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Callus induction and influence of biotic and abiotic elicitation on active constituents of *Portulaca oleracea* L. calli induced *in vitro*

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Abstract

Purslane (*Portulaca oleracea* L.) is a plant that is distributed widely around the world. It contains many secondary metabolites that are used in treatment of many diseases. The present study was conducted to investigate the effect of plant growth regulators on calli induction, and the subsequent experiments were conducted to investigate the influence of biotic (*Fusarium oxysporum*) and abiotic elicitation (Mannitol) on active constituents of *P. oleracea* . The results showed that the best calli induced from the stem explant of *P. oleracea* on Murashige and Skoog's medium with benzyl adenine 1 mg / L and 2,4-dichlorophenoxyacetic acid 0.25 mg / L. On the other hand, Alkaloids became 280 mg / g in a treated calli with 1 % filtrate of fungal and (100 mil Mol) mannitol after being 140 mg / g in dry powder of calli. Flavonoids became 340 mg / g in a treated calli with 1 % filtrate of fungal with 150 mM mannitol after being 180 mg / g in dry powder of calli. Tannins became 180 mg / g in a treated calli with 1.5% filtrate of fungal only after being 180 mg / g in dry powder of calli. Elicitation studies have a promising effect in increasing yields of the secondary metabolites in *P. oleracea* .

Keywords: *Portulaca oleracea*, tissue culture, benzyl adenine (BA), 2,4-dichlorophenoxyacetic acid (2,4-D), elicitation, secondary metabolites, medicinal plants

Introduction

Purslane (Portulaca oleracea) is a common weed that belongs to the Portulacaceae family. It is an annual, succulent summer plant that grows in cosmopolitan areas [1]. It is distributed around the world and is widely used as a medicinal and potherb herb in Asia, Europe, and the Mediterranean region [2].

It is a medicinal plant that includes a variety of bioactive chemicals such as cerebrosides, flavonoids, and alkaloids, all of which have been shown to have cytotoxic properties in the face of cancer cell lines of human [3] and are used in folk medicine in several countries. As a human diet, it is added to soups and salads around the Mediterranean and tropical Asian countries [4], flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins, and minerals were extracted from it [5]. It has high nutritional value as it contains an omega-3 fatty acid [6]. In addition, *P. oleracea* has pharmacological uses such as hepato-protective, neuroprotective, anti-inflammatory, antimicrobial, antidiabetic, antioxidant, anticancer, and antihypertensive actions [7], anti-asthmatic [8], and decreasing renal histological damage and enhancing renal function parameters [9]. Potassium, magnesium, calcium, phosphorus, iron, and other dietary minerals can be found in it [10]. The biosynthesized *P. oleracea* silver nitrate nanoparticles (AgNPs) may be considered a fungicide to protect different plants against phytopathogenic fungi [11].

Generally, an intermediate ratio of auxin and cytokinin helps callus induction, while a high ratio of auxin to cytokinin or cytokinin to auxin stimulates root and shoot regeneration, respectively [12]. Auxin and cytokinin are usually used to induce callus because they promote cell growth by stimulating cell division

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and elongation through synergistic, antagonistic, and additive interactions [13]. Auxin (2,4-dichlorophenoxyacetic acid; 2,4-D) has been widely used either alone or combined with cytokinin, especially 6-benzylaminopurine (BAP), to stimulate callus induction and obtain bioactive compounds in vitro. However, the concentration and combination of these regulators must be defined for each species [14]. The addition of trace amounts of elicitors to plant cell culture systems in vitro increases the manufacture of defensive secondary metabolites [15]. Elicitors are compounds that play a significant role in biosynthetic pathways for the enhanced manufacture of commercially important compounds and include exogenous elicitors substances of pathogen origin and endogenous elicitors compounds that are released from plants by the action of the pathogen [16]. The present study aimed to determine the best treatment and the best explant for callus induction and to investigate the influence of biotic (Fusarium oxysporum) and abiotic elicitation (Mannitol) on active constituents of *P. oleracea* .

Material and methods

This study was carried in the Plant Tissue Culture Unit in the Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt from 2020-2021. *P. oleracea* seeds were collected from Cairo waste land habitat.

1- Surface sterilization of seeds for studied plant

The seeds of *P. oleracea* plant were soaked in 70 % ethanol for 3 minutes before being rinsed with sterile distilled water to eliminate all traces of the alcohol. Secondly, seeds were immersed in 10 %, 15 %, and 20 % sodium hypochlorite solution with 1 drop of tween 20 for different periods of time (10, 15, 20, and 25 minutes) for each treatment. This was followed by five rinses with sterile distilled water to remove all traces of disinfected and detergent agents.

2-Germination and explant preparation

Sterilized seeds of *P. oleracea* were germinated on growth regulator free [17] medium, basal salts fortified with 30 g/l sucrose (1/2 MS and total MS medium). About 5 seeds were put in each jar containing 35 ml of culture media. For each duration, 3 jars were used for each treatment. The cultures were kept at room temperature (25 ± 2 oC), illuminated by cool fluorescent lambs with a photoperiod of 16 hours. The obtained 4-5-week-old seedlings were employed as sources of stem segments (1 cm2), leaves, and root segments (1 cm2).

3- Preparation of growth regulator hormones

Cytokinin like benzyl adenine (BA) was dissolved in HCl and auxins like 2,4 dichlorophenoxy acetic acid (2,4D) was dissolved in alcohol. A concentration of 1 mg/ml stock solutions of each hormone was prepared individually by dissolving 100 mg of each hormone in 2 ml of 1 M HCl, and distilled water at approximately 45 oC was then added to make a final volume of 100 ml of stock solution of each hormone. Stock solutions were stored at 2- 4ºC.

4-Treatments for calli induction

For calli induction of *P. oleracea* , explants were separately cultured on MS culture medium supplemented with BA (0, 1, 2 and 3 mg / l) in combination with 2,4-D (0.0, 0.25, 0.5, and 0.75 mg / l) to create 16 different treatments (Table 1). The conical containers were incubated at 25 ±2 ºC with a photoperiod of 16 h light / 8 h dark every day. The data for callus induction from tested explants of *P. oleracea* were recorded after six weeks.

Table 1. Various 2,4-D and BA treatments for callus formation from leaf, stem and root segments explants seedlings from *P. oleracea* .

	2.4D (mg / l)			
BA (mg /l)	**0.0**	**0.25**	**0.5**	**0.75**
0.0	1	2	3	4
1	5	6	7	8
2	9	10	11	12
3	13	14	15	16

5- Calli treatment with biotic elicitor and abiotic elicitor

One-month-old calli pieces (approximately 250 mg) obtained from stem segments and grown on MS medium enriched with BA (1 mg / l) and 2,4-D (0.25 mg / l) for one month. then transferred to jars which contain 35 ml of culture media supplemented with biotic elicitor (Fusarium oxysporum) and abiotic elicitor (mannitol) to construct 15 distinct formulations in addition to the control treatment as illustrated in Table 2.

Table 2. Concentration of fungal filtrate and mannitol added to MS culture media as biotic and abiotic elicitors

		Biotic elicitation with autoclaved *Fusarium oxysporum* filtrate			
		0.0%	**1.0%**	**1.5%**	**2%**
Abiotic elicitation with mannitol	**0.0 mM**	1	2	3	4
	50 mM	5	6	7	8
	100 mM	9	10	11	12
	150 mM	13	14	15	16

6-Determination of secondary metabolites

a- Total alkaloids

[18] method was used to calculate total alkaloids five grammes of the (mother plant or callus) were weighed into a 250 ml beaker, and 200 ml of 10 % acetic acid in ethanol were added, covered, and left to stand for four hours. This was filtered, and the extract was concentrated to one-quarter of its original volume in a water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until it precipitated. After allowing the entire solution to settle, the precipitate was collected and washed with weak ammonium hydroxide before being filtered. The alkaloid was dried, weighed, and expressed as mg/g dry plant material in the residue.

b-Total tannins

According to [19], total tannins were evaluated by gravimetry. One gramme of (mother plant or callus) was cooked in two batches of 100 ml acetone: water for one hour (1:1). Filtration was done, and the combined filtrates were diluted to 250 mL with distilled water before being heated to boiling again. Tannins were then precipitated as copper tannate from the extract by adding 30 mL of a 15 % aqueous solution of copper acetate. The precipitate was collected on ashless filter paper, rinsed with water until it was clear of copper acetate, and then fired in a porcelain crucible that had been pre-ignited to a fixed weight. A few drops of nitric acid were added to the residue and the weight was reset to a constant. The amount of tannins was calculated as mg/g dry plant material using the weight of the resultant copper oxide.

c- Total flavonoids

Total flavonoids were calculated using the [20] method. At room temperature, two grammes of (mother plant or callus) were extracted many times with 100 ml of 80 percent aqueous methanol. The entire solution was filtered using filter paper number 42 (125 mm), after which the filtrate was transferred to a crucible and evaporated to dryness over a water bath until a consistent weight was attained. The flavonoids were measured in milligrams per gramme of dried plant material.

7- Statistical Analysis

The means of three analytical replications are used to calculate all analytic values. Analysis of variance (ANOVA) was used to determine significance by using SPSS software (version 18.0), where $P< 0.05$ is considered significant.

Results and discussion

The results showed that a suitable method to sterilize seeds achieved the highest percentage for survive (86 %) at10 % of sodium hypochlorite for (25 minutes) on total MS.

Callus induction

In order to induce callus formation from various *P. oleracea* explants, 2,4-D alone or in combination with BA in various concentrations was added to MS culture media, and the results were taken after one month of growth. Amongst the leaf, stem and root explants cultured for callus induction, stem segments explants achieved the largest production of calli from each treatment, followed by leaf explants, and finally, roots segments explants, which achieved the lowest callus production.

Several studies aimed to determine the effect of different explants on calls induction. Some studies have reported that the leaf explant is suitable for callus induction [21].

On the other hand, several studies reported that a balance between auxin and cytokinin hormone in the culture medium is an essential for callus induction, which act as synergistically to improve cell division as acritical process for callus induction [22] and [23].

With respect to the morphological characteristics of calli obtained, the results illustrated in Tables 3,4, 5, and 6 show that the percentage of calli induction, the vigor of growth ranged from weak to vigorous; the color differed from white, green, light green to brown; the texture was spongy or compact, and the surfaces of calli were smooth or nodular. It seemed that callus morphology was tissue and growth regulator treatment dependent. The different types of explants produced calli with different morphological characteristics in many cases. Also, the same growth regulator treatment

applied to various explants of *P. oleracea* resulted in the formation of calli differing in their morphology. The results indicated that each callus obtained was a direct result of the interaction of both the type of explant and the growth regulators applied. Calli from the three types of explants used can be seen in Figure (1).

Callus can be divided into two types: compact and friable [24]. Compact calli are typically rigid, with discrete structures that may be split into individual components, whereas friable calli are typically soft. The reasons responsible for the observed morphological changes in the calli are still unknown [25].

Table 3. Some morphological characteristics of calli obtained from leaves explants of *P. oleracea* in vitro

Table 3. Some morphological characteristics of calli obtained from leaves explants of *P. oleracea in vitro*
No callus (-), Doubt (±), Week growth (+), medium growth (++), vigorous growth (+++).

BA	2,4-D	Callus induction (%)	Growth	Color	Surface	texture
	0.0	-	-	-	-	-
	0.25	-	-	-	-	-
	0.50	100 %	+++	White	Nodular	Compact
0	**0.75**	50 %	++	White	Nodular	Compact
1	**0.0**	40 %	±	White	Smooth	Compact
	0.25	80 %	+++	White	Nodular	Spongy
	0.50	90 %	+++	White	Nodular	Spongy
	0.75	90 %	+++	White	Smooth	Compact
2	**0.0**	-	-	-	-	-
	0.25	80 %	++	White	Smooth	Compact
	0.50	100 %	++	White	Smooth	Compact
	0.75	100 %	+++	White	Smooth	Compact
3	**0.0**	-	-	-	-	-
	0.25	80 %	++	White	Smooth	Compact
	0.50	70 %	+	White	Smooth	Compact
	0.75	70 %	+	White	Smooth	Compact

Table 4. Some morphological characteristics of calli obtained from stem segments explants of *P. oleracea in vitro*

BA	2,4-D	Callus induction (%)	Growth	Color	Surface	texture
0	**0.0**	-	-	-	-	-
	0.25	90 %	+	Brown	Nodular	Spongy
	0.5	60 %	++	White	Nodular	Compact
	0.75	40 %	+	Brown	Smooth	Spongy
1	**0.0**	60 %	+	Green	Nodular	Compact
	0.25	100 %	+++	light green	Nodular	Spongy
	0.5	90 %	+++	White	Smooth	Compact
	0.75	70 %	+++	White	Nodular	Spongy
	0.0	40 %	++	Green	Nodular	Compact
2	**0.25**	60 %	++	light green	Nodular	Spongy
	0.5	70 %	+++	light green	Nodular	Spongy
	0.75	100 %	+++	light green	Nodular	Spongy
	0.0	50 %	+	White	Smooth	Compact
3	**0.25**	100 %	+++	White	Nodular	Spongy
	0.5	90 %	++	light green	Nodular	Compact
	0.75	90 %	++	White	Smooth	Compact

Table 5. Some morphological characteristics of calli obtained from root segments of *P. oleracea in vitro*

BA	2,4-D	Callus induction (%)	Growth	Color	Surface	texture
0	**0.0**	-	-	-	-	-
	0.25	-	-	-	-	-
	0.5	30 %	±	Brown	Smooth	Compact

	0.75	-	-	-	-	-
1	**0.0**	-	-	-	-	-
	0.25	80 %	+	White	Smooth	Compact
	0.5	-	-	-	-	-
	0.75	-	-	-	-	-
2	**0.0**	-	-	-	-	-
	0.25	30 %	+	White	Smooth	Spongy
	0.5	60 %	+++	Light green	Smooth	Spongy
	0.75	-	-	-	-	-
3	**0.0**	-	-	-	-	-
	0.25	50 %	+	White	Smooth	Compact
	0.5	-	-	-	-	-
	0.75	-	-	-	-	-

The results illustrated in Table 6 show, in general, that the amount of calli produced depended largely on both the type of explant and the concentration of the growth regulators added to the culture media. It is to be taken into consideration that 1 mg / l BA in combination with 0.25 mg / l 2,4D achieved the best treatment for callus production in three different explants. The maximum percentage of callus induction, callus fresh weight, and callus dry weight of fresh weight were recorded by stem segments as (100 %, 1.74 g, and 0.09 g), respectively, followed by the leaf (100 %, 0.86 g, and 0.07 g), respectively, then the root segments (80 %, 0.55 g and 0.03 g), respectively. The data obtained in the present study, showed the effect of growth regulators concentration in the production of the maximum amount of calli from each type of explant. However, this may be explained on the bases of the variation on the nature of the cells and tissues used for induction of calli and the interaction between the exogenous and endogenous concentrations of growth substances, which may be characteristic for each type of explant.

The previous studies on *P. oleracea* showed the best medium and explant for callus induction.[26], reported the combination of 10 M Indole 3-butyric acid (IBA) and 10 or 5 M BAP was found to be effective for callus induction from leaves. The medium containing 1 mg / l BAP and 1 mg / l 2, 4-D hormones produced more callus from hairy roots.[27] illustrated that 3.0 mg / l 2, 4-D combined with 0.5 mg / l kin achieved the highest percentage callus induction, callus fresh weight, and callus dry weight from leaves explant [28]. The best callus initiation achieved by nodal segments on MS medium containing 2.0 mg / l BA and 2.0 mg / l NAA [29]. The combination of 2 mg / l BAP and 0.5 mg / l 2,4-D gave the best result for percentage callus induction, fresh weight, and dry weight by using leaves explant. However, 1 mg / l BAP and 0.5 mg / l 2,4-D were recorded the best by shoot tip explants [30]. Stem explants achieved the highest callus percentage, fresh and dry weight of calluses on a medium containing 0.5 mg / l BAP and0.5 mg / l NAA [31].

Results of the phytochemical analysis of the present study have shown that Total alkaloids were found in amounts ranging from 10 mg / g and 60 mg / g plant dry wt. in general. It seems that calli obtained from leaves accumulated the maximum amount of total alkaloids (60 mg / g dry wt.), more than stem segments (50 mg / g dry wt.) and root segments (30 mg / g dry wt.). This study agrees with [32] who showed that leaf discs produced more hypericin alkaloid in vitro than stem segments. With respect to total flavonoids, it ranged between 90 mg / g and 220 mg / g dry weight in general. The least amount was recorded in the mother plant tissues, in contrast, the amount obtained from the three types of calli achieved the highest amount of total flavonoids. Root segments and stem segments contained nearly double the amount of total flavonoids, that were accumulated in the mother plant tissues (Figure 3). [33] reported that to enhance the production of flavonoids, callus culture is one of the possibilities of novel biotechnological technologies for ensuring more homogeneous and stable flavonoid production year-round under controlled environmental conditions.

¬In the present study, the data recorded show that the leaf and root of the mother plant achieved the highest amounts of total tannins (90 and 80 mg / g dry wt.), respectively. On the other hand, calli obtained from stem segments contained nearly threefold the amount of total tannin that was accumulated in the stem of mother plant tissues (Figure 2).

Table 6. Effect of type of explant and various growth regulator treatments on fresh and dry weights of calli induced from various explants of *P. oleracea* after six weeks

Treatment (mg/l)		Origin of callus (Type of explant)					
		Leaf		Stem		Root	
BA	2,4-D	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
0	0.0	0.00	0.00	0.00	0.00	0	0
	0.25	0.00	0.00	0.08	0.01	0	0
	0. 5	*0.86	0.7*	1.05*	0.06*	0	0
	0.75	0.32	0.02	1.07*	0.07*	0	0
	0.0	0.02	0.01	0.25	0.01	0	0
1	0.25	0.28	0.02	1. 74*	0.09*	*0.55	*0.03
	0. 5	*0.50	*0.03	0.28	0.01	0	0
	0.75	*0.50	*0.03	0.22	*0.05	0	0
2	0.0	0.00	0.00	0.05	0.01	0	0
	0.25	0.33	0.01	0.32	*0.03	0.17	*0.02
	0. 5	0.37	0.02*	0.46	*0.03	0.23	*0.02
	0.75	0.130*	0.10*	0.130*	0.010*	0.443*	0.040*
	0.0	0.00	0.00	0.00	0.00	0.00	0.00
3	0.25	0.153*	.0117*	0.130*	0.017*	0.333*	0.023*
	0.5	0.320*	0.030*	0.157*	0.017*	0.143*	0.010*
	0.75	0.205*	.020*	0.217*	0.020*	0.733 *	0.07*

Each value is a mean of three determinations. * mean significant value. where $P < 0.05$ is considered significant.

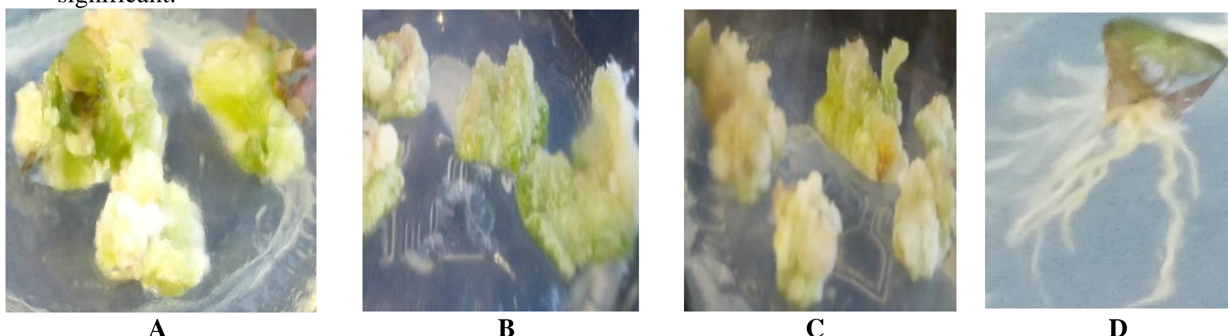

Figure 1. Callus formation from stem segments (A), (B) leaves and root segments (C) of *P. oleracea* (D) response leaves by formation roots on MS free.

Phytochemical analysis of mother plant and calli

In general, that the type of explant has its own impact on the biosynthesis or accumulation of various classes of secondary metabolites, probably due to the biological, anatomical, and biochemical nature of the cells from which the culture was initiated.

The results obtained in this study may partially (at least) agree with the earlier results, which indicated the enhanced synthesis of different classes of plant secondary metabolites via callus [25] and [34].

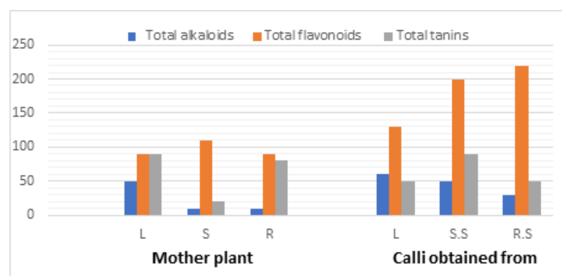

Figure 2. Amount of some total secondary metabolites (mg / g plant dry wt) of mother plant and calli obtained from various explants of *P. oleracea in vitro.* L= Leaf, S= stem, L= Leaf, S.S = Stem segments, R.S = Root segments.

Biotic and abiotic elicitation of calli

(1) Effect on calli morphology

Tables 7, 8 and 9 and Figure 3 show the influence of biotic elicitation (the addition of autoclaved fungal filtrates to the callus inducing culture medium) and abiotic elicitation (the addition of mannitol to the callus inducing media) on callus morphology (vigour of growth, colour, and texture). The results obtained showed that fungal filtrate reduced the vigor of growth of *P. oleracea* callus by 2 % while, mannitol treatments of 50 and 100 mM also reduced the callus growth. When fungal filtrate and mannitol were combined, callus formation was reduced from moderate to mild. except for 50 mM and 2 % fungal filtrate interaction, which gave the vigor growth as compared to all treatments.

Results, in the current study, revealed that the color of the callus and its uniformity varied from uniform to patch according to treatment, but it is difficult to connect the color of the callus to a particular treatment. However, the color varied from green to greenish and from yellow to creamy to brownish.

All the calli obtained had either a smooth or nodular surface, and all the calli obtained had a compact texture. It was obvious that mannitol produced a smooth callus and a compact resembling the control one, and fungal filtrate resulted in nodular callus and a compact, but Mannitol-fungal filtrate interaction, in general, the development of compact and smooth calli. The observed alterations in calli development, colour, and texture could have been caused by elicitors altering the metabolism of the cultured cells. Many secondary metabolites are pigmented compounds. according to [35].

2-Effect on callus growth

Data in Table 10 showed that the fresh weight of the callus increased from 1.14 gram in control to 1.63 gram in treated calli with 2 % fungal filtrate interaction with 50 mM mannitol, but 2 % of fungal filtrate alone affected the growth of the callus, where the fresh weight of the callus decreased to (1.0 gram, while 150 mM mannitol with 1 % fungal filtrate showed the minimum of fresh weight (0.620 gram).

Table 11 shows that the calli that revealed the highest fresh weight (1.63 g) also revealed the highest dry weight (0.15 g). The treatment with 150 mM mannitol and 1 % fungal filtrate yielded the lowest dry weight (0.05 g).

This agrees with studies [36] and [35] that proved that the stress of osmotic formed by low mannitol concentration improved callus growth and the results disagree with the results that were recorded by [37] found that when compared to control, fungal biotic elicitation had a detrimental influence on both callus fresh and dry weights. The reported results could be due to overexpression of particular genes, which has activated the expression of several downstream genes, resulting in better stress tolerance in plants [38].

Table 7. Effect of biotic and abiotic elicitation on vigor of growth of *P. oleracea* calli

Mannitol	Fungal filtrate			
	0.0 %	1 %	1.5 %	2 %
0.0 mM	++	++	++	+
50 mM	+	++	+	+++
100 mM	++	+	++	+
150 mM	+	+	+	++

+(Mild growth), ++ (Medium growth), +++ (Vigorous growth)

Table 8. Effect of biotic and abiotic elicitation on color of *P. oleracea* calli

	Fungal filtrate			
Mannitol	**0.0 %**	**1 %**	**1.5 %**	**2 %**
0.0 mM	G/U	Gsh / P	Gsh / P	Gsh / P
50 mM	Y/U	Cr / U	Cr / U	Y/U
100 mM	Cr/U	Cr / P	Cr / U	Cr / P
150 mM	Cr/U	Gsh/p	Cr/ U	Bsh / p

Colors: G(Green), Gsh(Greenish), Y (Yellow), Bsh(Brownish), Cr(Creamy), U(Uniform), P(Patchy).

Table 9. Effect of biotic and abiotic elicitation on texture and surface of calli of *P. oleracea*

Mannitol	Fungal filtrate			
	0.0 %	**1 %**	**1.5 %**	**2 %**
0.0 mM	C/Sm	C/Nr	C/Nr	C/Nr
50 mM	C/Sm	C/ Sm	C/ Sm	C/Sm
100 mM	C/Sm	C/ Sm	C/Sm	C/ Sm
150 Mm	C/Sm	C/Nr	C/Nr	C/Nr

Texture: C (Compact) and Surface: Nr (Nodular), Sm (Smooth).

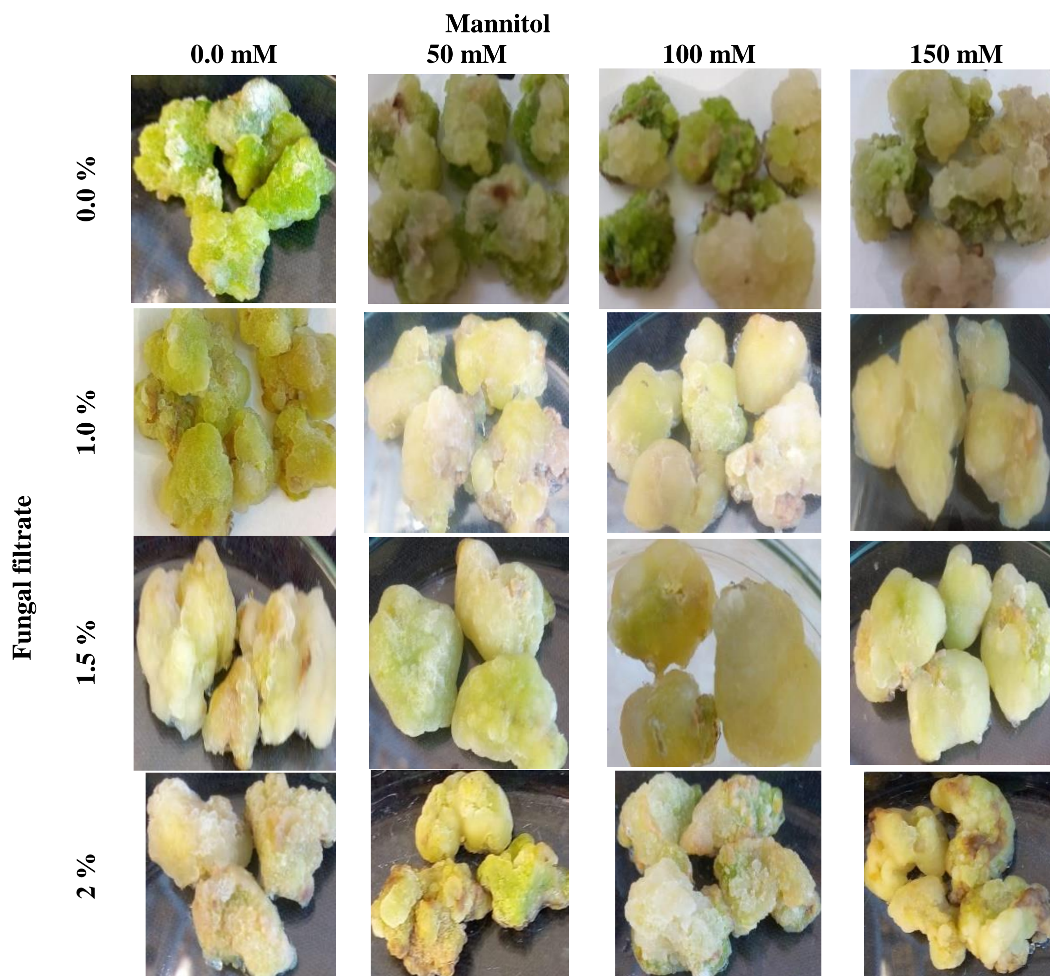

Figure 3. Calli of *P. oleracea* produced under biotic and abiotic elicitation.

Table 10. Effect of biotic and abiotic elicitation on fresh weight (g) of calli of *P. oleracea* after 6 weeks of cultured

Mannitol	Fungal filtrate			
	0.0 %	1.0 %	1.5 %	2.0 %
0.0 mM	1.07	1.225	1.33	1.0
50 mM	.950*	1.44	.87*	1.63*
100 mM	1.26	.817*	1.12	.882
150 mM	1.0	.620*	.820*	1.11

Each value is a mean of three determinations. * Mean significant value. where $P < 0.05$ is considered significant.

Table 11. Effect of biotic and abiotic elicitation on dry weight (g) of callus of *P. oleracea*

Mannitol	Fungal filtrate			
	0.0 %	1.0 %	1.5 %	2.0 %
0.0 mM	.09	.10	.12	.09
50 mM	.08*	.13	.07*	.15*
100 mM	.10	.07*	.09	.07
150 mM	.09	.05*	.07*	0.09

Each value is a mean of three determinations. * Mean significant value. where $P < 0.05$ is considered significant.

Effect of elicitation on some total active secondary metabolites.

In terms of alkaloids, the results in Figure 4 reveal that the amount of alkaloids in calli ranged from 100 to 280 mg / g callus dry wt. In response to 1 percent, 1.5 percent, and 2 percent, respectively, treatment with autoclaved fungal filtrate increased alkaloid content from 140 mg in control calli to 240, 200, and 180 mg / g callus dry wt. The higher concentrations of fungal filtrate suppressed alkaloid biosynthesis or accumulation. The amount of alkaloids found in calli treated with 100 mM mannitol, which is regarded appropriate for alkaloid biosynthesis, was 240 mg / g callus dry wt., but higher concentrations hindered alkaloid biosynthesis or accumulation. Treatment with 1 percent autoclaved fungal filtrate with 100 mM mannitol resulted in the maximum accumulation of alkaloids (280 mg / g callus dry wt.), a value that means the treatment increased the amount of alkaloids detected in control calli by twofold. It's likely that the combination of the two eliciting factors at these concentrations was optimal, resulting in some form of synergism that activated alkaloids production [35]. Treatments with 0.1 percent fungal filtrate combined with 150 mM mannitol and 1.5 percent fungal filtrate combined with 100 mM mannitol, on the other hand, were harsh enough to reduce alkaloids to 100 mg / g callus dry wt. In general, determining the optimal mix and concentration of elicitors may have a good impact on the biosynthesis of alkaloids by *P. oleracea* calli in vitro. Although the treatments used had good and negative impacts on alkaloids in some cases, they had no effect in others (Figure 4). The amounts of flavonoid were shown in Figure 5 ranged from 160 mg / g to the highest value of 340 mg/g callus dry wt. in a treated calli with 150 mM mannitol+1 % fungal filtrate. It is mentioned that all the treatments had markedly positive effects, i.e., amounts of flavonoid higher than the corresponding control, except for 1 % fungal filtrate alone and 1.5 % combination with 50 mM mannitol treatments. In Figure 6, the amount of tannins increased from 50 mg / g in control calli to a high of 180 mg / g, in calli treated with 1.5 % fungal filtrate alone. The results showed that all of the treatments produced significantly more tannins than the comparable control. Both biotic and abiotic therapies, alone or in combination, had a favorable effect on secondary metabolism and the buildup of alkaloids, flavonoids, and tannins.

Often, the use of elicitors aids in the release of the metabolite into the medium, which serves as an additional biotechnological advantage [39]. The results of this study suggested that biotic elicitation may boost secondary metabolite biosynthesis in callus cultures, which may agree with prior observations reported in the same plant study and other plant investigations by [40],[41], [42], [43], [44], [45], [46] and [35].

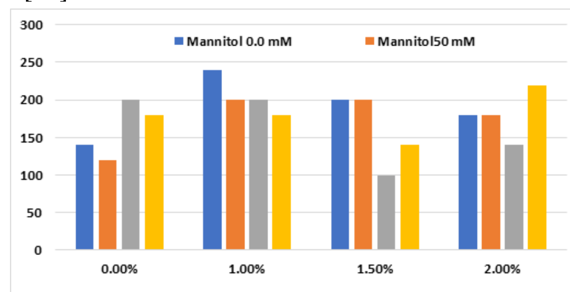

Figure 4. Effect of biotic and abiotic elicitation on callus contents of total alkaloids (mg / g callus dry wt.) of *P. oleracea*.

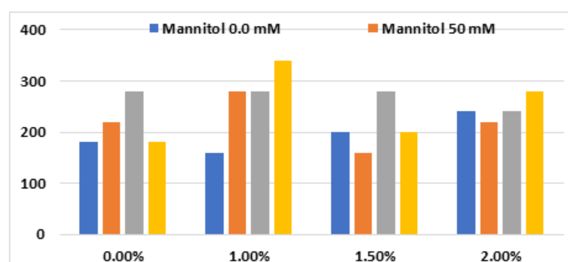

Figure 5. Effect of biotic and abiotic elicitation on total flavonoid (mg / g callus dry wt.) of calli originally from stem segments of *P. oleracea*.

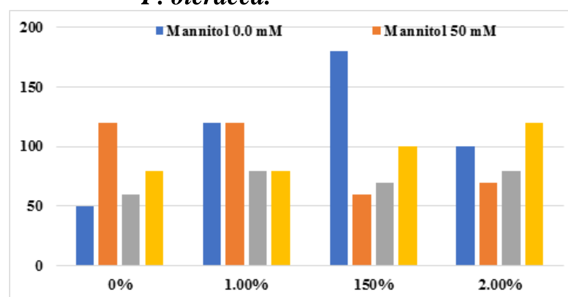

Figure 6. Effect of biotic and abiotic elicitation on callus contents of total tannins mg / g callus dry wt.) of *P. oleracea*.

Conclusion

It is to be taken into consideration that 1 mg / l combination with 0.25 mg / l achieved the best treatment for callus production in three different explants. The maximum % callus induction, callus

fresh weight, and callus dry weight of fresh weight were recorded by stem segments as (100 %, 1.74 g, and.09 g), respectively, followed by the leaf (100 % ,0.86 g, and 0.07 g), respectively, then the root segments (80 %, 0.55 g and 0.03 g), respectively .The obtained results showed that the best calli induced from the stem explant of *P. oleracea* on MS medium with Benzyl Adenine (BA) 1 mg / L and 2,4-dichlorophenoxyacetic acid (2,4- D) 0.25 mg / L. On the other hand, the alkaloids in dry calli powder were raised from 140 mg/g to 280 mg/g after treatment with 1 % fungal filtrate and 100 mM mannitol. Flavonoids increased from 180 mg / g in dry calli powder to 340 mg / g in calli treated with 1 % fungal filtrate containing 150 mM mannitol. Tannin levels increased from 50 mg / g in dry calli powder to 180 mg / g in calli treated only with 1.5 percent fungal filtrate. Elicitation studies show promise in improving secondary metabolite production in *P. oleracea* .

References

1. Singh, K.P., Effect of temperature and light on seed germination of two ecotypes of Portulaca oleracea L. New Phytologist 72,289–295 (1973).
2. Uddin, M. K., Juraimi, A. S., Hossain, Md.S., Un Nahar, M. A., Ali,M. E and Rahman, M. M., Purslane weed (Portulaca oleracea): A prospective plant source of nutrition, Omega-3 Fatty Acid, and antioxidant attributes. Scientific World Journal, (2014) http://dx.doi.org/10.1155/2014/951019.
3. Zheng, G, Y., Qu, L. P., Yue, X. Q., Gu, W., Zhang, H and Xin, H. L., Portulaca erebroside A induces apoptosis via activation of the mitochondrial death pathway in human liver cancer HCCLM3 cells," Phytochemistry Letters, 7 (1),77–84(2014).
4. Tabatabaei, F. S. R., Rashno, M., Ghaderi, S and Askaripour, M., The Aqueous Extract of Portulaca Oleracea Ameliorates Neurobehavioral Dysfunction and Hyperglycemia Related to Streptozotocin-Diabetes Induced in Ovariectomized Rats. Iranian journal of pharmaceutical research ,15(2),561-71 (2016).
5. Abd El-Aziz, H. A., Sobhy, M. H., Ahmed, K. A., Abd El-Hameed. A. K., Rahman Z.A and Hassan, W.A., Chemical and remedial effects of purslane (portulaca oleracea) plant. Life Science Journal,11(6) (2014).
6. Zhou, Y. X., Xin, H. L., Rahman, K., Wang, S, J., Peng, C and Zhang, H., Review Article Portulaca oleracea L.: A Review of Phytochemistry and Pharmacological effects. BioMed Research International, Article ID 925631, 11 pages (2015).
7. Chugh, V., Mishra, V., Dwivedi, S. Vand Sharma, K. D., Purslane (Portulaca oleracea L.): An underutilized wonder plant with potential pharmacological value. The Pharma Innovation Pharmaceutical Journal,8(6), 236-246(2019).
8. Mohammad, R, K., Akbar, A and Majid, K., Anti-Asthmatic Effects of Portulaca Oleracea and its Constituents, a Review. Journal of Pharmacopuncture,22(3),122-130(2019).
9. Sadidi, M., Bakhtiyari, M and Alirezaei, A., Effects of the Portulaca oleracea Extract on Gentamicin-Induced Nephrotoxicity in Male Rats. Iran Red Crescent Medical Journal ,21(2), e83785(2019).
10. Srivastava, R., Srivastava, V. and Singh, A., Multipurpose Benefits of an Underexplored Species Purslane (Portulaca oleracea L.): A Critical Review. Environmental Management,(2021). https://doi.org/10.1007/s00267-021-01456-z.
11. Al-Otibi, F., Alfuzan, S. A., Alharbi, R. I., Al-Askar, A. A., AL-Otaibi, R. M., Al Subaie, H. F and Moubayed, N. M., Comparative Study of Antifungal Activity of Two Preparations of Green Silver Nanoparticles from Portulaca oleracea Extract. Saudi Journal of Biological Science, (2022). https://doi.org/10.1016/j.sjbs.2021.12.056
12. Skoog, F and Miller, C.O., Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Society Experimental Biology,54,118–130(1957).
13. Coenen, C. and Lomax, T., Auxin-cytokinin interactions in higher plants. Old problems and new tools. Trends in Plant Scince,2(9), 351-356(1997).
14. Loredo-Carrilo, S. E., Santos-Diaz, M. L., Lyeva, E. and Santos-Diaz, M. S., Establishment of callus from Pyrostegia venusta (Ker Gawl. Miers) and effect of abiotic stress on flavonoids and sterols accumulation. Journal of Plant. Biochemistry Biotechnology, 22 (3), 312-318(2013).
15. Radman, R., Saez, T., Bucke, C. and Keshavarz, T., Elicitation of plant and microbial systems. Biotechnology Applied Biochemistry, 37,91-102(2003).

16. Angelova, Z., Georgiev, S and Roos, W., Elicitation of Plants. Biotechnology. & Biotechnological Equipment, 20(2), 72-83(2006).
17. Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant, 15, 473-497(1962).
18. Harborne, J. B., Phytochemical Methods. Aguide to modern techniques of plant analysis. Chapman and Hall, London(1984).
19. Ali, A. A., Ross, S. A., Mesbah, M. K., and El-Moghazy, S. A., Phytochemical study of Limonium axillare (Forssk.). Bulletin Faculty Pharmacy Cairo University, 29, 59-62(1991).
20. Bohm, B. A and Kocipai-Abyazan, R., Flavonoids and condensed tannins from leaves of Hawailan Vaccinium vaticulatum and V. calycinium. Pacific Science,48, 458-463(1974).
21. Dhar, U. and Joshi,M.,Efficient plant regeneration protocol through callus for Saussurea obvallata (DC .) Edgew . (Asteraceae): effect of explant type, age and plant growth regulators. Plant Cell Reports, 24, 195–200 (2005).
22. Roy, J and Banerjee, N., Induction of callus and plant regeneration from shoot-tip explants of Dendrobium fimbriatum Lindl. var. oculatum Hk. f. Biology Scientia Horticulture, 97(3–4),333–340(2003).
23. Johri, M.M and Mitra, D., Action of plant hormones. Current Scince,80(2), (2001).
24. Bhatia,S., Sharma, K., Dahiya ,R and Bera ,T., Modern applications of plant biotechnology in pharmaceutical sciences. Academic Press, London(2015).
25. Bodede, O., Shaik, S. and Moodley, R., Evaluation of bioactive flavanols and ent-kaurenes in the 2, 4-dichlorophenoxyacetic acid-induced calli of Senegalia nigrescens using FTIR and GC–MS. Journal of Plant Biochemistry and Biotechnology, 1-8(2021).
26. Safdari, Y and Kazemitabar, S. K., Plant tissue culture study on two different races of purslane (Portulaca oleracea L.). African Journal of Biotechnology, 8 (21), 5906-5912(2009).
27. Pirian, K. H., Callus induction in hairy root of Portulaca oleracea. Iranian Journal Medicinal and Aromatic Plant, 30(64),231-238(2014).
28. Oraibi,V. A. G, A., AlShammari, A. A., Mohsien, R, A and Obaid, W.J., Investigation the Antibacterial Activity of Portulaca oleracea L. Tissue Cultures in vitro. Journal of Pharmaceutical Research International,18(5), 1-7(2017).
29. Al-Bakr, R. A., Propagation of Portulaca oleracea L. and level of Active Compounds in Callus Culture. International Journal of Enhanced Research in Science, Technology & Engineering, 7 (2).33-43(2018).
30. Heidargholinezhad, F., Moradi, H., Karimi, M and Akbarpour, V., Callus induction in Portulaca oleracea L. by different hormone concentrations and explant types. Nova Biologica Reperta 6(2): 176-183(2019).
31. Saffaryazdi, A., A. Ganjeali, A., Farhoosh, R and Cheniany, M., Culture optimization for In Vitro callogenesis in Purslane (Portulaca oleracea) and effect of yeast extract on antioxidant compounds. Journal of Horticultural Science, 34(1): 107-118. (2020).
32. Ayan, A. K., Çirak, C., Kevseroglu, K and Sökmen, A., Effects of explant types and different concentrations of sucrose and phytoharmones on plant regeneration and hypericin content in Hypericum perforatum L. Turkish journal. of agriculture and forestry, 29(3), 197-204(2005).
33. Bharati, A. J and Bansal, Y. K., In vitro production of flavonoids: a review. World Journal of Pharmacy and Pharmaceutical Sciences, 3(6), 508-533(2014).
34. Vignesh, A., Selvakumar, S. and Vasanth, K., Comparative LC-MS analysis of bioactive compounds, antioxidants and antibacterial activity from leaf and callus extracts of Saraca asoca. Phytomedicine Plus Journal, 2(1), 100167(2022).
35. Ebad,F.A., Hussein,E.A and Hussein ,N.A., Impact of biotic and abiotic elicitation on morphology, growth, active constituents and antibacterial activity of Solanum nigrum(L.)calli induced in vitro. Egyptian Journal Desert Research, 67 (1): 47-63(2017).
36. Hussein, E. A. and Aqlan, E. M., Callus culture of Flaveria trinervea, growth curve, phytochemical screening and antibacterial activity. Egyptian Journal. Biotechnology, 26, 76-91(2007).
37. El-Nabarawya, M. A., El-Kafafia, S. H. and M. A. Hamzab, M.A., The effect of some factors on stimulating the growth and production of active substances in Zingiber officinale callus cultures. Annals Agriculture Science, 60 (1), 1-9(2015).
38. Peleg, Z., Apse, M. P. and Blumwald, E., Engineering salinity and water-stress tolerance in

crop plants. Getting closer to the field. Advanced Botanical Research, 57, 407 - 432(2011).

39. Kaur, G., Prakash, P., Srivastava, R., and Verma, P. C., Enhanced Secondary Metabolite Production in Hairy Root Cultures Through Biotic and Abiotic Elicitors. Plant Cell and Tissue Differentiation and Secondary Metabolites: Fundamentals and Applications, 625-660(2021).
40. Karwasara, V., Tomar, P. and Dixit, V., Influence of fungal elicitation on glycyrrhizin production in transformed cell cultures of Abrus precatorius Linn. Pharmacognosy Magazine, 7(28): 307–313(2011).
41. Swaroopa, G., Anuradha, M. and Pullaiah, T., Elicitation of forskolin in suspension cultures of Coleus forskohlii (wild.) Briq. using elicitors of fungal origin. Curr. Trends Biotechnology Pharmacy, 7, 755–762(2013).
42. Baldi, A., Srivastava, A. K., Bisaria, V. S., Fungal elicitors for enhanced production of secondary metabolites in plant cell suspension cultures. In symbiotic fungi, soil biology. Varma, A., Kharkwal, A.C., Eds.; Springer-Verlag: Berlin/Heidelberg, Germany, 18, 373–380(2009).
43. Mendhulkar, V. D. and Vakil, M. M. A., Chitosan and Aspergillus Niger mediated elicitation of total flavonoids in suspension culture of Andrographis paniculata (Burm. f.). Nees. International Journal of Pharma and Bio Sciences, 4, 731–740(2013).
44. Tahsili, J., Sharifi, M., Safaie, N., Esmaeilzadeh-Bahabadi, S. and Behmanesh, M., Induction of lignans and phenolic compounds in cell culture of Linum album by culture filtrate of Fusarium graminearum. Journal Plant Interact., 9, 412–417(2013).
45. Gadzovska, S. S., Tusevski, O., Maury, S., Hano, C., Delaunay, A., Chabbert, B. M, Lamblin, F., Laine, E., Joseph, C. and Hagege, D., Fungal elicitor-mediated enhancement in phenylpropanoid and naphtodianthrone contents of Hypericum perforatum L. cell cultures. Plant Cell Tissue Organ Culture, 122,213–226(2015).
46. Ebrahimi, M. A. and Zarinpanjeh, N., Bio-elicitation of β-carboline alkaloids in cell suspension culture of Peganum harmala L. Journal Medicinal Plants, 14 (55), 43-57(2015).