POTENTIAL OF HORMONES COMBINATION ON CALLUGENSIS OF ANISE SEEDS AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

***In vitro,* a culture experiment was initiated from the seeds of *Pimpinella anisum* L. to investigate callugensis (regeneration and rooting) and antioxidant formation. Three hormones with three different combinations were used in this experiment; Benzylaminopurine (BAP) in three concentrations (0.5, 1, 1.5, and 2 mg L^{-1}) alone and combinations with 2,4-dichlorophenoxyacetic acid (2,4-D) (0.5, 1, 1.5, and 2 mg L^{-1}), or combination with indole butyric acid (IBA) (1 and 1.5 mg L^{-1}). A completely randomized design (CRD) was used with three replicates followed by Fisher's LSD test ($P < 0.05$). The results revealed that Murashige and Skoog (MS) medium fortified by (0.5 mg L^{-1}BAP and 1 mg L^{-1} 2,4-D) was significantly the best combination for improving the fresh weight of 23.66 g and total phenolics of 536.5 mg. Furthermore, a combination of 1.5 mg L^{-1} BAP and 1.5 mg L^{-1}IBA gave the best dry weight of 1.8267 g. While applied (2 mg L^{-1} BAP and 1.5 mg L^{-1} IBA increases 2,2-diphenyl 1-picrylhydrazyl (DPPH) estimation method by 71.06%. It was concluded that binary combinations were efficient to maximize phenolics resulted in augmenting antioxidant potentiality.**

Keywords: *Anise; Callus; Phenolic; Benzyl amino purine; Indole butyric acid.*

INTRODUCTION

Pimpinella anisum L. (aniseed or anise) is an aromatic plant that belongs to the family of Apiaceous that originated from different geographical zones in Asia such as Iran, India, and the Caucasus. It is considered one of the oldest plants utilized by the human in Greece, Rome,

Egypt, and the Middle East [1]. *P. anisum* has brown fruits and white flowers and approximately 2% of its weight is essential oil [1]. Moreover, it sources as a worldwide medical and spice crop in European and North American countries [2]. Seeds of apiaceous species are a valuable source of different classes of metabolites, essential oil, coumarins, flavonoid compounds, phenolics, lipids, and nutrients [1]. Likewise, this crop had been embedded in the International Pharmacopoeia due to its medical values. Some factors such as the growing season and agronomical practices could affect the phenolic content in some crops [3]. Furthermore, the genotypic variation could influence some active compounds in aniseed [4]. Umbel extracted from Aniseed had revealed to be possessed active phenolic acids that resulted in antioxidant potentiality [5]. Thus, the technique of phenolic formation is essential where callus biomass produced *in vitro* gave the highest quantity of phenolic compounds. Total phenolic content (TPC) in shoot callus of anise was significantly varied from a minimum of benzylaminopurine (BAP) and *α*-napthaleneacetic acid (NAA) combination to a maximum of BAP and NAA combination [6]. In *Trachyspermum Ammi*, the combinations 0.25 mg L^{-1} BAP with 2 mg L^{-1} 2,4-D concentrations proved that optimal for producing augmented callus, and also was more active on callus' weight, volume, and color. The best explant based on callus weight was cotyledon explant, and for callus, volume was shoot explant. The prior result was shown that the effective hormone combination and explant (2 mg L^{-1} of 2,4-D with 0.25 mg L^{-1} of BAP) were more active on callus induction [7]. Benzylaminopurine with NAA and indoleacetic acid increased the number of shoots that were regenerated in fennel [6] and anise [8]. On sage, *Salvia officinalis* L., (Neamah and Almehemdi [9] found that the callus inducted from cotyledon gave the highest activity of growth and development with absorbance reached 0.375, 0.422, and 0.562, when extracted by hexane, ethyl acetate, and methanol, respectively. Some active compounds could be increased by adding more glucose in MS. Herewith, their growth activity would be increased also as mentioned by Ameen and Mohamed [10]. However, we hypothesized that BAP and indole butyric acid (IBA) combination could give the best weight and antioxidant activity.

To our knowledge, no references indicated that hormones combination may affect the regeneration and rooting of aniseed seeds and their antioxidant activity; thus, this research was conducted to investigate the effects of different hormone combinations on callus induction of *P. anisum* L. [11-13]. Regarding three different hormones combination were employed to investigate the effect of some hormone's combination on callugensis and its effects on antioxidant activity. This study was started to better understand the potential of these hormones' combination to produce more quantity.

MATERIALS AND METHOD

Plant Material and Callus Induction

Our experiment was accomplished at the Lab of Tissue Culture, Faculty of Agriculture, Damanhour University, Egypt. Seeds of *P. anisum* L. were obtained from Isis industrial company (Cairo, Egypt). Seeds were surface-sterilized with freshly preparing the 0.1 % (w/v) NaOCL up to 20 min. Then, they were washed in sterile distilled water four times. For callus induction disinfested seeds were cultured on (Murashige and Skoog [14] compact medium supplemented plant growth regulators (PGR) by using BAP (Sigma Aldrich) in three concentrations (0.5, 1, 1.5, and 2 mg L^{-1}) alone or combinations with 2,4-D (0.5, 1, 1.5 and 2 mg L^{-1}) (Sigma Aldrich). The third treatment is BAP in three concentrations (0.5, 1, 1.5, and 2 mg L^{-1}) in combination with IBA (Sigma Aldrich) (1 and 1.5 mg L^{-1}) with 30 mg L^{-1} of sucrose (Duchefa Biochemie, The Netherlands) (Table 1). The hydrogen potential (pH) of the medium was adjusted at 5.8 before adding 0.8% (w/v) agar (Duchefa Biochemie, The Netherlands), and it then was autoclaved at 121°C for 15 min and allowed to induce callus under laboratory-maintained conditions at 25±2°C for 3 weeks. After callus cultures were collected, the samples were prepared for extraction (three replicates each treatment).

Table 1. The different combinations of BAP, 2,4-D, and IBA on seed callus induction of aniseed

Treatment's Number	BAP (mg L^{-1})	2,4-D (mg L^{-1})	IBA (mg L^{-1})
1	0.5	0	0
2	1	0	0
3	1.5	0	0
4	2	0	0
5	0.5	0.5	0
6	0.5	1	0
7	0.5	1.5	0
8	0.5	2	0
9	1	0.5	0
10	1	1	0
11	1	1.5	0
12	1	2	0
13	0.5	0	1
14	1	0	1
15	1.5	0	1
16	2	0	1
17	0.5	0	1.5
18	1	0	1.5
19	1.5	0	1.5
20	2	0	1.5

BAP= Benzylaminopurine, 2, 4-D= Dichlorophenoxyacetic acid, IBA= Indole-3-butyric acid

Preparation of Callus Extractions

The dried samples were grounded to powder using a mortar and pestle. After 72 h, the extraction process was done using the continuous hot percolation method with petroleum ether, methanol, and water, and then the samples were stored for further investigation.

Assessment of Antioxidant Activity

The DPPH Radical Scavenging Activity assay was accomplished according to Zhu et al. [15]. In short, 2 mL of DPPH solution (0.1 mM in methanol) was mixed with 2 mL of each sample at various levels (50, 100, 150, 200, 250, and 300 μg mL^{-1} in methanol). The reaction components were mixed well and darkly brood for 30 min. at room temperature. The solution absorbance was determined at 517 nm. The free radical scavenging activity of each fractioned solution was determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical was assessed using the following formula:

$$[\text{DPPH}\ (\%)\ = \frac{Dss-Dsa}{Dss} \times 100]$$

Dss is standing for (the absorbance of the control), and Dsa is (the absorbance of the sample).

Total Polyphenols

The total phenolic content was calculated via the Folin-Ciocalteu method [16,17]. The reaction components in all treatments were combined with 0.1 mL of methanolic extract, 7.9 mL of distilled water, 0.2 mL of the Folin-Ciocalteu's reagent (Sigma, Germany), and 1.5 mL of 20% sodium carbonate. The resultant solution was mingled and allowed to stand for 2 hours. The absorbance was measured at 765 nm using a UV-Spectrophotometer (Shimadzu UV-1700, Japan) [18,19]. The total phenolics were calculated as Gallic acid equivalents (GAE) per gram dry weight. All treatments combined from three plant regulators levels are shown in Table 1 [16, 20,11].

Statistical Design

The statistical analysis of the obtained data was carried out using the Minitab program (Chicago, USA) [12,21]. Moreover, CRD design was used followed by Fisher's LSD at $P < 0.05$ using CoStat® software (version 6.451).

RESULTS

Fresh weight (FW) and Dry weight (DW)

Callus initiated from seeds were cultured on the MS medium solidified with BAP and 2, 4-D or BAP and IBA after 28 days of brood under the condition of darkness. There was a significant difference among the treatments' weight of the obtained callus from seed ($P < 0.05$) (Table 2, Figs. 1 & 2). Meanwhile, LSD analysis indicated that there was a significant difference between explants including based on callus weight. However, Table 2 suggests that the best combination was when MS medium fortified by 0.5 mg L^{-1} of BAP and 1 mg L^{-1} of 2,4-D which gave the highest fresh weight of 23.66 g, followed by 1.5 mg L^{-1} BAP and 1 mg L^{-1} IBA combination that achieved 17.78 g; while the combination of 2 mg L^{-1} BAP and 1 mg L^{-1} IBA produced only 2.24 g (Table 2, Figs. 1 & 3).

Table 2. Fresh, dry weight, and color of aniseed as affected by different combinations of BAP, 2, 4-D, and IBA on seed callus

Treatments	BAP (mg L^{-1})	2,4- D (mg L^{-1})	IBA (mg L^{-1})	Fresh weight (g)	Mean (FW)	SD (FW)	Dry weight (g)	Mean (DW)	SD (DW)	Callus color
1	0.5	0	0	4.707	4.7	0.21	0.7000	0.7	0.07	brown
2	1	0	0	5.343	5.3	0.17	0.8933	0.9	0.04	brown
3	1.5	0	0	3.423	3.4	0.02	0.5300	0.5	0.04	brown
4	2	0	0	7.093	7.1	0.10	0.9333	0.9	0.05	brown
5	0.5	0.5	0	14.140	14	0.03	1.1467	1.1	0.02	yellow
6	0.5	1	0	23.660	23	0.7	1.2700	1.3	0.06	yellow
7	0.5	1.5	0	6.623	6.6	0.5	0.6767	0.7	0.07	yellow
8	0.5	2	0	5.930	5.9	0.06	0.7200	0.7	0.06	yellow
9	1	0.5	0	12.067	12	0.8	0.7700	0.8	0.04	yellow
10	1	1	0	3.123	3.1	0.2	0.4067	0.4	0.05	yellow
11	1	1.5	0	6.743	6.7	0.5	0.6967	0.7	0.01	yellow
12	1	2	0	2.837	2.8	0.04	0.3133	0.3	0.02	yellow
13	0.5	0	1	12.993	13	1.8	1.3267	1.3	0.02	brown
14	1	0	1	13.397	13	1.3	1.1833	1.2	0.09	brown
15	1.5	0	1	17.780	18	0.5	1.5100	1.5	0.02	brown
16	2	0	1	2.240	2.2	0.4	0.3033	0.3	0.04	brown
17	0.5	0	1.5	4.297	4.2	0.2	0.7200	0.7	0.01	brown
18	1	0	1.5	3.503	3.5	0.5	0.5333	0.5	0.03	brown
19	1.5	0	1.5	13.840	14	1.7	1.8267	1.8	0.06	brown
20	2	0	1.5	4.590	4.6	0.4	0.7733	0.8	0.03	brown

BAP= Benzylaminopurine, 2, 4-D= Dichlorophenoxyacetic acid, IBA= Indole-3-butyric acid, P < 0.05

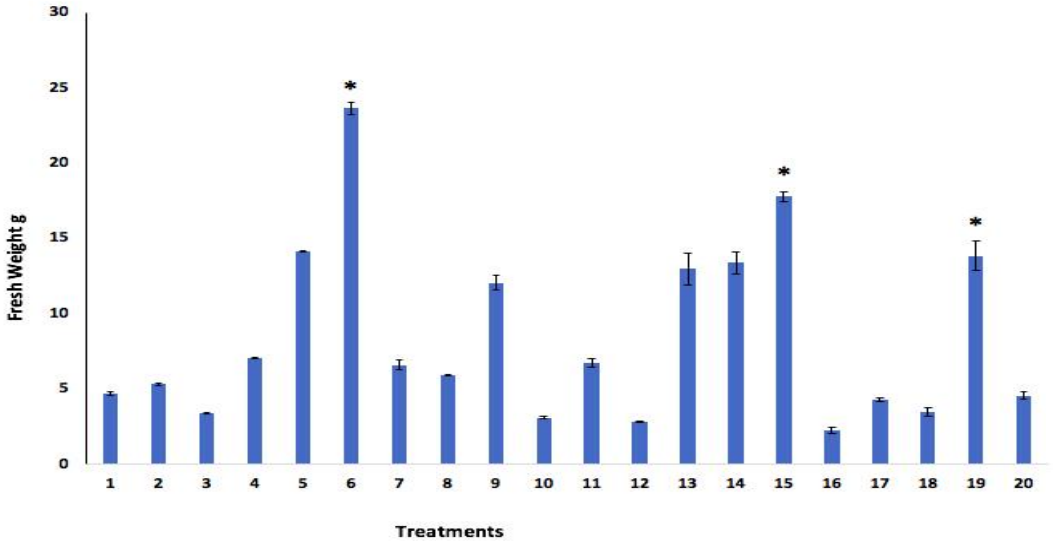

Fig. 1. Fresh weight of aniseed as affected by different combinations of BAP, 2, 4-D, and IBA on seed callus (*P* < 0.05)

The callus-induced aniseed seeds were developed a normal callus on MS growth regulator medium after 60 days. The seed of aniseed induced the largest amount of callus. The data observed that a large amount of proliferating callus dry weight (as shown in Table 2) is the medium with 1.5 mg L^{-1} BAP and 1.5 mg L^{-1} IBA of 1.8267g, followed by 1.5 mg L^{-1} BAP and 1 mg L^{-1} IBA of 1.51 g (Figs. 2 & 4). While the lowest dry biomass was 0.3033g achieved under 2 mg L^{-1} of BAP and 1 mg L^{-1} of IBA. These combinations are commonly used to induce callus. The solid agar MS medium

supplemented with plant growth regulators, IBA, BAP, and 2, 4-D was used as a formation medium. Using sub-culturing, the cultures were developed as calli culture separate the target combinations of three growth regulators above. As indicated in Table 2, the callus was compact and had brown color for BAP alone while used combinations between BAP with 2, 4-D gave friable yellow callus. Still, it was compact brown color in the other treatments (Fig. 5).

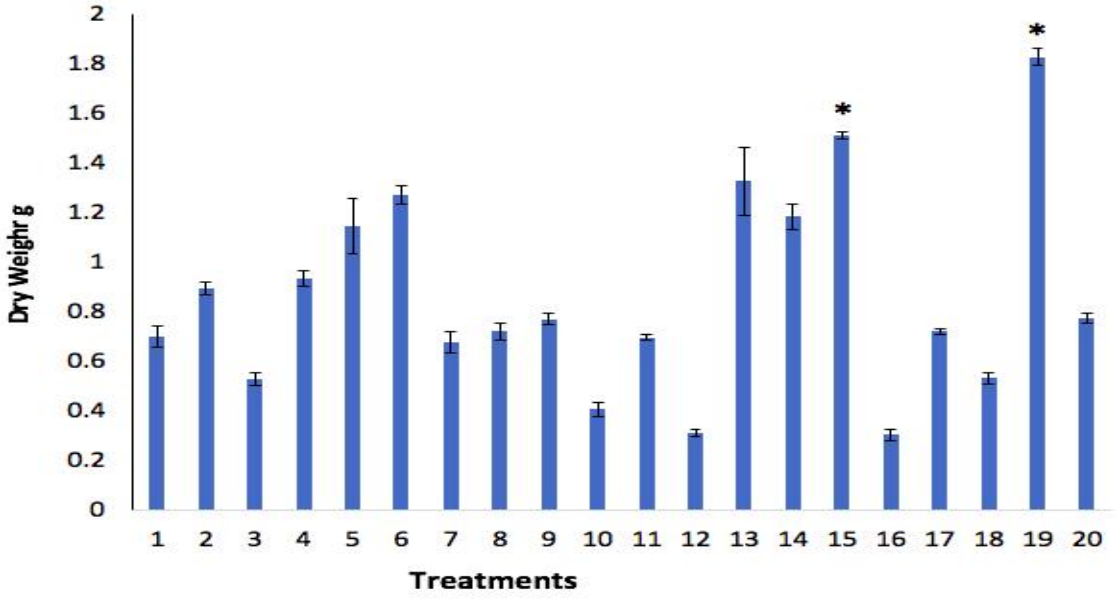

Fig. 2. Dry weight of aniseed as affected by different combinations of BAP, 2, 4-D, and IBA on seed callus ($P < 0.05$)

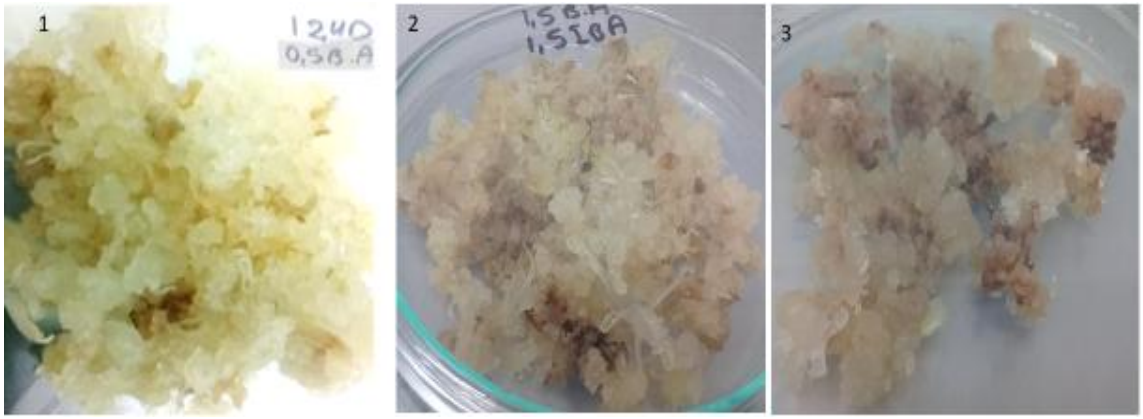

Fig. 3. Callus fresh weight: 1 is the largest weight (0.5 mg L^{-1} BAP and 1 mg L^{-1} 2,4 D), 2 is medium weight (1.5 mg L^{-1} BAP and 1.5 mg L^{-1} IBA) , 3 is lowest weight (2 mg L^{-1} BAP and 1 mg L^{-1} IBA)

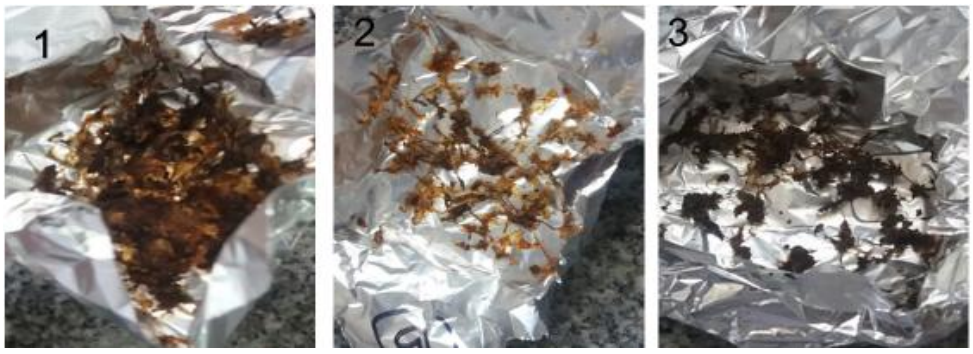

Fig. 4. Callus dry weight for different MS medium: 1 is the largest weight (1.5 mg L^{-1} BAP and 1.5 mg L^{-1} IBA), 2 is the medium weight treat (1.5 mg L^{-1} BAP and 1 mg L^{-1} IBA), 3 is the lowest weight Treatment (2 mg L^{-1} BAP and 1 mg L^{-1} IBA)

Fig. 5. Callus Color morphology as affected by used BAP alone and with different combinations of 2, 4-D, and IBA of aniseed

Table 3. Total phenolic and DPPH as affected by different combinations of BAP, 2, 4-D, and IBA on seed callus of aniseed

Treatments	BAP mg L^{-1}	2,4- D mg L^{-1}	IBA mg L^{-1}	Total phenolic content	Mean (TPC)	SD (TPC)	DPPH	Mean (DPPH)	SD (DPPH)
1	0.5	0	0	343.17	343	23.2	53.25	53.2	0.76
2	1	0	0	289.42	289	6.3	56.40	56.4	2.0
3	1.5	0	0	417.33	417	9.2	62.77	62.8	2.1
4	2	0	0	384.00	384	30.9	51.66	51.6	4.8
5	0.5	0.5	0	464.83	464	21	53.44	53.4	5.2
6	0.5	1	0	536.50	536	8.7	53.15	53.1	2.7
7	0.5	1.5	0	279.00	279	11.1	51.88	51.9	2.2
8	0.5	2	0	336.50	336	12.7	52.61	52.6	2.8
9	1	0.5	0	325.67	325	0.7	53.87	53.8	2.4
10	1	1	0	467.33	467	9.04	51.88	51.9	3.05
11	1	1.5	0	351.08	351	20.1	55.09	55	5.2
12	1	2	0	377.33	377	4.3	55.15	55.1	3.1
13	0.5	0	1	290.67	290	6.9	54.49	54.5	3.8
14	1	0	1	346.50	346	18.4	58.39	58.3	1.7
15	1.5	0	1	416.08	416	3.1	54.76	54.7	6.8
16	2	0	1	289.83	289	3.14	54.20	54.2	2.1
17	0.5	0	1.5	176.10	176	13.4	50.29	50.3	4.0
18	1	0	1.5	266.50	266	7.8	53.08	53.1	5.3
19	1.5	0	1.5	305.25	305	10.2	63.31	63.3	1.4
20	2	0	1.5	445.67	445	10.1	71.06	71.1	2.9

BAP= Benzylaminopurine, 2, 4-D= Dichlorophenoxyacetic acid, IBA= Indole-3-butyric acid, $P < 0.05$

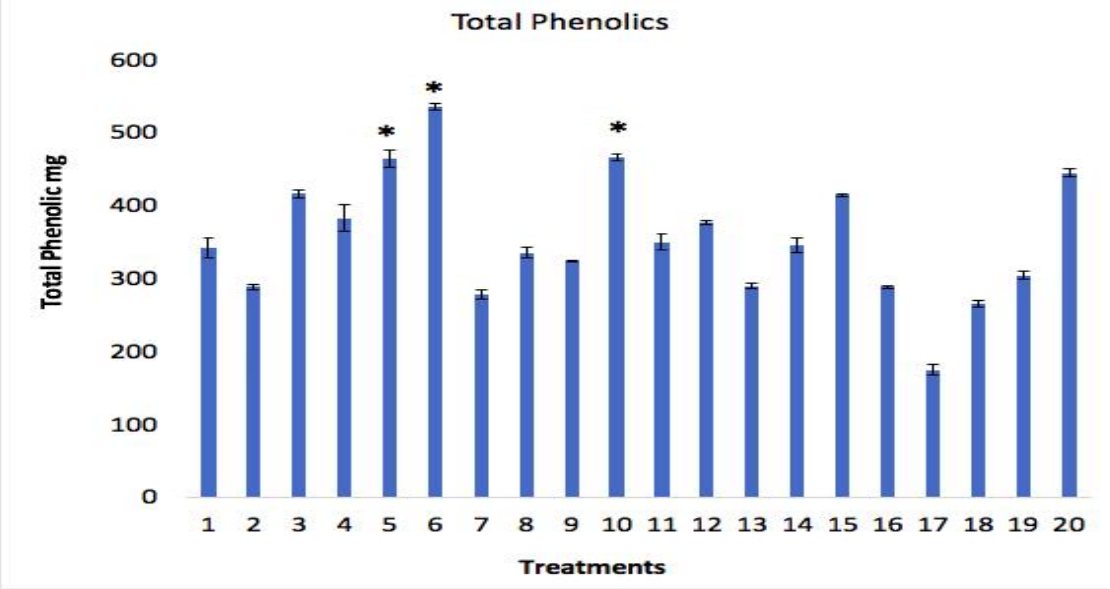

Fig. 6. Total phenolic as affected by different combinations of BAP, 2, 4-D, and IBA on seed callus of aniseed

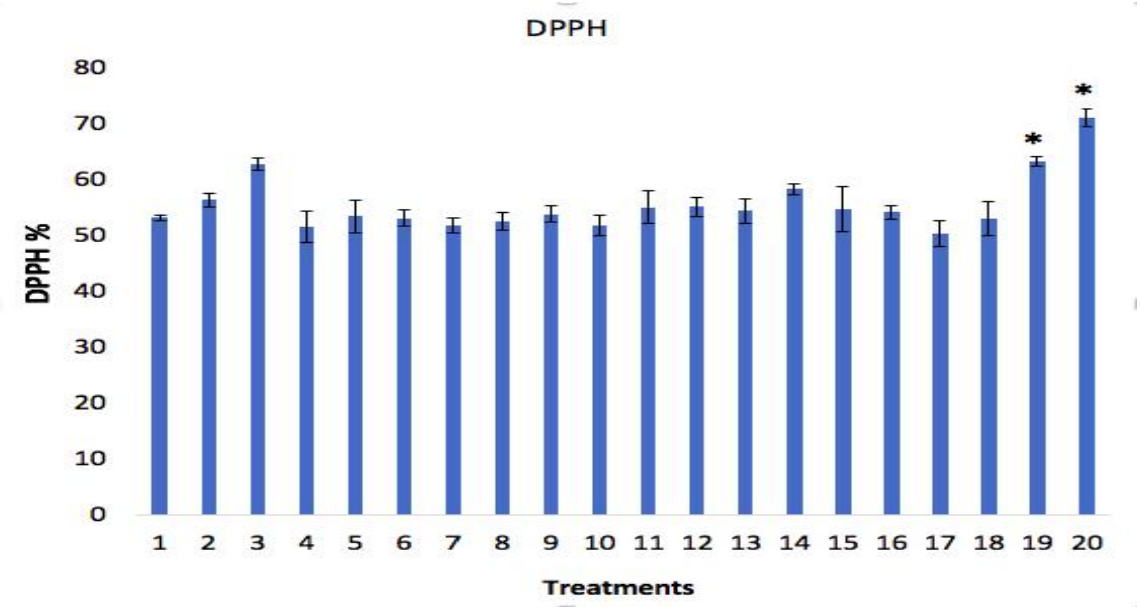

Fig. 7. Total DPPH as affected by different combinations of BAP, 2, 4-D, and IBA on seed callus of aniseed

Total Phenolic Content (TPC)

Data presented in Table 3 are the mean of three replicates. Total Phenolic content was significantly varied from a minimum of 176.10 mg GAE g^{-1} dry weight (DW) on 0.5 mg L^{-1} BAP and 1.5 mg L^{-1} IBA and 1 mg L^{-1} BAP and 1.5 mg L^{-1} IBA of 266.50 mg GAE g^{-1} to a maximum of

536.50 mg GAE g^{-1} DW on 0.5 mg L^{-1} BAP and 1 mg L^{-1} 2,4-D closely followed by 467.33 mg GAE g^{-1} DW on 1 mg L^{-1} BAP and 1 mg L^{-1} 2,4-D (Table 3 & Fig. 6). These differences in TPC showed the effect of growth regulators on the production of phenolic compounds in tissue culture. This effect was also visible through different callus color and callus morphology since there was creamy, yellowish, and brownish colored callus with friable or compact morphology.

Antioxidant Activity

The methanol extract of dried callus is obtained from various growth regulators combinations and evaluated for total antioxidants. The reactivity of callus extract of *P. anisum* was measured with DPPH, a stable free radical. DPPH locks onto one electron as a free radical scavenger was presented. Consequently, the absorptivity is decreased. Therefore, the resulted coloration was associated with electrons that would be released. The DPPH radical scavenging (%) activity is revealed in Tables 3 and Fig. 7. Thus, *P. anisum* callus extract exerted an inhibition of 71.06% for applying 2 mg L^{-1} BAP + 1.5 mg L^{-1} IBA in media, followed by 63.31% for 1.5 mg L^{-1} BAP + 1 mg L^{-1} IBA. While the lowest inhibition was obtained from the addition of 0.5 mg L^{-1} BAP + 1.5 mg L^{-1} IBA that gave DPPH 50.29% (Tables 3 & Fig. 7).

DISCUSSION

This experiment aims to get a better insight into the consequences of hormone combinations on Anise's weight, antioxidant activity, and morphological characters. In past, the studies were focused on aniseed plants [22,13]. However, in this study, fresh, dry weight, total phenolics, and DPPH were all elevated.

Cytokinins and auxins commonly are the most used as growth hormones for plant regeneration [23]. Also, benzylaminopurine (cytokinins) is one of the hormones known to affect different phases of development and floral initiation by affecting the flower mutation, increase the organ number and increase flower development [24]. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a plant growth hormone and well known to be used as a herbicide in high doses. In low doses, however, 2,4-D uses in promoting and developing the plant, chemical analogues of auxin, a plant growth hormone. Similarly, indole butyric acid is one of the auxin groups that plays role in plant growth and development [25].

In our results, hormones combined were added to each treatment to investigate the potential of these hormones on the plants' regeneration and antioxidant activity. Interestingly, the data showed that MS medium fortified by (0.5 mg L^{-1} BAP +1 mg L^{-1} 2,4-D) was significantly the best combination for improving the fresh weight of 23.66 g and total phenolics of 536.5 mg. Furthermore, a combination of 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} IBA gave the best dry weight of 1.8267 g. While applied (2 mg L^{-1} BAP+1.5 mg L^{-1} IBA) increases DPPH estimation method of 71.06% (Tables 1 & 2, Figs. 1-7).

The same conclusion was proposed by Zheng et al. [26] when the experiment was done on *Thermopsis lanceolate.* Our findings match a past finding by [27-29] which proposed that protein amounts and amino acid were elevated in oak and tea shoots and antioxidant parameters were affected in plants treated with bioregulators. Previously, Ha et al. [30] suggested that the synthesis of antioxidant enzymes can be controlled by cytokinin.

Prior studies focused on organogenesis or somatic embryogenesis from the shoot, root tissue cells for micropropagation of the plant species by culturing different plant parts from divergent organs under target conditions [31]. Shoot and root could be formed as growth regulating constituents when added to medium based on their concentrations, especially auxin and cytokinin [32]. Herewith, regeneration had registered from these callus tissues that were initiated from seeds of *P. anisum* L. Seed could form callus when cultured on MS medium solidified with IAA combined with kinetin and BAP. Furthermore, calli were formed on a medium fortified with 2,4-D alone or with kinetin. These calli also achieved a rise in a medium enriched with BAP combined with or IAA, and BAP as suitable concentration applied. In our study, regeneration has been adequately reported from these calli produced from seeds of

P. anisum L. These calli tissues were shown to have antioxidant activity because of the higher total phenolic compounds. Additionally, several studies have shown that calli compounds have antioxidant activity of calli compounds [9,10]. Moreover, the accumulation of phenylpropanoids shown to have antioxidant activity in the study of Reichling et al. [33] which calli may possess antioxidants such as pseudoisoeugenol, which produced in tissue culture from L-phenylalanine, trans-cinnamic acid, and p-coumaric acid [22]. Also, solvent properties could be affected by DPPH activity via the effect on the type of extracted constituents from callus [10]. Furthermore, some nutritional chemicals such as monosaccharides that fortified MS could be produced by more active compounds.

Liu et al. [23] showed MS medium (3.0 mg L^{-1} 2,4-D and 1.0 mg L^{-1} BAP) gives 100% callus induction ratio; and it was suggested that the high concentration of 2,4-D could cause callus browning. Moreover, they proposed that BAP was essential during the induction of buds of *R. hybrida*. Due to the inhibitory effect of 2,4-D in high concentrations, it is wise to use low concentrations in the callus induction process [23]. Also, (Moitreyee et al. [34] suggested that MS medium has better growth results when supplemented with high auxin and low cytokinin. Moreover, it was reported that the best combination that gives a better callus induction ratio is 2,4-D and kinetin. Additionally, it has been previously reported in other studies that BAP and NAA (Naphthalene acetic acid) give the best results in callus induction [34].

CONCLUSION

Anise seed *in vitro* cultures is a rich source of phenolics. Thus, antioxidant potentiality seems to be associated with total phenolic that is affected by growth hormones such as auxins and cytokinin. In-depth investigations are required to characterize active phenolic compounds that possess the antioxidant. Applicably, active phenolic compounds can be given for seed germination regulation and augmenting antioxidation phenolics for application in nutraceutics. It could be proved that these data obtained from a combination of BAP (cytokinin) with auxins as 2,4-D or IBA was beneficial to induce callus from anise seeds. Therefore, the ongoing studies on active constituents would be valuable for effective antioxidation that formed from anise seeds callus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rocha L, Fernandes CP. Aniseed (*Pimpinella anisum, Apiaceae*) Oils. In Essential Oils in Food Preservation, Flavor and Safety: Elsevier. 2016;209-213.
2. Tepe AS, Tepe B. Traditional use, biological activity potential and toxicity of *Pimpinella* species. Ind. Crops Prod. 2015; 69:153-166.
3. Kara N. Yield, quality, and growing degree days of anise (*Pimpinella anisum* L.) under different agronomic practices. Turk. J. For Agric. 2015;39(6):1014-1022.
4. Arslan N, Gürbüz B, Sarihan EO, Bayrak A, Gümüşçü A. Variation in essential oil content and composition in Turkish anise (*Pimpinella anisum* L.*)* populations. Turk. J. For Agric. 2004;28(3):173-177.
5. Shyu YS, Lin JT, Chang YT, Chiang CJ, Yang DJ. Evaluation of antioxidant ability of ethanolic extract from dill (*Anethum graveolens* L.) flower. Food Chemistry. 2009;115(2):515-521.
6. Saxena S, Kothari P, Rathore S, Ishan-Ulla-Khan R. Organogenesis in fennel (*Foeniculum vulgare Mill*.). International J. Seed Spices. 2012a;2(2):1-4.
7. Fazeli-Nasab B. The effect of explant, BAP and 2, 4-D on callus induction of *Trachyspermum ammi*. *Potravinarstvo.*2018;12(1):578-586.
8. Saxena SN, Ishan KU, Saxena R. Organogenesis in anise (*Pimpinella anisum L.*). J. Spices Aromat. Crops. 2012b;21(1): 59-63.
9. Neamah S, Almehemdi A. Extraction of natural compounds from callus inducted of common sage plant *Salvia officinalis L.* and their evaluation of antioxidant activity. Iraqi J. Agric. Sci. 2017;48(6):1541-1548.

10. Ameen SK, Mohamed HI. Influence of succharides on secondary metabolites production from leaves induced callus of *Salvia officinalis in vitro*. Anbar J. Agri. Sci. 2010;8(4):236-255.
11. Prakkash MJ, Ragunathan R, Jesteena J. Evaluation of bioactive compounds from *Jasminum polyanthum* and its medicinal properties. J. Drug Deliv. Ther. 2019; 9(2):303-310.
Zanobini A. Using MINITAB software for teaching measurement uncertainty. Paper read at Journal of Physics: Conference Series; 2015.
12. Martin R, Schilling G, Reichling J. Studies on the biosynthesis of pseudoisoeugenols in tissue cultures of *Pimpinella anisum*. *Z. Naturforsch.* C. 1988;43(5-6):328-336.
13. Sonmez C. Effect of phosphorus fertilizer on some yield components and quality of different anise (*Pimpinella anisum* L.) populations. Turkish J. Field Crop. 2018; 23(2):100-106.
14. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 1962; 15(3):473-497.
15. Zhu K, Zhou H, Qian H. Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. Process Biochem. 2006;41(6):1296-1302.
16. Noreen H, Semmar N, Farman M, McCullagh JS. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. Asian Pac. J. Trop. Med. 2017; 10(8):792-801.
17. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16(3):144-158.
18. Prabha S, Rao M, Kumar MR. Evaluation of in vitro Antioxidant, Antibacterial and Anticancer activities of leaf extracts of *Cleome rutidosperma*. Res. J. Pharm. Technol. 2017;10(8):2492-2496.
19. Umavandhana R, Jayanthi S. Analysis of Phytochemical compounds and DPPH radical scavenging activity of *Dictyota dichotoma* and *Halimeda macroloba*. Phytochem Anal. 2018;11(8):3440-3444.
20. Tiwari P, Patel RK. Estimation of total phenolics and flavonoids and antioxidant potential of Ashwagandharishta prepared by traditional and modern methods. Asian J. Pharm. Ana. 2013;3(4):147-152.
21. Martins N, Barros L, Santos-Buelga C, Ferreira IC. Antioxidant potential of two Apiaceae plant extracts: A comparative study focused on the phenolic composition. Ind. Crop Prod. 2016;79:188-194.
22. Snedecor G, Cochran W. Statistical methods. 11th. Iowa State College Press. Ames, Iowa, US. 1990;369-375.
23. Liu J, Feng H, Ma Y. Effects of different plant hormones on callus induction and plant regeneration of miniature roses (*Rosa hybrida* L.). Hortic. Int. J. 2018;2:201-206.
24. Venglat S, Sawhney VK. Benzylaminopurine induces phenocopies of floral meristem and organ identity mutants in wild-type *Arabidopsis* plants. Planta. 1996; 198(3):480-487.
25. Ludwig-Müller J. Indole-3-butyric acid in plant growth and development. Plant Growth Regul. 2000;32(2):219-230.
26. Zheng XF, Wang XD, Zhang CQ, Wang EJ. Study on the optimum combinations of plant hormone in callus culture of Thermopsis lanceolata. Zhong Yao. Cai. 2013; 36(10):1555-1559.
27. Olaiya C, Osonubi O. Effects of pre-sowing seed treatments on tomato (*Lycopersicon esculentum* (*L.*) Mill) seedling emergence. Int. J. Eng. Technol. 2009;1(4):321-323.
28. Nandi S, Palni L. Chemical manipulation of dormancy in tea shoots and associated biochemical changes. J. Plant. Crops. 1995; 23:52-58.
29. El-Gaied LF, Abu El-Heba GA, El-Sherif NA. Effect of growth hormones on some antioxidant parameters and gene expression in tomato. *GM Crops Food.* 2013;4(1):67-73.
30. Ha S, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, P LS. Tran. Cytokinins: metabolism and function in plant adaptation to environmental stresses. Trends Plant Sci. 2012;17(3):172-179.

31. Henshaw G, O'Hara J, Webb K. Morphogenetic studies in plant tissue cultures. Symposium of the British Society for Cell Biology. England: Cambridge University Press. 1982;231–251.
32. Ooms G, Hooykaas PJ, Moolenaar G, Schilperoort RA. Crown gall plant tumors of abnormal morphology, induced by *Agrobacterium tumefaciens* carrying mutated octopine Ti plasmids; analysis of T-DNA functions. Gene. 1981;14(1-2): 33-50.
33. Reichling J, Martin R, Thron U. Production and accumulation of phenylpropanoids in tissue and organ cultures of *Pimpinella anisum*. *Z.* Naturforsch. 1988;43(1-2):42-46.
34. Moitreyee S, Karuna S, Singh S. Effect of culture media and growth hormones on callus induction in *Aquilaria malaccensis Lam*., a medicinally and commercially important tree species of North East India. Asian J. Biol. Sci. 2013;6(2):96-105.