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EFFECT OF CARBON SOURCES AND TYROSINE ON THE ACCUMULATION OF BIOACTIVE COMPOUND (PYRETHRINS) IN *CHRYSANTHEMUM HORTORUM*

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SUMMARY

The existing study held in the Plant Tissue Culture Laboratory, University of Tikrit, Iraq, sought to increase the production of some secondary metabolite compounds in tissue cultures of *Chrysanthemum hortorum* Hort. cv. 'Dwarf White.' Inducing callus by culturing the bases of young leaf explants on the MS medium received supplementation of different concentrations of Benzyl adenine (BA) (0, 1.5, and 2 mg L^{-1}) and Indole-3-acetic acid (IAA) (0.0, 0.5, and 1.5 mg L^{-1}). Tyrosine addition at different concentrations (0, 30, 60, and 80 mg L^{-1}) and sucrose at 30, 60, 80, and 100 g L^{-1} concentrations ensued. In addition, fructose and glucose applications at 90.0, 60.0, 30.0, and 120.0 g L^{-1} transpired for the callus growth. The combination of 2.0 mg L^{-1} BA + 1.5 mg L^{-1} IAA gave the highest average fresh and dry weights of callus, reaching 1.8 and 5.72 mg, respectively. The best treatment was 60 g L^{-1}, which recorded the maximum pyrethrin concentration, amounting to 2.134 µg/ml DW. The treatment of 90 g L^{-1} fructose + 60 mg L^{-1} tyrosine was more effective in increasing pyrethrin production in the callus, reaching 3.175 µg/ml DW. The treatment of 90 g L^{-1} glucose + 60 mg L^{-1} tyrosine was recorded with the utmost pyrethrin concentration, reaching 3.346 µg/ml DW. The treatment of 90 g L^{-1} glucose + 80 mg L^{-1} tyrosine provided 2.826 µg/ml DW of pyrethrin.

Keywords: *Chrysanthemum hortorum,* secondary metabolites, explants, carbon sources, pyrethrins, tyrosine, callus, in vitro

Key findings: Callus induction by culturing the base of young leaf explants on the MS medium had BA at 0, 1.5, and 2 mg L^{-1} and IAA at 0.0, 0.5, and 1.5 mg L^{-1} supplementations. The results showed that the combination of BA at a concentration of 2.0 and 1.5 mg L^{-1} of IAA gave the highest average fresh and dry weights and the maximum percentage of callus. The above combination served to maintain induced callus. Secondary metabolite compounds gained estimation by the HPLC device.

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INTRODUCTION

Adding some stimuli to the cell system helps increase the biosynthesis of some cellular compounds that can attain dividing into biotic and abiotic stimuli depending on their nature. Some studies have indicated the possibility of inducing callus production of active substances, such as increasing the production of alkaloids, saturated alkanes, flavonoids, and esters in plant cells when using abiotic stress factors causing stress (Ibrahim, 2022; Ewhayid *et al*., 2023).

An increase in the concentration of the type of sugar added to the nutrient medium beyond the specified amount constitutes stress in the tissue, and this, in turn, stimulates the production of some bioactive substances, accelerating the withdrawal process and the absorption of alkaloids metabolizing products. In addition to their contribution to constructing primary and secondary products involved in the structural pathways, they produce specific compounds in plants, which can be their estimation (Park *et al*., 2008; Ibrahim *et al*., 2022).

The production of some secondary products has limits, in most cases, from its natural sources only and is unobtainable industrially. Additionally, it can be hard to obtain a compound with high purity. The *Chrysanthemum hortorum* Hort. cv. 'Dwarf White' belongs to the Compositae family. This plant came from the cross between the two wild species, *Chrysanthemum morifolium* Ramat. × *Chrysanthemum indicum* L. These two species originated in China and Japan, from which all the current cultivars of multi-shaped and colored flowers originated (Chakravarty, 1976). The scientific name change ensued in 1987, from *Chrysanthemum morifolium* to *Dendranthema grandiflora*, but the old scientific name is still in use (Anderson, 1987).

The *Chrysanthemum* genus includes more than 150–200 species (Superfloralretailing, 2007; Kemper, 2016). Ahmed and Obaid (2016) indicated that when they added the sucrose to the medium at four different levels (80, 60, 40, and 20 g L^{-1}), it led to an increase in the amount of the Vindoline compound produced from the callus of *Catharanthus roseus* L. plant. The possibility of stimulating the cultured explant to increase the production of secondary compounds soars after exposure to biotic and abiotic stresses (Davuluri *et al*., 2005). The micropropagation by callus induction from leaf culture was a difficult step for In vitro propagation. Various physical and biological factors, including media, play a role during pyrethrin compound production from *Chrysanthemum hortorum* Hort. cv. 'Dwarf White' plant in vitro propagation (Silva *et al*., 2005). The presented study strived to determine the production of secondary metabolite compounds from callus and the quantitative and qualitative detection of the produced pyrethrins by the high-performance liquid chromatography (HPLC) device.

MATERIALS AND METHODS

The pertinent study commenced in 2022–2023 at the Plant Tissue Culture Laboratory of the College of Agriculture, University of Tikrit, Tikrit, Iraq.

Sources of explants

This study used explants taken from imported seedlings of the Dwarf White Hort. The *Chrysanthemum hortorum*, obtained from spring nurseries in the Diyala province, incurred planting of stalks 15 cm in diameter containing 5–6 vegetative shoots (Figure 1). These planted pots were placed in the laboratory under the light until use. The bases of the young leaves, appearing to be rectangular, had dimensions of 0.8 mm × 0.3 mm, as shown in Figure 2.

Sterilization of explant surface

The petioles of young leaves sustained plucking from the Chrysanthemum. Placing explants directly in a sodium hypochlorite (NaOCl) solution with an active substance concentration of 1.05%, received two to three drops of the

Figure 1. Dwarf *Chrysanthemum hortorum* Hort. seedlings, "Dwarf White" cultivar.

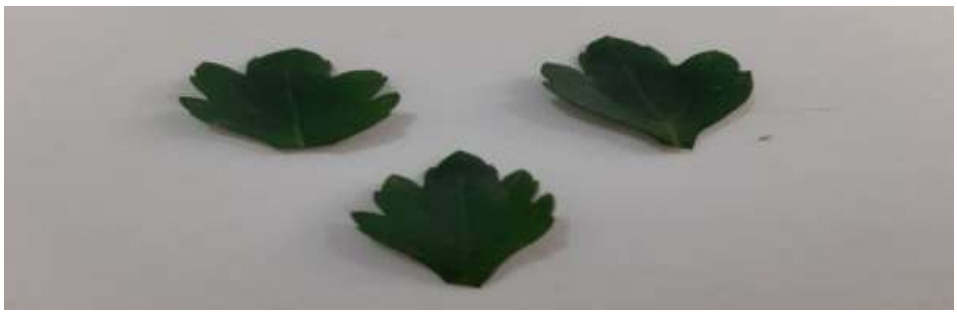

Figure 2. Petiole of young leaves taken from the *Chrysanthemum morifolium* plant.

diffuser (Tween-20) for 30 min. Afterward, these parts underwent washing with sterile distilled water four times.

Preparation of the nutrient medium

In this study, a nutritional medium had the composition of MS salts obtained from Himedia, India, for use at a concentration of 4.33 g L^{-1} in powder form. Combining growth regulators, auxins, and cytokinins into the medium at the required concentrations was according to the objective of the experiment. The pH adjusted to 5.7–5.8 had two solutions, sodium hydroxide NaOH and hydrochloric acid 0.1 N HCl. Agar's inclusion was at a concentration of 7 g L^{-1}, followed by polyvinyl pyrrolidone (PVP) at a 2 g L^{-1} concentration. Sterilizing culture tubes, glass vials, and conical flasks with their contents used an autoclave under a pressure of 1.05 kg/cm^2 and a temperature of 121 °C for 20 min.

Effect of cytokinin BA and auxin IAA on the callus induction

Young leaf petioles planted in a horizontally rectangular shape on the nutrient medium consisted of MS salts supplemented with cytokinin (benzyl adenine) BA at concentrations of 0, 1.5, and 2 mg L^{-1} and concentrations of auxin (Indole-3-acetic acid) IAA at concentrations of 1.5, 0.5, and 0.3 mg L^{-1} to induce primary callus from the stalks of young leaves. The data recording focused on the percentage of callus induction (%), fresh weight of callus (g), dry weight of callus (g), and average water content (%).

Effect of sucrose, fructose, and glucose tyrosine on growth

In this experiment, callus developed and induced from basal parts of young leaves only had an approximate weight of 100 mg of callus taken and cultured on the nutrient medium supplied with MS salts and the components of the nutrient medium. The best concentration of the growth regulator BA (2 mg L^{-1}) with the presence of IAA at a constant concentration of 1.5 mg L^{-1} attained the addition of different concentrations of tyrosine (0, 30, 60, and 80 mg L^{-1}), sucrose levels at concentrations of 30, 60, 80, and 100 g L^{-1}), and fructose and glucose at concentrations of 90, 60, 30, and 120 g L^{-1} each proceeded induction for callus growth.

Fresh and dry weights and percentage of callus

HPLC analysis for pyrethrin continued in the chrysanthemum extract. The standard mixture of six constituents of pyrethrin, separated on the FLC (Fast Liquid Chromatographic) column, was under the optimum separation condition.
Column: C-18, 3µm particle size (50 mm × 4.6 mm I.D)
Mobile phase: Acetonitrile 0.01M potassium phosphates buffer pH 4.5 (75:25, V/V), Detection UV set at 280 nm, and flow rate 1.0 ml/min.
Temperature: Ambient 25 °C., injection volume 20 µl

Statistical analysis

The factorial experiments used a completely randomized design (CRD). The data analysis utilized the SAS (2002). The treatment means' comparison employed the Least Significant Difference (LSD) Test under a 5% probability level. Each treatment included 10 replicates, each containing one explant.

RESULTS AND DISCUSSION

The results showed the effect of treatment with cytokinins, auxins BA, and IAA on the gravimetric rate in fresh weight (FW) and dry weight (DW), the percentage of callus induction (%), and the percentage of callus water content (WC). The interaction indicated the treatment between the concentration of 2 mg L^{-1} BA and 1.5 mg L^{-1} IAA, the highest rate of fresh and dry weights, reaching 1.8 and 5.72 g, respectively, with a significant difference from all other interaction treatments. The interaction treatment between 0 mg L^{-1} BA + 0.5 mg L^{-1} IAA and 1.5 mg L^{-1} BA + 0.3 mg L^{-1} IAA gave the lowest means for the fresh and dry weights. Following it was the effect on the average fresh and dry weights, which reached 4.77 and 0.99 g, respectively, when the treatment was 2 mg L^{-1} BA and 0.5 mg L^{-1} IAA. It was not significantly different from the interaction treatment (1.5 mg L^{-1} BA and 1.5 mg L^{-1} IAA) for the callus percentage formed from leaf petioles grown in nutrient media and incubated in the dark. Notably, from the same table, the proportion rose with increasing concentrations of BA and IAA to 2 mg L^{-1} BA and 1.5 mg L^{-1} IAA in the medium.

It reached a maximum of 95.93% up to the last concentration of 2 mg L^{-1} BA and 0.5 mg L^{-1} IAA, with a significant difference for all treatments. The lowest percentage was in the combination treatment of 1.5 mg L^{-1} BA and 0.3 mg L^{-1} IAA, which reached 55.31%. From the table, the different concentrations of BA and IAA added to the medium significantly affected the average percentage of water content (%).

The concentration of 2 mg L^{-1} BA and 1.5 mg L^{-1} IAA was significantly superior in achieving the highest percentage of water content (%), which reached 78.67%. The least time required for callus induction was 18.00 days. The two combination treatments, 2 mg L^{-1} BA + 0.5 mg L^{-1} IAA and 1.5 mg L^{-1} BA + 1.5 mg L^{-1} IAA, did not differ significantly in percentage water content (%). A substantial decrease was visible in the percentage of the water content of callus, reaching 47.11% when lowering the BA concentration in the medium (0.0 mg L^{-1} BA + 0.5 mg L^{-1} IAA) during the maximum period of 23.00 days. The cause for the superiority of the high concentration of 2 mg L^{-1} BA + 1.5 mg L^{-1} IAA in the average fresh and dry weights may be due to the callus

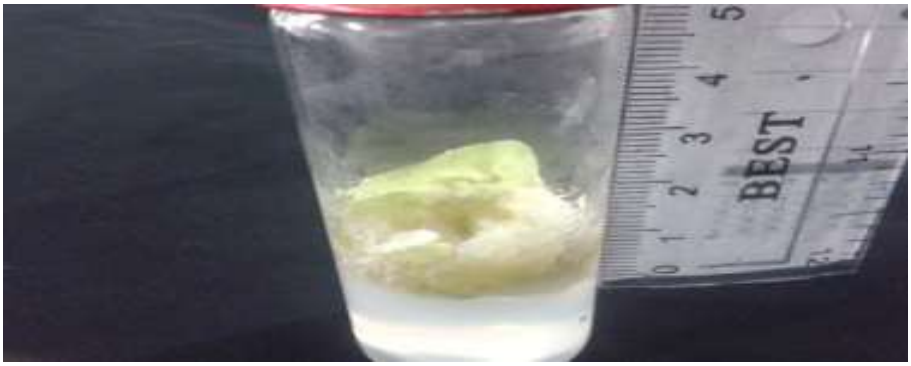

Figure 3. Process of subculture of the primary callus tissue (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" propagation in MS medium supplemented with 2.0 mg L^{-1} BA supplemented with 1.5 mg L^{-1} IAA for seven weeks in the dark.

induction achieving the immense callus mass in the shortest time required for its formation.

The above concentration of BA is the optimal concentration in increasing cell division of the cultivated plant part and stimulating it to form a callus, as well as the role of auxin IAA in energizing the formation of RNA necessary to build enzymes and proteins affecting growth (Kamruzzaman *et al*., 2015; Aziz *et al*., 2016; Safana *et al*., 2022). The nutrient medium supplemented with 5 mg L^{-1} BA added with 2,4-D at a concentration of 1.0 mg L^{-1} gave the best callus formation. Also notable was the use of high concentrations of BA and low concentrations of 2,4-D increased in the induced callus (Shinoyama *et al*., 2004; Al-Drisi *et al*., 2022a; Hussain *et al*., 2023).

The results showed the formation of a granular callus characterized by its transparent green and bright green colors and friable texture produced from the primary callus induction from the culture of the bases of young leaves of the *Chrysanthemum morifolium* plant, Dwarf white variety (Figure 1). The MS medium acquired supplementation with different combinations (0, 1.5, and 2 mg L^{-1}) of BA and (0.5, 1.5, and 0.3 mg L^{-1}) of IAA for seven weeks of incubation in the dark. The reason for incubating the cultures in the dark may refer to its role in inhibiting the oxidation enzymes of phenolic substances (Trigiano and Gray, 2005). The callus mass began to divide and grow with the formation of loose green granules that wholly covered all the basal explants of the leaf at all concentrations, with the difference in the amount of callus produced depending on the type and concentration of cytokinins and auxins (Ramdan *et al*., 2014, Kazmi *et al*., 2015). These results agreed with the findings of many studies (Abd-Elaleem *et al*., 2009; Taiz and Zeiger, 2010), with further confirmation from Shinoyama *et al.* (2004). The primary callus formation induced from the basal explants of young leaves of *Chrysanthemum hortorum* plant 'White Dwarf' cultivar in the MS medium supplemented with 2.0 mg L^{-1} BA + 1.5 mg L^{-1} IAA appears in Figure 1.

Effect of sucrose and tyrosine on the fresh weight of callus

A culture of approximately 20 ± 150 mg of induced callus tissue began from the culture of young leaf base explants of the White Dwarf cultivar in the MS media prepared with different concentrations of sucrose (30, 60, 80, and100 g L^{-1}) and fructose and glucose (30, 60, 90, and 120 g L^{-1}) (Figure 3). It gained a combination with tyrosine acid (0, 30, 60, and 80 mg L^{-1}) and the supplementation of 2 mg L^{-1} BA and 1.5 mg L^{-1} IAA at a constant concentration after seven weeks of incubation in the light. The results in Table 1 show the statistical analysis related to the effect of these sucrose levels on the fresh weight (FW) and the dry weight (DW). The rate of fresh callus weight decreased significantly with the

Table 1. Effect of different concentrations of sucrose and tyrosine on the average fresh weight of callus (mg) induced from the bases of young leaves of the seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after five weeks of culture.

Sucrose (mg L^{-1})	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	267.00	545.62	465.07	448.19	435.97
60	329.11	152.11	367.83	416.61	316.42
80	398.64	368.25	245.33	232.19	311.10
100	297.78	294.29	294.25	292.27	294.65
$LSD_{0.05}$	169.16				
Means (mg)	323.13	340.07	350.62	347.32	
$LSD_{0.05}$	84.58				

increase in the added concentrations of sucrose. It gave the prepared MS medium at a concentration of 30 g L^{-1} sucrose the highest average fresh weight of the callus, which reached 435.97 mg. The average fresh weight of callus, decreased by increasing the concentrations of sucrose to 60, 80, and 100 g L^{-1}, was 316.42, 311.10, and 294.65 mg, respectively.

The MS medium prepared with a concentration of 60 mg L^{-1} of tyrosine gave the maximum average fresh weight of 350.62 mg and differed significantly from the rest of the treatments (Table 1). The lowest value was in the control treatment, which gave an average fresh weight of 323.13 mg. Thus, sucrose added to the medium is an indispensable energy source that plays an essential role in the cell division of plant tissues (Al-Drisi *et al*., 2022b). The results of this study agreed with the findings found by Ikeda *et al*. (1976) in the callus of the tobacco plant, Mizukami *et al*. (1977) in the callus of *Lithospermum erythrorhizon*, and Nessler (1982) in the callus of *Papaver somniferum*. Table 1 shows the interaction between the concentrations of sucrose and the amino acid tyrosine. An increase emerged in the average fresh callus weight grown on the MS medium supplied with 30 g L^{-1} sucrose + 30 mg L^{-1} tyrosine, which amounted to 545.62 mg.

Effect of sucrose and tyrosine on the dry weight of callus

The dry callus weight decreased significantly with increasing concentrations of added sucrose (Table 2). It gave the prepared MS medium at 30 g L^{-1} sucrose the highest average callus dry weight, reaching 36.81 mg. The callus average dry weight decreased with rising concentrations of sucrose to 60, 80, and 100 g L^{-1} at 26.15, 24.44, and 24.37 mg L^{-1}, respectively. The same table indicates that the MS medium supplied with 30 mg L^{-1} of tyrosine recorded the utmost mean dry weight of 29.78 mg and differed significantly from the rest of the treatments. The lowest value was in the control treatment, with an average fresh weight of 25.64 mg. The interaction between the concentrations of sucrose and the amino acid tyrosine in Table 2 signified an increase in the average weight of dry callus in the MS medium supplied with 30 g L^{-1} of sucrose + 30 mg L^{-1} tyrosine, amounting to 47.59 mg. It was markedly different, compared with the rest of the treatments, except for the MS treatment supplied with 30 g L^{-1} sucrose + 60 mg L^{-1} tyrosine, resulting in 44.80 mg.

The lowest value was in the MS medium equipped with 60 g L^{-1} sucrose + 30 g L^{-1} tyrosine, which amounted to 18.58 mg. It differed significantly from the rest of the treatments except for the medium supplemented with 80 g L^{-1} sucrose + 60 g L^{-1} tyrosine (19.5 and 19.0 mg, respectively). Adding sucrose to the nutrient media is an essential energy source for cell division in plant tissues (Huang and Liu, 2002). The possibility of stimulating cultivated explants to increase the production of secondary compounds increases after exposure to biotic and abiotic stress, which revitalizes producing plant chemical compounds (Davuluri *et al*., 2005).

Table 2. Effect of different concentrations of sucrose and tyrosine on the average dry weight of callus (mg) induced from the bases of young leaves of the seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after seven weeks of culture.

Sucrose (mg L^{-1})	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	20.54	47.59	44.80	34.32	36.81
60	27.33	18.58	28.01	30.69	26.15
80	29.81	28.39	20.30	19.25	24.44
100	25.64	24.56	25.67	21.60	24.37
$LSD_{0.05}$	5.82				
Means (mg)	25.83	29.78	29.70	26.47	
$LSD_{0.05}$	2.91				

Table 3. Effect of different concentrations of fructose and tyrosine on the average fresh weight of callus (mg) induced from the bases of young leaves of (C*hrysanthemum hortorum* Hort.) cv. "Dwarf White" cultured on MS medium after seven weeks.

Fructose (mg L-1)	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	441.09	662.32	559.42	476.04	534.71
60	261.41	158.21	361.43	414.61	298.91
90	314.60	363.75	316.25	363.69	339.57
120	396.49	343.38	306.59	352.45	349.73
$LSD_{0.05}$	152.15				
Means (mg)	353.40	381.91	385.92	401.70	
$LSD_{0.05}$	76.07				

Effect of fructose and tyrosine on the fresh weight of callus after seven weeks

The results in Table 3 indicate the excellence of the MS medium supplied with 30 mg L^{-1} fructose, recording the highest average callus fresh weight of 534.71 mg. It did not differ significantly with the concentration of 120 g L^{-1} fructose, which recorded 349.73 mg. The 60 g L^{-1} fructose concentration provided an average callus fresh weight of 298.91 mg. Results in Table 3 showed the superiority of tyrosine at a concentration of 80 mg L^{-1} with an average callus fresh weight of 401.70 mg. It differed insignificantly with the 60 mg L^{-1} tyrosine, which gave 385.92 mg. The lowest average of fresh callus weight was 353.40 mg in the control treatment. On the effect of the interaction between the concentrations of fructose and tyrosine added to the nutrient medium, outcomes from the same table showed the excellence of the nutrient medium supplied with 30 g L^{-1} fructose + 30 mg L^{-1} tyrosine. It gave an average weight of fresh callus at 662.32 mg. The lowest in the medium resulted from the 60 g L^{-1} fructose + 30 mg L^{-1} tyrosine, recording 158.21 mg. The difference in the callus average fresh and dry weights may be because carbohydrate concentration plays crucial roles at different stages in the plant tissue culture process (Amiri and Kazemitabar, 2011; Al-Razan, 2018).

Effect of fructose and tyrosine on fresh weight of callus after five weeks

The findings implied the superiority of the nutrient medium supplied with 30 g L^{-1} fructose (Table 4). It exhibited the callus' highest average dry weight of 42.53 mg, which was not significantly different from 120 g L^{-1} fructose treatment. The lowest values were 32.07 and 32.21 mg when treated with 60 and 90 g L^{-1} fructose, respectively. The results showed the excellence of tyrosine in the medium at a concentration of 60 mg L^{-1}, recording a callus maximum average dry weight of 36.76 mg. The concentrations of 0

Table 4. Effect of different concentrations of fructose and tyrosine on the average dry weight of callus (mg) induced from the bases of young leaves of the seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after seven weeks of culture.

Fructose (mg L^{-1})	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	40.18	45.53	42.75	41.65	42.53
60	29.84	20.41	38.65	39.39	32.07
90	31.96	32.05	27.99	31.83	32.21
120	34.26	32.58	37.65	31.50	33.99
$LSD_{0.05}$	7.61				
Means (mg)	35.31	32.64	36.76	36.09	
$LSD_{0.05}$	3.80				

Table 5. Effect of different concentrations of glucose and tyrosine on the average fresh weight of callus (mg) induced from the bases of young leaves of the seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" cultured on MS medium after seven weeks.

Glucose (mg L^{-1})	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	418.18	597.82	506.75	490.44	503.30
60	268.91	478.82	421.93	356.51	381.54
90	416.80	325.15	261.61	308.69	328.06
120	404.70	474.32	230.63	290.55	350.05
$LSD_{0.05}$	150.15				
Means (mg)	377.15	469.03	365.62	351.15	
$LSD_{0.05}$	75.07				

and 30 mg L^{-1} tyrosine gave average dry weights of callus at 35.31 and 32.64 mg, respectively. Concerning the effect of the interaction between the fructose and tyrosine concentration on the average dry weight of the callus, the outcomes showed that the nutrient medium supplied with 30 g L^{-1} fructose + 30 mg L^{-1} tyrosine was superior, recording the highest average dry weight of the callus at 45.53 mg. The nutrient medium supplied with 60 g L^{-1} fructose + 60 mg L^{-1} tyrosine provided the lowest value, reaching 20.41 mg. Moreover, the nutrient medium with 60 g L^{-1} fructose and 80 mg L^{-1} tyrosine displayed an average dry weight of callus of 39.39 mg, and the medium with 90 g L^{-1} fructose + 30 mg L^{-1} tyrosine recorded an average callus dry weight of 27.99 mg.

Effect of glucose and tyrosine on the fresh weight of callus after five weeks

The results indicated the advantage of the nutrient medium supplied with 30 g L^{-1} glucose, showing the highest average callus fresh weight of 503.30 mg (Table 5). The concentrations at 60, 90, and 120 g L^{-1} glucose recorded average callus fresh weights of 381.54, 328.06, and 350.05 mg, respectively. The same table showed that the concentration of 30 mg L^{-1} of the amino acid tyrosine excelled, recording the highest average callus fresh weight, amounting to 469.03 mg. The results of the interaction between glucose and tyrosine indicate the excellence of the medium supplied with 30 g L^{-1} glucose + 30 mg L^{-1} tyrosine, indicating the maximum rate of callus fresh weight at 597.82 mg. The lowest value emerged in the medium supplied with 90 g L^{-1} glucose + 60 mg L^{-1} of tyrosine, giving a 261.61 mg callus fresh weight.

Effect of glucose and tyrosine on dry weight of callus after seven weeks

A significant decrease was evident in the average callus dry weight of callus induced by increasing the added glucose concentrations

(Table 6). The MS medium supplied with 30 g L^{-1} glucose recorded the highest average callus dry weight at 46.78 mg. The callus' average dry weight decreased with increasing glucose at 120, 90, and 60 g L^{-1}, displaying 32.48, 31.48, and 31.55 mg, respectively. Table 6 also indicates that the MS medium supplied with 30 mg L^{-1} tyrosine recorded the highest mean dry weight of 41.12 mg. It differed significantly from the rest of the treatments. The lowest value was in the control treatment, at an average fresh weight of 31.89 mg. It varied substantially from the rest of the treatments. As for the interaction between the concentrations of the glucose and the tyrosine, as shown in the same table, an increase in the average of callus dry weight occurred in the MS medium supplied with 30 g L^{-1} glucose + 30 mg L^{-1} tyrosine, amounting to 58.18 mg. It significantly contrasted with the rest of the treatments. The lowest value was in the MS medium supplied with 120 g L^{-1} glucose + 60 mg L^{-1} tyrosine at 22.99 mg. It indicates that the callus multiplication increases with the elevation of glucose concentration in the MS medium (Gibson, 2000).

Effect of glucose and tyrosine on the production of the pyrethrin compound

The results estimated by the Mass HPLC device indicate that the interaction treatment of 90 g L^{-1} glucose + 60 g L^{-1} tyrosine was superior to other treatments (Table 7). These recorded the highest pyrethrin compound concentration, which reached 3.346 µg/ml dry weight. Meanwhile, the interaction between treatments of 90 g L^{-1} glucose + 80 mg L^{-1} tyrosine provided the pyrethrin compound content of 2.826 µg/ml dry weight. It did not differ significantly from the rest of the treatment interactions. The lowest content of pyrethrin compound reached 1.955 µg/ml dry weight when treated with 30 g L^{-1} glucose + 0 mg L^{-1} tyrosine. This result is consistent with the findings of the study observed by DiCosmo and Towers (1984).

Table 6. Effect of different glucose and tyrosine concentrations on the average dry weight of callus (mg) induced from the bases of young leaves of the seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after seven weeks of culture.

Glucose (mg L^{-1})	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	35.02	58.18	52.78	41.15	46.78
60	25.27	39.42	36.09	29.15	32.48
90	34.09	29.79	30.39	31.65	31.48
120	33.19	37.08	22.99	32.96	31.55
$LSD_{0.05}$	3.75				
Means (mg)	31.89	41.12	35.57	33.73	
$LSD_{0.05}$	3.75				

Table 7. Effect of different glucose and tyrosine concentrations on the production of Pyrethrin I (µg/ml dry weight) of induced callus from the bases of young leaves of seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after eight weeks of culture (primary weight 200 mg).

Glucose (mg L^{-1})	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	1.955	2.958	2.150	2.057	2.280
60	2.323	2.187	1.763	1.986	2.065
90	2.222	1.994	3.346	2.826	2.602
120	1.969	1.987	2.089	2.238	2.071
$LSD_{0.05}$	0.767				
Means (mg)	2.118	2.281	2.342	2.277	
$LSD_{0.05}$	0.384				0.383

Effect of sucrose and tyrosine on the production of secondary metabolites

The results showed an increase in pyrethrin extract content from the callus of the growing apex of the Dwarf White cultivar plant in response to increased concentrations of sucrose and N-tyrosine added to the MS medium (Table 8). The MS medium supplied with 30 g L^{-1} sucrose + 30 mg L^{-1} tyrosine caused the pyrethrin compound production when elevating the concentrations of tyrosine and sucrose added to the medium. The lowest value recorded resulted in the control treatment at 1.580 µg/ml dry weight of pyrethrin. The highest value was in the MS medium supplied with 30 mg L^{-1} tyrosine, reaching 3.147 µg/ml dry weight of pyrethrin. The response decreased with an elevation of the sucrose concentration to 120 g L^{-1}, amounting to 1.265 µg/ml dry weight of pyrethrin. The solutions, 60 g L^{-1} sucrose + 0 mg L^{-1} tyrosine, recorded the highest value for the pyrethrin compound, amounting to 2.134 µg/ml dry weight.

Effect of fructose and tyrosine on the production of secondary metabolites

The response of the callus to producing secondary compounds decreased with an increase in the tyrosine concentration added to the medium (Table 9). The low tyrosine concentration (0 mg L^{-1}) gave a minimum of 2.164 mg. However, the concentrations of secondary compounds in the callus varied according to the concentration of fructose added. The highest pyrethrin compound content emerged when adding the treatment of 90 g L^{-1} fructose + 60 mg L^{-1} tyrosine at 3.175 µg/ml dry weight. It did not differ significantly from the rest of the concentrations. The concentrations, 30 g L^{-1} fructose + 60 mg L^{-1} tyrosine, recorded the lowest value of 2.958 µg/ml dry weight for pyrethrin.

Table 8. Effect of different concentrations of sucrose and tyrosine on the production of Pyrethrin (µg g^{-1} dry weight) of induced callus from the bases of young leaves of seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after eight weeks of culture (primary weight 200 mg).

Sucrose (mg L-1)	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	1.580	3.147	1.327	1.739	1.962
60	2.134	1.863	1.427	1.886	1.824
90	1.910	1.687	1.844	1.198	1.659
120	1.265	1.400	1.420	1.869	1.488
$LSD_{0.05}$	0.752				
Means (mg)	1.719	2.023	1.504	1.687	
$LSD_{0.05}$	0.376				0.756

Table 9. Effect of different concentrations of fructose and tyrosine on the production of Pyrethrin (µg g-1 dry weight) of induced callus from the bases of young leaves of seedling (*Chrysanthemum hortorum* Hort.) cv. "Dwarf White" grown on MS medium after eight weeks of culture (primary weight 200 mg).

Fructose (mg L-1)	Tyrosine (mg L^{-1})				Means (mg)
	0	30	60	80	
30	2.164	2.958	2.197	2.329	2.413
60	2.178	2.459	2.979	1.978	2.398
90	2.115	2.114	3.175	1.856	2.315
120	2.218	2.511	2.129	1.926	2.197
$LSD_{0.05}$	0.609				
Means (mg)	2.169	2.512	2.620	2.023	
$LSD_{0.05}$	0.305				0.304

CONCLUSIONS

Induction of callus tissues led to an increase in the production of secondary metabolite compounds by preparing the medium containing callus to the concentrations of different types of carbon sources (sucrose, fructose, and glucose) and some chemical additives to stimulate and produce pyrethrins from the bases of young leaves of *Chrysanthemum hortorum* Hort. cv. 'Dwarf White' in vitro. The highest concentration of these additions in the medium led to an enhanced pyrethrin compound production. In the presented study, the elicitation of pyrethrin may refer to the specificity of concentration, type of elicitor, and treatment duration. The results showed that the combination of 2.0 mg L^{-1} BA and 1.5 mg L^{-1} IAA recorded the maximum average fresh and dry weights of callus. The utmost response to callus induction was 95.93%.

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