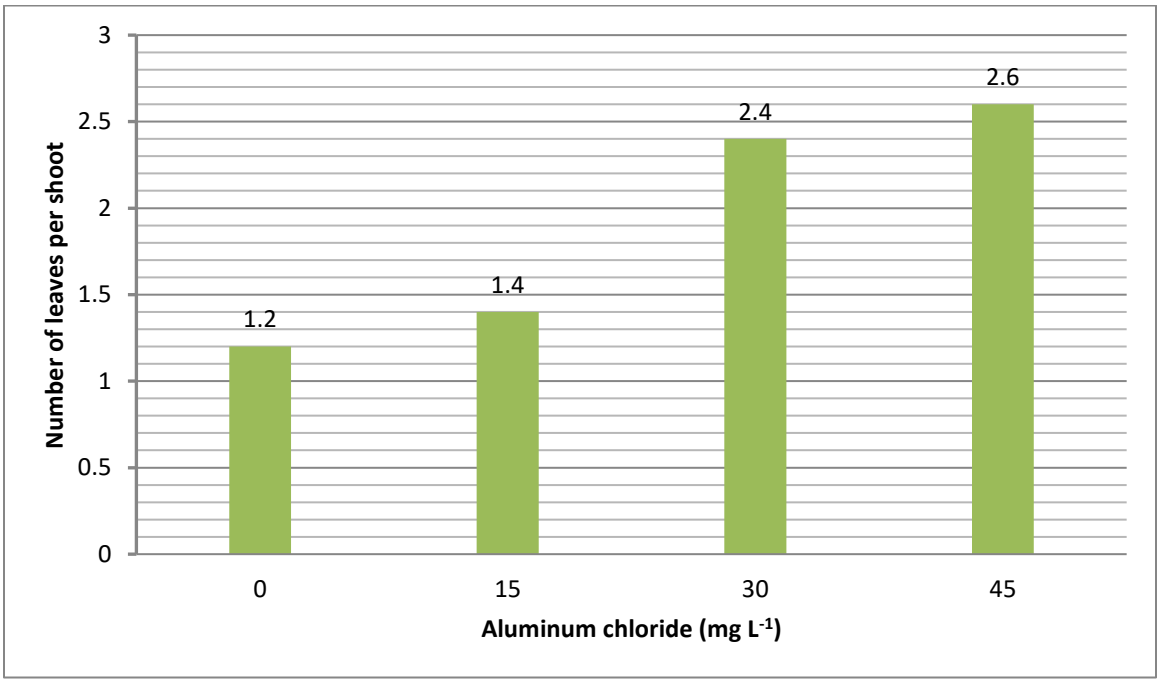

Figure (7): Effect of Aluminum chloride on number of leaves per shoot after eight weeks of culture (R-LSD 0.05= 0.67).