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PLANT GROWTH REGULATORS, LICORICE EXTRACT, AND SALTS USED IN MEDIA FOR MICROPROPAGATION OF *GARDENIA JASMINOIDES*

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SUMMARY

The study for improving the multiplication and rooting systems of *Gardenia jasminoides* cv. veitchii ensued in 2020–2021 at the Department of Horticulture, College of Agriculture, University of Kerbala, Kerbala, Iraq. The first experiment comprised multiplication, using Benzyl adenine (BA) at the concentrations of 0, 2, 4, and 6 mg L^{-1} and licorice extract at the concentrations of 0, 1, 2, and 3 g L^{-1}. The second experiment involved the rooting system, including cultivation of gardenia, shoots on MS media with different formulations, i.e., MS media with half strength of basal salts, and a) IBA (0, 0.5, 1, 1.5 mg L^{-1}), b) licorice extract concentrations (0, 1, 2, 3 g L^{-1}), MS medium with the full strength of basal salts, c) IBA (0, 0.5, 1, 1.5 mg L^{-1}), and d) licorice extract (0.1, 2, 3 g L^{-1}). The results showed the superiority of benzyl adenine (4 mg L^{-1}) in achieving the highest number of shoots per plant, and the concentration of 2 mg L^{-1} showed superior in giving the maximum shoot length and the number of leaves per plant. The licorice extract (2 g L^{-1}) achieved the highest number of shoots per plant, while the concentration of 3 gm L^{-1} proved superior in reaching the maximum shoot length and number of leaves per plant. The Indole 3-butyric acid- IBA (1 mg L^{-1}) revealed the highest rooting percentage, root length, and root dry weight, with IBA (2 mg L^{-1}) being the highest root number. Also, licorice extract (3 g L^{-1}) supplemented to the media with half strength of basal salts displayed superior rooting percentage, root length, and root dry weight.

Keywords: plant growth regulators, licorice extract, micropropagation, Gardenia veitchii, in vitro

Key findings: Benzyl adenine - BA (4 mg L^{-1}) produced the maximum number of shoots per plant, and BA at 2 mg L^{-1} generated the most number of leaves and shoot length. Adding 2 g L^{-1} of licorice extract yielded the highest number of shoots plant^{-1}, with licorice at the 3 g m L^{-1} producing more leaves and shoot length. The IBA (1 mg L^{-1}) had the highest rooting percentage, length, and dry weight. In half-strength salt media plus licorice extract at the 3 g L^{-1} increased the number of roots, root length, and dry weight.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the essential cereal crops worldwide. It is commonly used for human consumption, feeds for animals and poultry, and starch and oils production for cooking. Egypt could increase its maize production by horizontal expansion, growing

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The gardenia is a tender evergreen shrub with amazingly fragrant flowers and shiny, dark-green leaves belonging to the family Rubiaceae. The genus Gardenia includes about 200 species and *Gardenia jasminoides* with the cultivars Ellis and Veitchii as one of the most popular species (Xiao *et al*., 2017). The gardenia is one of the indoor plants that bloom abundantly with everlasting greenery, grown inside buildings, balconies, and shaded home gardens, and serves as a valuable breeding plant. However, few varieties can stay outside because of their sensitivity to high summer heat and direct sunlight (Abdullah *et al*., 2003). Also very popular for its beautiful flowers and aromatic scent, its economic importance relates to its use in the perfume and dye industries because of its crocetin content (Phatak, 2015). Crocetin is a natural apocarotenoid dicarboxylic acid found in the crocus flower, together with its glycoside, crocin, and *Gardenia jasminoides* fruits (Ichi *et al*., 1995; Umigai *et al*., 2011). The height of the gardenia ranges from 1–2 m, with its white aromatic flowers and the spear-shaped leaves reaching in length to 10 cm.

The gardenia tree needs strong indirect light to get good flowers, and temperatures range between 15 °C – 25 °C (He *et al.*, 2006). Gardenia requires vegetative propagation because its sexual propagation gives undesirable and genetically differentiated plants. For gardenia plant propagation, the tissue culture technique is one of the practical applications due to its unique characteristics compared with the traditional propagation. Micropropagation allows the production of large numbers of plants within a short period. Plants that are difficult to propagate by traditional methods can be multiplied easily, in addition to the propagation throughout the year, without being restricted to the growing season for the rooting and the acclimatization stage (Shukri and Muaykal, 2013).

The type and concentration of plant growth regulators added to the media are the determining factors in the accomplishment of plant micropropagation. Cytokinins (benzyl adenine - BA) and auxins (Indole 3-butyric acid - IBA) are the most used for this purpose. The induction of shoot multiplication can consist of the following: a) by the stimulation of lateral buds by eliminating the apical dominance by adding Cytokinins to the media, b) by the medium of the formation forming transverse lateral buds that develop into new branches shoots, c) through the transformation converting the cells into somatic embryos, and d) %that the plants give rise to produced new branch shoots without turning them into embryos in the term called organogenesis (Devlin and Witham, 1983). Several studies suggested that it would be possible to produce new gardenia plants by removing various plant elements of explants, such as the shoot tips and nodes, and culturing them before replanting in a medium that had been specially prepared (Salim and Hamza, 2017). Gardenia multiplication can proceed without adding cytokinins (free hormone) to the media medium, however, with a low rate of success, the concentration of 2 mg L^{-1} exceeded and gave the highest detection rate of bud proliferation and number of shoots. The prepared MS media included a half-strength MS salt medium with a rooting rate of 100%, compared with the full-strength MS medium. Bashi (2010) studied the effects of plant growth regulators on the propagation of gardenia plants in vitro, and his findings revealed that benzyl adenine (BA 2 mg L^{-1}) showed significantly superior to other treatments and control, and the auxin - Indole 3-butyric acid (IBA - 2 mg L^{-1}) achieved 95% of rooting.

Several studies revealed that some plant extracts have an encouraging effect in vegetative, root, and flower growth, as well as, many plant yields because these extracts contain essential nutrients that participate in metabolic processes and perform important functions, with their deficiency also causing physiological imbalance as a result of low content of specific nutrients. In addition, it possesses a plethora of phytochemical substances, each of which varies in kind, quality, and quantity depending on the specific species, plant parts explants, stages of plant growth, and environmental conditions (Zulfiqar *et al*., 2020). Therefore, in the recent global trend, plant extracts utilization as an alternative material for industrial chemical compounds commenced to preserve the environment and avoid side effects.

Licorice (*Glycyrrhiza glabra*) extract has high nutritional and protective efficacy as it is one of the natural compounds derived from plants used in the traditional, industrial, and agricultural aspects, and also one of the modern trends used in the nutrition of various plants (Hammoud, 2011). Licorice contains many chemical compounds, and the most crucial ones are glycyrrhizin and citric acid, which work as plant hormones that enhance proteins and improve growth rate (Al-Ajili, 2005). Licorice contains the most required essential elements, i.e., PO_4, Mg, Fe, Mn, Zn, and Pb, as well as, proteins, amino acids, and sugars. Licorice extract works like GA3 as it contains the same constructivist initiator, which stimulates germination and helps in cell division and elongation, and the vegetative system by improving flowering and yield characteristics (Al-Jawari, 2002).

Many studies have occurred to improve the propagation efficiency in terms of its good ability to grow, including the study carried out on treating the cuttings of the chrysanthemum plant with different types and concentrations of plant extracts. The superiority of licorice extract proved to be significant in the fresh and dry weight rates compared with garlic extract (Al-Ma'athid, 2010). Licorice extract, added to the propagation media of the apricot plant, led to a significant enhancement in the modification of the shoot and root system compared with the control treatment (Cefana, 2013).

Al-Hafothy and Hassan's (2018) findings revealed the effects of several types of vegetable extraction on the separation and identification of some compounds in *Cassia acutifolia*. However, the superiority of licorice prevailed compared with other extracts. The importance of adding plant extracts to the food media MS medium used in textile farms comes from their accessibility and cheapness compared with the manufactured materials and the increased efficiency of the food media MS medium used in accurate multiplication, micropropagation, and the adaptation acclimation of the resulting in vitro propagated plants in field agriculture. Therefore, the progressive study aimed to assess the efficiency of plant growth regulators and interaction with licorice extract on the micropropagation of *Gardenia Jasminoides* cv. veitchii plants.

MATERIALS AND METHODS

The shoot tips of vegetative shoots (1–2 cm) separated from the *Gardenia jasminoides* cv. veitchii at the age of two years contains one node per piece to use for establishment. The plants gained sterilization by immersing them in 3% sodium hypochlorite solution for 15 min and then washed with double distilled water (DD water) three times to remove traces of the sterile material from the explants. Then, cutting both ends of sterilized shoot tips in MS solid media (Murashige and Skoog, 1962) for placement in earlier prepared different concentrations of benzyl adenine (BA) (0.0, 2.0, 4.0, and 6.0 mg L^{-1}) and licorice extracts (0.0, 1.0, 2.0, and 3.0 g L^{-1}) followed. Recording of the research traits regarding the number and length of shoots and the number of leaves for each vegetative shoot progressed after 30 days as the incubated cultures remained in an incubation room of 25 °C. . For the rooting stage, the resulting plants from the multiplication stage (ranges between 2–3 cm) proceeded to transfer to four types of nutrient media (Table 1). Keeping the gardenia plants in the incubation room under the same conditions as before, recording the rooting data regarding germination percentage, root number, length, and dry weight of the rooting system took place after 30 days.

Plant acclimatization

Taking out the gardenia plants from the planting bottles continued with their roots washed with double distilled water from any residual trace of agar and sugars. Then their planting in anvils containing soil consisting of sand and peat at a ratio of 1:1 ensued for two weeks for acclimatization and later transferred to the greenhouse.

Table 1. Nutrients MS media used for the rooting stage of *Gardenia* (*G. Jasminoides* cv. Veitchii).

No.	Media formulation
1	MS media with full basal salt strength prepared with different concentrations of (Indole 3-butyric acid - IBA) (0.0, 0.5, 1.0, and 2.0 mg L^{-1}), and benzyl adenine - BA @ 0.1 mg L^{-1}.
2	MS media with half basal salt strength prepared with different concentrations of (Indole 3-butyric acid - IBA) (0.0, 0.5, 1.0, and 2.0mg L^{-1}), and benzyl adenine - BA @ 0.1 mg L^{-1}
3	MS media with full strength of salt prepared with different concentrations of licorice extract (0.0, 1.0, 2.0, and 3.0 g L^{-1})
4	MS media with half strength of basal salt prepared with different concentrations of licorice extract (0.0, 1.0, 2.0, and 3.0g L^{-1}).

Statistical analysis

Experimental data analysis transpired for the factorial completely randomized design in 10 replications. The use of analyses of variances conducted LSD tests for mean separation ($P < 0.05$) (Gomez and Gomez, 1990).

RESULTS

Adding BA and licorice extract to the nutrients of MS media showed a superior response in the average number of shoots of *Gardenia jasminoides* cv. veitchii (Table 2). For four concentrations of BA (0.0, 2.0, 4.0, and 6.0 mg L^{-1}), the shoots in gardenia plants ranged from 2.33 to 3.46. However, on average, the BA concentration of 4 mg L^{-1} achieved the highest number of shoots $plant^{-1}$ (3.46) compared with the control with the low number of shoots (2.33) in gardenia plants. For licorice four extracts (0.0, 1.0, 2.0, and 3.0g L^{-1}), the shoots in gardenia plants ranged from 1.99 to 3.83. However, on average, the licorice extract at the concentration of 2 g L^{-1} also showed superiority by achieving the highest number of shoots $plant^{-1}$ (3.83) compared with the control treatment having the lowest number of shoots (1.99). In the interaction of BA concentrations and licorice four extracts, the combination of BA (4 mg L^{-1}) and licorice extract (2 g L^{-1}) achieved the highest rate of shoots $plant^{-1}$ (4.30) compared with the lowest rate (1.00) obtained in control with zero concentration of BA and licorice extract.

For the average length of gardenia shoots, data in Table 3 indicated that the BA concentration of 2 mg L^{-1} achieved the highest shoot length (3.26 cm), followed by the concentrations of 4 and 6 mg L^{-1} with 2.78 cm and 2.29 cm, respectively. However, the minimum shoot length showed for the control (2.10 cm). Still, on average, the licorice extract at the concentration of 3 g L^{-1} also showed superiority by getting the maximum shoot length (3.10 cm), followed by the extract concentration of 2 g L^{-1} (2.70 cm), compared with the control treatment with minimum shoot length (2.23 cm). As for the interaction between BA and licorice concentrations, the BA concentration of 2 mg L^{-1} of BA and 3 g L^{-1} of licorice extract revealed the highest average shoot length (3.75 cm) compared with the minimum shoot length obtained in media-free BA and licorice.

For leaves per plant in *Gardenia jasminoides* cv. veitchii, the various concentrations of BA and licorice extract revealed significant differences (Table 4). However, on average, the BA concentration of 2 mg L^{-1} exhibited significantly superior and achieved the highest number of leaves per plant (6.49), followed by a decrease in response with increased concentrations of 4 and 6 mg L^{-1}, reaching 5.01 and 4.68 leaves per plant, respectively, compared with the control treatment with the least number of leaves per plant (2.38). The licorice extract also amended the leaf number in gardenia, with the concentration of 3 g L^{-1} displaying superiority in achieving the highest rate of leaves $plant^{-1}$ (5.61), which did not differ significantly from the concentration of 2 g L^{-1} (5.10 leaves $plant^{-1}$), compared with the lowest rate of leaves obtained of the control (3.84 leave $plant^{-1}$). As for the interaction effects, the nutrient medium prepared with the combination of BA (2 mg L^{-1}) and licorice extract (2 g L^{-1}) outperformed other treatments in achieving the highest rate of leaves $plant^{-1}$ (7.85), followed by the interaction of BA (2 mg L^{-1}) and licorice extract (3 g L^{-1}) with 7.60 leaves per plant. However, the control treatment with zero concentration of BA and licorice extract resulted in the least number of leaves per plant (2.10).

Other factors also affected the growth and development traits of the gardenia plants, which revealed a reflection on the course of metabolic processes within the growing plants. However, among all these factors, the licorice extract with its different concentrations had an excellent effect on the expression of various characters. The reason may refer to the mineral elements contained in the extract, such as P, Mg, Fe, Mn, Zn, and Co, as well as, other materials, such as, proteins, amino acids, and carbohydrates that have a vital role in the photosynthesis process and subsequently in plant growth and development (Musa *et al*., 2002).

The increase in growth traits can also refer to the licorice extract, which contains auxins and cytokinins that stimulate physiological processes that delay leaf aging and increase the total chlorophyll content. Chlorophyll is essential in photosynthesis, which is why this extract is beneficial (Desoky *et al*., 2019). The present results also agreed with the findings of Hamza (2011), who found a superiority in the mean values of the studied

Table 2. Effect of benzyl adenine and licorice extract on the number of shoots of the *Gardenia jasminoides* cv. veitchii plants.

Licorice Extract (g L^{-1})	Benzyl Adenine (BA mg L^{-1})				Means
	0.0	2.0	4.0	6.0	
0.0	1.00	1.50	3.10	2.37	1.99
1.0	1.31	2.27	3.25	2.68	2.37
2.0	3.15	3.80	4.30	4.09	3.83
3.0	3.85	3.50	3.20	3.62	3.54
Means	2.33	2.76	3.46	3.19	
$LSD_{0.05}$ BA = 0.31, $LSD_{0.05}$ LE = 0.21, $LSD_{0.05}$ BA ×LE = 0.20					

Table 3. Effect of benzyl adenine and licorice extract on the shoot length of the *Gardenia jasminoides* cv. veitchii plants.

Licorice Extract (g L^{-1})	Benzyl Adenine (BA mg L^{-1})				Means
	0.0	2.0	4.0	6.0	
0.0	1.20	3.15	2.40	2.17	2.23
1.0	1.80	2.60	3.14	2.10	2.41
2.0	2.60	3.56	2.40	2.25	2.70
3.0	2.82	3.75	3.19	2.65	3.10
Means	2.10	3.26	2.78	2.29	
$LSD_{0.05}$ BA = 0.12, $LSD_{0.05}$ LE = 0.20, $LSD_{0.05}$ BA × LE = 0.13					

Table 4. Effect of benzyl adenine and licorice extract on the number of leaves per plant of the *Gardenia jasminoides* cv. veitchii.

Licorice Extract (g L^{-1})	Benzyl Adenine (BA mg L^{-1})				Means
	0.0	2.0	4.0	6.0	
0.0	2.10	6.12	4.00	3.15	3.84
1.0	2.30	4.42	5.18	4.20	4.02
2.0	2.45	7.85	4.75	5.35	5.10
3.0	2.68	7.60	6.13	6.05	5.61
Means	2.38	6.49	5.01	4.68	
$LSD_{0.05}$ BA = 0.21, $LSD_{0.05}$ LE = 0.22, $LSD_{0.05}$ BA x LE = 0.40					

characters by increasing the concentration of licorice extract added to the medium used for the multiplication of cucumber plants instead of sucrose. Cefana's (2013) findings also revealed an evident increase in the growth characteristics with an increased concentration of licorice extract supplied to the media of apricot plant propagation in vitro.

The effects of indole 3-butyric acid (IBA) at the concentration of 1.0 mg L^{-1} with half salt concentrations added to media, studied in the root system, including the rooting percentage, roots length, and dry weight with leading values of 88%, 3.26 cm, and 0.40 mg, respectively, compared with the control having the lowest average values (15%, 0.98, and 0.15 mg, respectively) (Figures 1 and 2). The IBA concentration of 2 mg L^{-1} achieved the highest average number of roots per plant (5.70) compared with the control treatment (1.16 roots $plant^{-1}$).

The IBA concentration (1 mg L^{-1}) with the full strength of salt added to MS medium showed superiority in achieving rooting percentage (75%), roots number (3.80), and dry weight of roots (0.30 mg), while the IBA concentration of 0.5 mg L^{-1} achieved the highest rate of root length (1.75 cm), compared with the control treatment with least values (10%, 0.58, and 0.06 mg), respectively (Figures 1 and 3). The licorice extract concentration(3 g L^{-1}) with half strength of the salt gave an excellent combination in improving the root traits (90%, 6.80 roots $plant^{-1}$, 3.45 cm, and 0.58 mg, respectively), compared with the control treatment with the lowest values (17%, 1.30 roots $plant^{-1}$, 0.80 cm, and 0.10 mg, respectively) (Figures 1 and 4).

The licorice extract concentration of 3 g L^{-1} with the full strength of salts displayed significantly superior in the root system for traits rooting percentage, the number of roots, and dry weight reaching 80%, 5.25 roots $plant^{-1}$, and 0.40 mg, respectively. Similarly, the licorice extract concentration of 2 mg L^{-1} achieved the highest rate of root length (2.01 cm) compared with the control treatment (12%, 0.70 roots $plant^{-1}$, 0.65 cm, and 0.05 mg, respectively) (Figures 1 and 5).

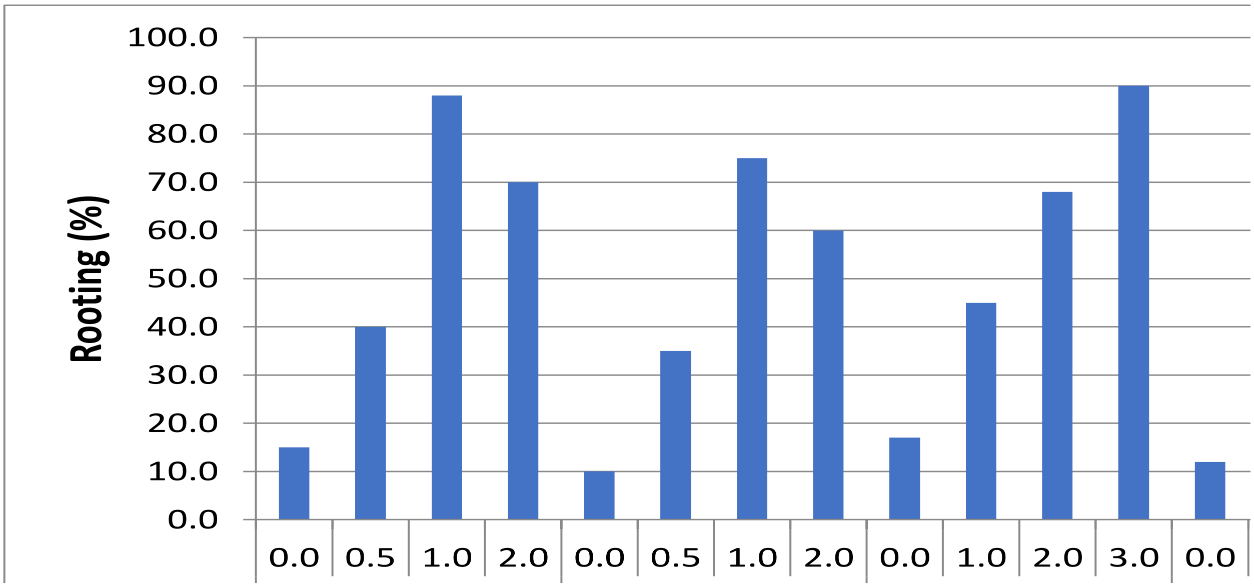

Figure 1. Effect of licorice extract and IBA concentration additives to MS media with half and full salt strength on rooting percentage of *Gardenia jasminoides* cv. veitchii.

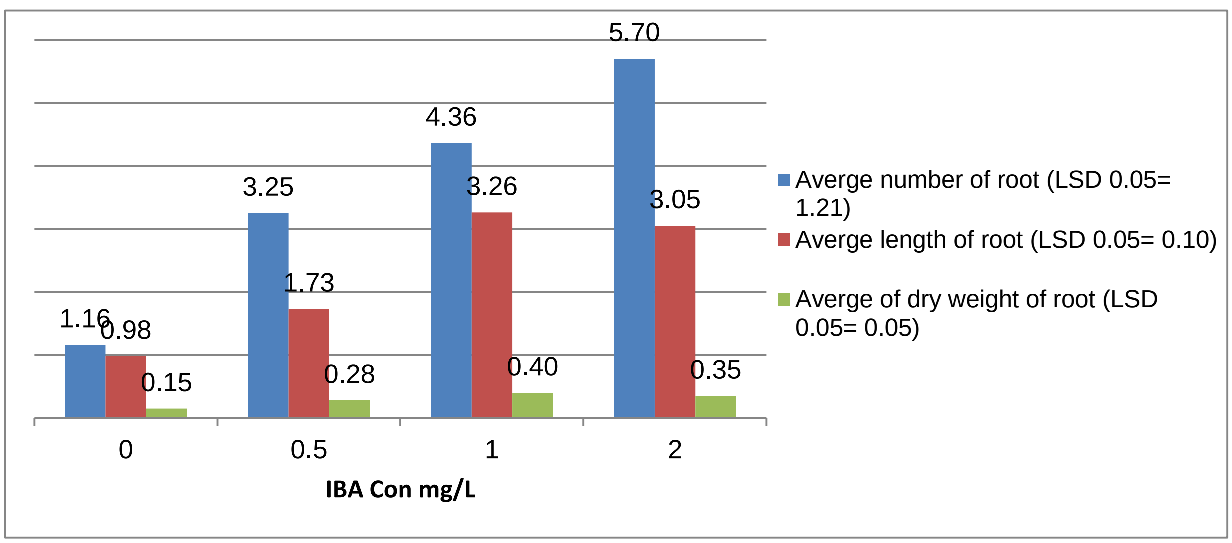

Figure 2. Effect of IBA concentration additives to MS media with half salt strength on the root traits.

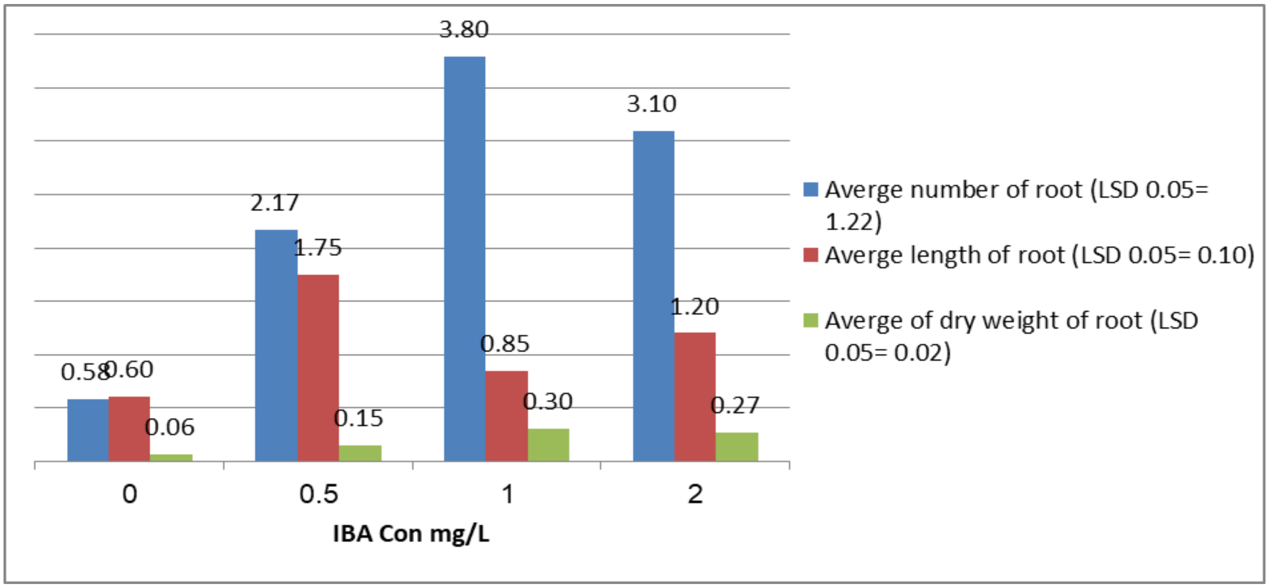

Figure 3. Effect of IBA concentration additives to MS media with full salt strength on the root traits.

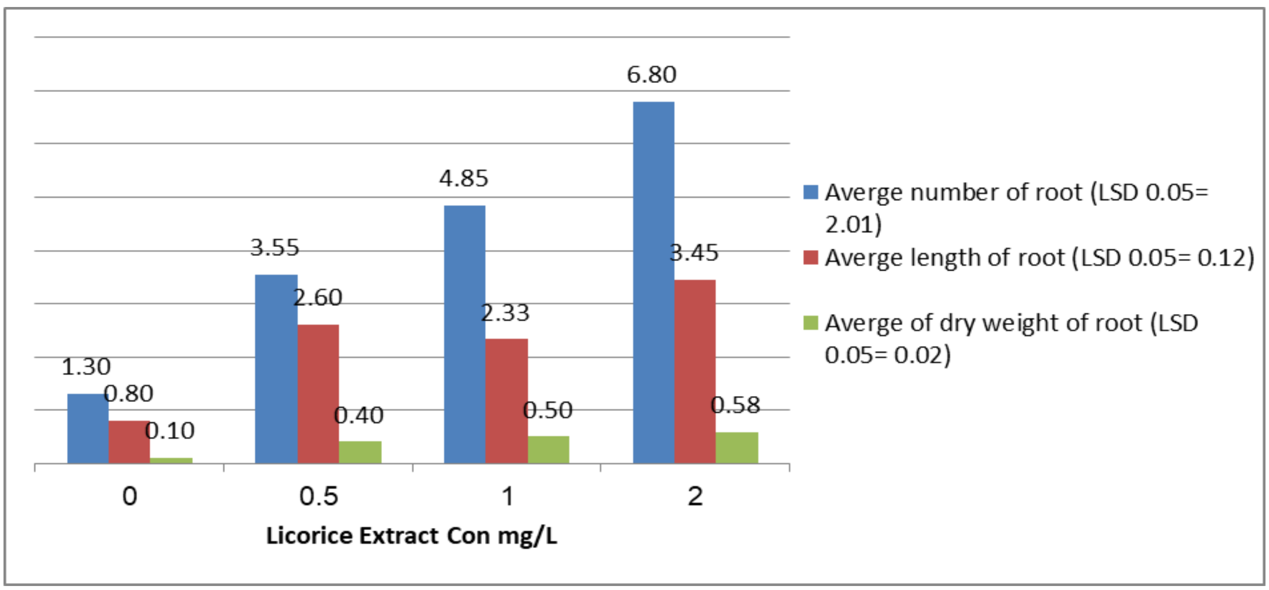

Figure 4. Effect of licorice extract concentration additives to MS media with half salt strength on the root traits.

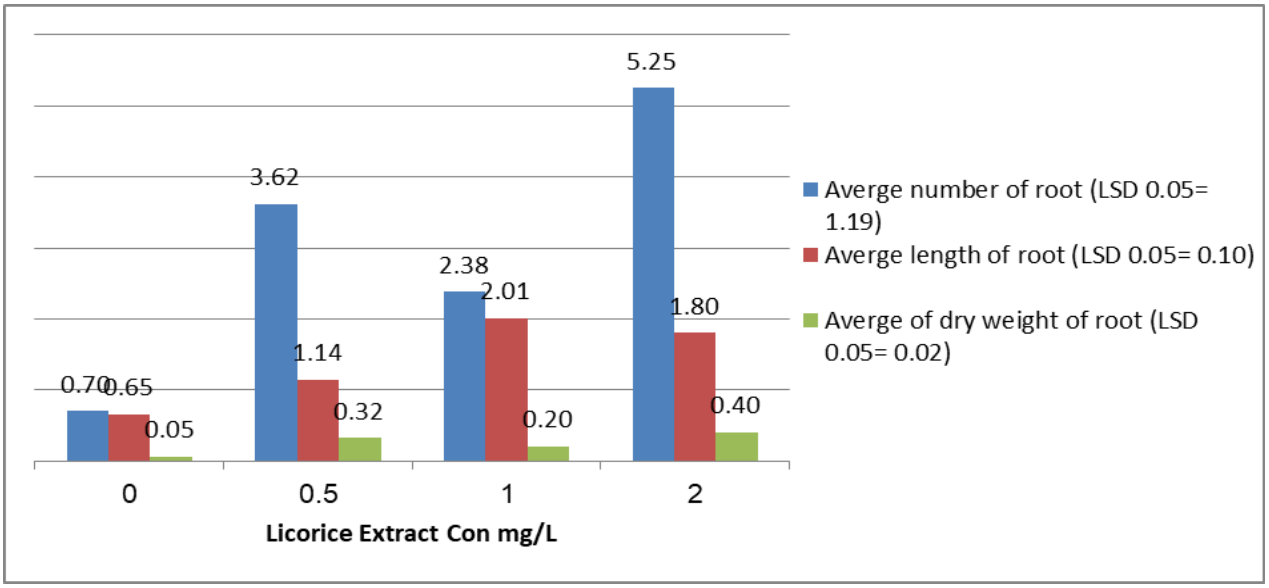

Figure 5. Effect of licorice extract concentration additives to MS media with full salt strength on the root traits.

DISCUSSION

In general, the BA treatment showed superior for the average of the various growth characteristics, which contained the number and length of multiplying shoots and the number of leaves for each plant, with the explanation pointing to the stimulator action of cytokinins in urging cells to divide and differentiate. The said action also results in the growth of buds into vegetative shoots. Many past studies also pointed out the vital role of cytokinins at appropriate concentrations in tissue culture, breaking the apical dominance and creating areas of attraction in the lateral buds, stimulating the transfer of nutrients to them, and stimulating the growth of the buds, thus exceeding the number of shoots (Howell and Lalland, 2003).

Several theories developed have tried to explain this phenomenon, including that the added cytokinins move from the bottom upwards through the axillary buds, thus canceling the effect of auxins formed in the terminal buds. In axillary buds, the cytokinins found in high concentrations impede their growth by inhibiting the differentiation of the lateral vascular tissues in these buds, thus its upward-moving will create a process of differentiation of the woody tissues and vascular bundles of the axillary buds to link with their counterparts in the stem. Cytokinins also facilitate the transfer of water and nutrients to these buds, consequently stimulating them to develop the side shoots, however their high levels may cause a decrease in growth due to disturbance in the vital processes within the tissues as a result of the hormonal imbalance (Hopkins and Huner, 1995; Alatar, 2015). Yet, that decrease does not necessarily mean the death of cells, but it usually results in growth impediment. The present results showed similarity and get supported by past studies in the propagation of gardenia plants (Bashi, 2010; Sayd *et al*., 2010).

The present results also revealed the superiority of indole 3-butyric acid (IBA) at a concentration of 1.5 mg L^{-1} for all the growth characteristics. It attributes to the fact that IBA is one of the auxins that encourages cell division and elongation, then stimulates the formation of roots from the cut areas, increasing the concentration of growth regulators, which leads to an enhancement in the root number and length to reach the optimum values. However, the higher concentration of auxins may lead to the inhibition of root initiators and reduce their number (Hartmann *et al*., 2010; Abdullah *et al*., 2003). Previous research also described the process of roots and sprouting shoots throughout the stage of multiplication for the *Digitalis lanata* plant (Ghanem *et al*., 2010) and *Catharanthus roses in vitro* (Al-Mukhtar and Muslim, 2019). The gardenia plants also showed the superiority of licorice extract in the average characteristics of the studied stem group by increasing its concentrations added to the medium. It can be due to the composition of licorice extract, which contains mevalonic acid, which works as a constructivist initiator of endogenous gibberellins that is important in the cell division and elongation processes (Zahir *et al*., 2005).

The licorice extract also raises the level of gibberellins of the plant, which stimulates the internal auxins level in the plant, in turn, leads to the emergence of roots, reflecting an increase in the rooting percentage, dry weight and an increase in the absorption of various nutrients, which improved plant growth (Taiz and Zeiger, 2006). The licorice extract's superiority can be due to the nutrients such as salts, vitamins, sugars, and organic acids that stimulate enzymes, cell division, and elongation, causing an enhancement in the rooting system (Hasan *et al*., 2021). These study results showed agreement with the findings of rooting in *Phoenix dactylifera* plants (Shareef and Al-Mayahi, 2010) and *Cassia acutifolia* plants (Murashige and Skoog, 1962). The improvement in the root traits in the medium with half strength compared with the same medium with full strength may refer to a decrease in the concentration of some elements that negatively affect the rooting ratio, number, and length of roots in the case of high concentrations, and this revealed analogy with the past findings about the rooting in gardenia plants (Sayd *et al*., 2010; Chuenboonngarma *et al*., 2011).

CONCLUSIONS

The benzyl adenine (BA) at a concentration of 4 mg L^{-1} achieved the highest number of shoots per plant, and BA at a concentration of 2 mg L^{-1} showed superior in giving the maximum shoot length and the number of leaves per plant in the *Gardenia jasminoides* cv. veitchii. The licorice extract at the concentration of 2 g L^{-1} achieved the highest number of shoots per plant, whereas, at the concentration of 3 g L^{-1}, it proved superior, acquiring the maximum shoot length and the number of leaves. The IBA at the concentration of 1 mg L^{-1} prepared for media with half salt strength exhibited superiority in showing the highest rooting percentage, root length, and root dry weight, and the IBA at the concentration of 2 mg L^{-1} achieved the maximum number of roots in gardenia plants. The licorice extract at the concentration of 3 g L^{-1} with half strength of salt displayed superiority for the rooting percentage, root number, root length, and root dry weight in gardenia plants.

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