Physical elicitation of *Rosmarinus officinalis* callus culture for production of antioxidants activity

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Abstract

In the present study, the free radical scavenging activity of calli of *Rosmarinus officinalis* in response to the treatment with physical eliciting factors was determined. The leaves of *Rosmarinus officinalis* were surface sterilized using Clorox. Calli were initiated from leaf explants using BA (1.0 and/or 2.0mg/l) and NAA (1.0, 2.0 and 4.0 mg/l) and 2,4-D (1 and /or 2.0 mg/l), alone or combined on MS culture media. Cultures were subcultured for 3 successive times (interval of 4 weeks). Calli which showed the best callus dry biomass were subjected to physical elicitation treatments (including microwave radiation powers of 100 and 200 watt for 30 and 60 seconds and five light qualities white, red, green, yellow and blue of 3000 lux intensities). The free radical scavenging activity of the treated calli was determined by the DPPH assay of antioxidant activity.

Results of the present study have revealed that using Clorox at 10% (v/v) for 15 min recorded the highest decontamination percentage (80%). impregnating the culture medium with NAA at 2.0 mg/L plus BAP at 1.0 mg/L had resulted in the maximum callus biomass (5.537 g/callus and 0.278 g/callus for fresh and dry weights respectively) at third subcultures which can be (statistically) considered significant over most of the other treatments tested. On the other hand, the least significant fresh and dry weights (1.295 g/callus and 0.019 g/callus respectively) were recorded in calli treated with MS hormone-free medium and MS medium supplemented with NAA at 1.5 mg/L plus BAP at 1.0 mg/L respectively.

It seems that both microwave and light quality treatments had significantly affected the fresh weight, dry weight of *R. officinalis* calli. The results obtained showed that the highest fresh and dry weights (2.49 g and 0.177 g respectively) were recorded in calli treated with 200 watts for 60 sec. while the lowest fresh and dry weights (1.617 g and 0.11 g respectively) were detected when callus culture were incubated under yellow or red lights respectively. There were some significant differences in response to many of the elicitation treatments with respect to antioxidant activity. For example, the highest significant antioxidant activity (314.326%) was detected when callus cultures were incubated under green light, whereas the lowest one (98.512%) was recorded when callus cultures were incubated at 3000 Lux. The results obtained indicate the possibility of improving callus growth and antioxidant activity using physical elicitation.

Keywords: *Rosmarinus officinalis,* callus culture, physical elicitation and antioxidants activity.

1. Introduction

Rosemary (*Rosmarinus officinalis* L., family Labiatae), a plant native to Mediterranean countries, is an evergreen shrub **(Browse, 1986).** Rosemary is also classified as a medicinal plant **(Hayashi *et al.,* 1987)**. The aromatic oil is used widely in the perfume industry. Rosemary is also used in food processing as a preservative **(Narasimha and Nigam, 1970).** *R. officinalis* usually has three basic function as raw materials. Firstly, it is used as spice products in food and beverage, secondly, the essential oil can be added into cosmetic production, lastly, in terms of numerous antioxidants, the plant or the essential oil is the materials of gastrointestinal ailments, which has great effect in various spasmodic conditions such as renal and biliary colic. So far, numerous pharmacological studies have suggested *Rosmarinus officinalis* L. may have a high therapeutic potential in inflammatory bowel diseases **(Minaiyan et al., 2011).**

Plant cells and callus cultures have been extensively used to explore the possibility of producing useful secondary metabolites through biotechnology methods **(Tabata and Fujita, 1985; Youssef, 1996; Mittler, 2002; Mohamed, 2005 and Chen and Chen, 2013)**. Numbers of research mentioned that light had induction of specific enzymes activity which are concerned with the formation of some secondary products **(George *et al.,* 2008; Ramawat and Mathur, 2007; Mahajan and Tuteja, 2005).** The influence of light quality on the formation of primary and secondary metabolites has been reported in *Cistanche deserticola* **(Jie *et al.,* 2003)** and *Perilla* plants

(Nishimura *et al.,* 2009). There has also been research into light quality induction of polysaccharide content in *Gelidium sesquipedale* **(Torres *et al*., 1995; Carmona *et al*., 1998)** and *Porphyridium cruentum* **(You and Barnett, 2004).** Also, significantly increase of catalase activity under red light contributed to scavenging of hydrogen peroxide generated by *Botrytis cinerea* infection **(Islam *et al*., 2011)**. **Guo *et al*., (2007)** indicating the stimulatory effect of blue light on the accumulation of plant phenolics *in vitro* cultures, with examples including flavonoids in *Saussurea medusa* calli and phenolic acids in shoots of *Ruta graveolens* **(Szopa *et al*., 2012).**

The objectives of this study were to investigate callus initiation, callus production and determination of anti-oxidants from *Rosmarinus officinalis* callus cultures affected by physical elicitors.

2. Material and Methods

This study was conducted in Tissue Culture Res. Lab., botany and microbiology department, faculty of science, Al-Azhar University, Cairo, Egypt, during the years from 2015 to 2017.

2.1. Plant materials

The mother plant of *Rosmarinus officinalis* was founded in plants Garden of botany and microbiology department, faculty of science, Al-Azhar University, Cairo, Egypt. The mother plant was used as a source of plant materials (leaves explants).

2.2. Culture medium and incubation conditions

The basal salts mixture of MS medium (Murashige and Skoog, 1962) supplemented with 25 g/L sucrose and pH adjusted to 5.7±1 was solidified with 7 g/L agar (Sigma). All the culture treatments were incubated in growth room under controlled conditions, where temperature was maintained at 24 ± 1°C day/night schedules and illumination intensity of 1500 lux using white cool fluorescent lamp (120 cm long 40 watts), the figure period was 16/8 controlled by electronic timer.

2.3. Callus initiation and growth

2.3.1. Effect of sodium hypochlorite (Clorox) and mercuric chloride on disinfection and survival percentages of *Rosmarinus officinalis* L.

Leaf disc explants (about 1 cm2) were initially subjected to hygienic soapy (Septol) water solution for 30 min, then to Dettol solution (3 %) for 40 min and rinsed with running tap water for one hour. Under aseptic condition in safety cabinet, leaf disc explants were immersed in ethanol (70 %) for one min. thereafter; they were surface disinfected with one of the disinfectant substances of Clorox (Sodium hypochlorite 5.25 %) or mercuric chloride ($HgCl_2$) as follow:

- Clorox at 10 % for 10 or 15 min.
- Mercuric chloride at 0.05 and 0.1 % for 7 min each.

All of the above used disinfectant solutions were provided with a few drops of tween-20 (polyxyethelen sorbite monolaurate) as an emulsifier (wetting agent). After the disinfection treatments, the explants rinsed in sterilized distilled water for three times to remove all traces of disinfectant substances, then they explanted vertically (one explants/tube) for four weeks in autoclaved media for initiating callus culture. At the end of the incubation period (four weeks) for each disinfection treatment, the following measurements were recorded:

- Disinfection percentage (%).
- Survival percentage (%).

Each treatment contained five replicates and 10 explants for each replicate.

2.3.2. Initiation of callus from leaf explants

Leaf cuttings of *Rosmarinus officinalis* were used as explants. The explants were cultured on MS medium supplemented with:

1- Control (hormone-free)
2- 2,4-D or NAA at 2.0 or 4.0 mg/L separately
3- 2,4-D or NAA at 2.0 or 4.0 mg/L + 1.0 mg/L BAP
4- 2,4-D or NAA at 2.0 or 4.0 mg/L + 2.0 mg/L BAP

All cultures were examined after 5 weeks of incubation at 25±2 ºC under 16 hrs. light and 8.0 hrs. Dark provided by cool florescent light intensity of 1500 lux.

2.3.3. *In vitro* callus growth

Calli initiated from leaf explants of *Rosmarinus officinalis* were divided into 1 gm fresh biomass parts and cultured on the surface of MS medium supplemented with combinations of an auxin and a cytokinin as the following:

1- Control (hormone-free)
2- NAA at 1.5, 2.0 or 4.0 mg/L + 1.0 mg/L BAP
3- NAA at 1.0 mg/L + 2.0 mg/L BAP

The formed fresh callus tissues resulting from each one of the above tested plant growth regulators treatments were aseptically removed, divided to one gram weight and re-cultured into fresh culture media of the corresponding components. This procedure was prolonged for 3 subcultures from (5-6) weeks interval. Each treatment consisted of ten replicates.

2.4. Physical elicitation treatments of *Rosmarinus officinalis* callus cultures

In this stage, calli produced from *Rosmarinus officinalis* cultures divided into 1 gm fresh biomass parts and cultured on the surface of MS medium supplemented the growth regulators formulation which resulted in the maximum callus fresh biomass (2.0 mg/L NAA + 1.0 mg/L BAP).

As regard microwave irradiation callus cultures were microwaved with 100 or 200 watts for 30 or 60 sec. The used microwave apparatus is a single-phase grounding 1.3 kw out-put 650 w at a frequency of 2450 MHz (Model: WP 100 AP 30-2).

As regard Light quality callus cultures were incubated under different types of light including:1500 Lux. white light, red light, green light, yellow light and red light.

At the end of elicitation treatments, callus fresh and dry weight were recorded and the dried callus were subjected to chemical investigations.

2.5. Chemical analysis

2.5.1. Extraction procedures

Air dried powdered samples (0.5 g, each) of the *in vitro* grown were separately mixed with 70 % methanol (3 X 10 ml, each) and shacked for 24h. Methanolic extract of each sample was filtered, evaporated under reduced pressure and the residue was used for determination.

2.5.2. Free radical scavenging activity (DPPH –RAS method)

The free radical scavenging activity of methanol extracts was measured by α, α-diphenyl-β- picryl-hydrazyl (DPPH) using the method described by **Brand-Williams et al. (1995).** The 0.1 mmol/l solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of extracts solution at concentration of 0.1 to 1.5 g/100ml. After 30 min absorbance was measured at 517 nm using spectrophotometer. Radical scavenging activity was expressed as inhibition percentage and was calculated using the formula:

$$\text{Radical scavenging Activity } \% = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \text{X } 100$$

3. Results and Discussion

3.1. Effect of mercuric chloride and Clorox on decontamination and survival percentage of leaf disc explants of *Rosmarinus officinalis*

As shown in table (1), the highest contamination percentage (70 %) was recorded when $HgCl_2$ was applied for 7 min, while the lowest significant contamination percentage (20 %) was detected when Clorox was used at 10 % for 15 min. The data revealed also that the contamination percentage was reduced with increasing duration of sterilization from 10 to 15 min. As for survival percentage, data indicated that the highest survival percentage (80 %) was recorded when Clorox was applied at 10 % for 15 min. while, the lowest survival percentage (30 %) was detected when mercuric chloride was used at 0.1 % for 10 min. Data in table (1) disclosed that the survival of explants was increased from 65 to 80 % when the time of application with 10 % Clorox was increased from 10 to 15 min.

As above-mentioned data, the variability's in the decontamination rates of *Rosmarinus officinalis* leaf disc explants as influenced by the various tested sterilization treatments under trials are corresponding with those earlier reviewed by numerous researches. Relating to the effect of the types of disinfectant substances, **Awad (1993)** remarked that subjecting the shoot tip explants of *Psidium guajava* to 0.5 % of NaOCl yielded the highest contamination percentage,

Referring to the effect of sterilization duration, **El-Sayed (2005)** reported that exposing the *Sequoia sempervirens* explants to Clorox at 30% for a longest period of sterilization (10 min) produced the highest survival rate (96.4 %), while the shortest time gave the minimum value (58.6 %). **Badoni and Chauhan (2010)** demonstrated that after sterilizing the *Solanum tuberosum* for three durations; 2, 5 and 8 min, the mortality rates were 70, 90 and 90 %, respectively in case of using HgCl2, while they were 80 %, 40 % and 50 %, respectively in case of using 1.0 % NaOCl. **(Al masoody and Stanica, 2015)** reported that exposing the leaf disc explants of *Rosmarinus officinalis* to sodium hypochlorite in a concentration of 0.75% for a longest period of sterilization (15 min) produced the highest survival rate (96.4 %).

Table 1. Effect of surface sterilization with different concentrations of NaOCl solution and $HgCl_2$ on the survival percentage of *Rosmarinus officinalis* leaf disc explants

Treatments	Conc.	Duration (min)	Contamination (%)	Survival (%)
$HgCl_2$	0.1gm %	7	70	30
$HgCl_2$	0.05gm %	7	60	40
Clorox	10 % (v/v)	10	35	65
Clorox	10 % (v/v)	15	20	80

3.2. Initiation of calli by plant growth regulators

As shown in **table (2), and Figure (1)** callus formation was varied according to the type and concentration of plant growth regulators used. Using combination of NAA (2.0 mg/L) and BAP (1.0 mg/L) leads to callus formation at the highest frequency while using the combination of 2,4-D (2.0 and 4.0 mg/L) and BAP (1.0 and 2.0 mg/L each) failed to induce callus (zero callusing). The data also revealed that using NAA individually at different concentration (2.0 and 4.0 mg/L) induced leaf explants to from callus at the same frequency. Using the auxin NAA (2.0 and 4.0 mg/L) with BAP at (1.0 and 2.0 mg/L each) was more effective than 2,4-D at the same concentration in inducing callus formation. **(Aref, 2014)** reported that using combination of NAA (2.0 mg/L) and BAP (2.0 mg/L) on *Dillenia indica* leads to callus formation at the highest frequency while using the combination of 2,4-D (1.0 mg/L) and kin (1.0 mg/L) failed to induce callus (zero callusing). **Abdellatef and Khalfalla, (2010)** on *Moringa oleifera* Lam., found that the explants failed to produce callus tissues in MS medium free of 2,4-D, they attributed that to the presence of 3,4-D was capable to induce callus tissues. They remarked also that, among the 2,4-D concentrations tested (0.1-3.0 mg/L), using 0.1, 0.5, 1.0 or 2.0 mg/L gave 100 % of callusing. Increasing the 2,4-D level to 3.0 mg/L significantly reduced the callusing rate to 84 %.

Table 2. Effect of plant growth regulators on callus initiation from leaf disc explants of *Rosmarinus officinalis*

Treatments (MS +)	Callus formation
MS (hormone-free)	++
2.0 mg/L 2,4-D	-
4.0 mg/L 2,4-D	-
2.0 mg/L NAA	+
4.0 mg/L NAA	+
2.0 mg/L 2,4-D + 1.0 mg/L BAP	-
4.0 mg/L 2,4-D + 1.0 mg/L BAP	-
2.0 mg/L NAA + 1.0 mg/L BAP	+++
4.0 mg/L NAA + 1.0 mg/L BAP	+++
2.0 mg/L 2,4-D + 2.0 mg/L BAP	-
4.0 mg/L 2,4-D + 2.0 mg/L BAP	-
2.0 mg/L NAA + 2.0 mg/L BAP	++
4.0 mg/L NAA + 2.0 mg/L BAP	+

- No callusing + small growth ++ Medium growth +++ vigorous growth

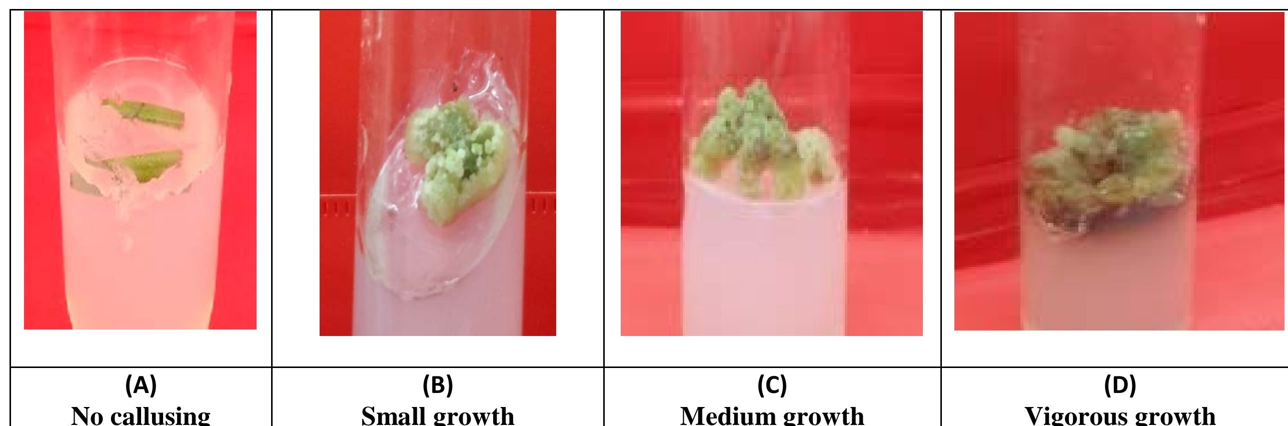

Figure (1): illustrating the vigor of callus growth (after 5 weeks), in response to various plant growth regulators treatments (A)- 2,4D at 2.0 or 4.0 mg/L, (B)- NAA 2.0 or 4.0 mg/L, (C)- NAA+BAP at 2.0 mg/L each and (D)- 2.0 or 4.0 mg/L NAA+BAP at 2 mg/L each.

3.3. Growth (as gram fresh weight) of calli in response to plant growth regulators and repeated subculturing of *Rosmarinus officinalis* cultures

As shown in **table (3) and figure (2)**, the effect of plant growth regulators on callus culture of *Rosmarinus officinalis* differed according to the number of subculture. During the first subculture the highest significant fresh weight (4.543g) was obtained when callus culture was grown on MS medium supplemented with 1.5 mg/L NAA + 1.0 mg/L BAP then (4.487 g) was obtained when callus culture was cultured on MS medium supplemented with 1.0 mg/L NAA + 2.0 mg/L BAP. During second and third subcultures, data showed that the highest significant fresh weight (4.445 and 5.537 g respectively) were obtained when callus culture was grown on MS medium supplemented with 2.0 mg/L NAA + 1.0 mg/L BAP. While the lowest significant fresh weight at first, second and third subcultures (2.28, 1.398 and 1.295g respectively) were detected when callus culture was allowed to grow on hormone-free MS medium. The results obtained in this study may more or less agree and confirm the observations obtained by other investigators who used different plant growth regulators alone or combined to induces callus formation from different plant species. For example, **Al masoody and Stanica, (2015)** reported that on *Rosmarinus officinalis* after the explants sterilization, the effect of six concentrations of BA and six concentrations of NAA and the overlap between them on the callus formation were studied. Highest callus size (mm3), callus fresh and dry weight (g) occurred at 0.2 mg/l. BA and 1.5 mg/l of NAA. **El-Shamy *et al.,* (2007)** on *Tanacetum parthenium*, subjected the explants to 2,4-D, NAA. BAP alone or in combination in different concentrations (0.0, 0.5 or 1.0 mg/L) and recorded that applying the combination of 1.0 mg/L NAA plus 1.0 mg/L BAP resulted in the highest rate of callusing (91.67 %). **Rawat *et al.,* (2013)** submitted the explants of *Aconitum violaceum* to 2,4-D at 0.5 – 1.0 µM or kin at 0.25 - 2.0 µM and remarked that employing the combination 5.0 µM 2,4-D plus 0.5 µM kin obviously increased the callus formation in the highest rates.

Table 3. Means of callus fresh weight of *Rosmarinus officinalis* explants as affected by different growth regulator and different light condition after first, second, third and fourth subcultures at 25±1 ºC

Treatments (MS +)	Callus fresh weight (g)		
	1st subculture	**2nd subculture**	**3rd subculture**
	Mean ± SE	Mean ± SE	Mean ± SE
MS (hormone-free)	2.28 ±0.172 c	1.398±0.0929 c	1.295±0.0333 d
1.5 mg/L NAA + 1.0 mg/L BAP	4.543±0.183 a	3.737± 0.168 b	4.342±0.224 b

2.0 mg/L NAA + 1.0 mg/L BAP	3.482±0.111 [b]	4.445±0.098 [a]	5.537±0.424 [a]
4.0 mg/L NAA + 1.0 mg/L BAP	3.26±0.271 [b]	3.688±0.266 [b]	4.253±0.132 [b]
1.0 mg/L NAA + 2.0 mg/L BAP	4.487 ±0.3 [a]	3.958 ±0.305 [ab]	3.503±0.171 [c]
P value	***	***	***
F ratio	18.595	33.239	44.491

Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, (Note: each Colum of means compared according to different treatments showed in first Colum).

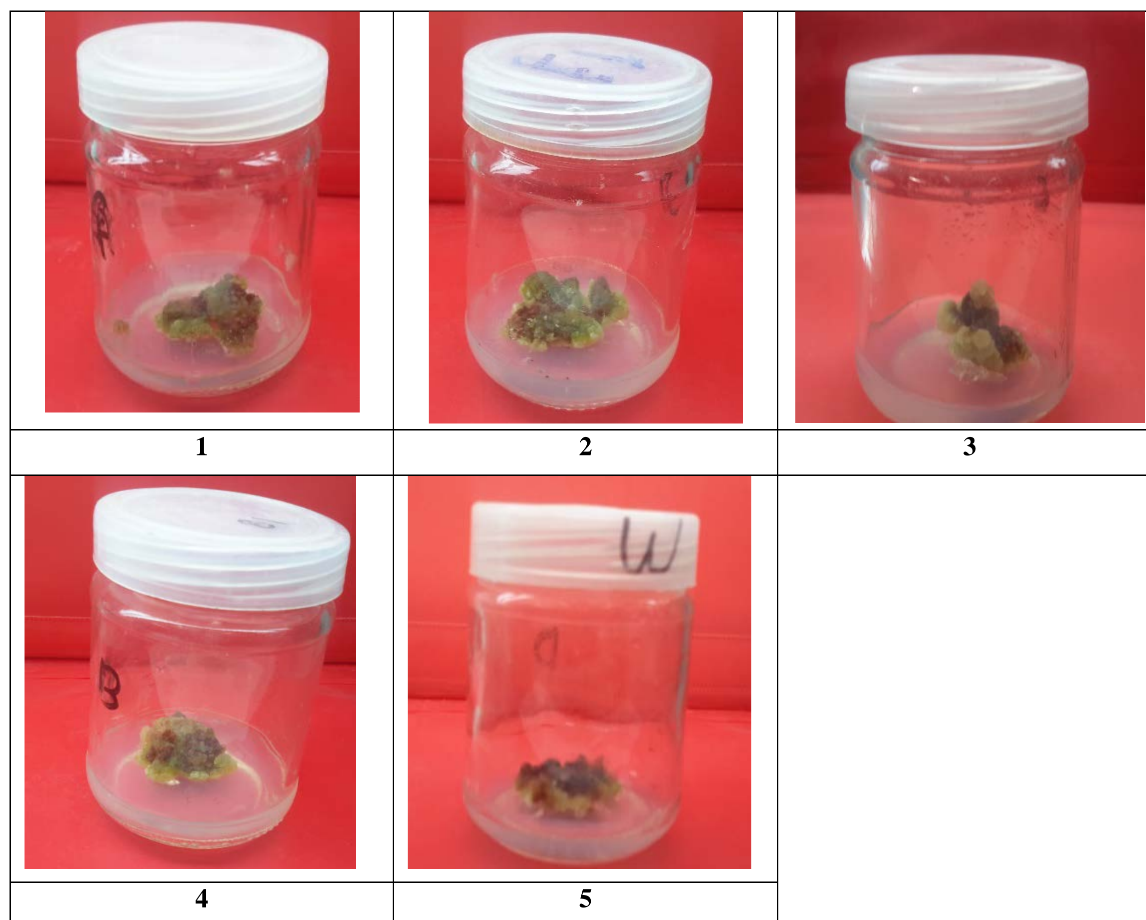

Figure (1): Callus culture of *Rosmarinus officinalis* produced from leaf disc explants on MS medium supplemented with 2.0 mg/L NAA + 2.0 mg/L 2.4D +2.0 mg/L BAP as affected by various plant growth regulators treatments at 1500 Lux and 25±1 ºC. (1 control = MS (hormone-free); 2 = culture produced from MS+1.5 mg/L NAA + 1.0 mg/L BAP; 3= culture produced from MS+2.0 mg/L NAA + 1.0 mg/L BAP; 4= culture produced from MS+4.0 mg/L NAA + 1.0 mg/L BAP; 5= culture produced from MS+1.0 mg/L NAA + 2.0 mg/L BAP).

3.4. Growth (as gram dry weight) of calli in response to plant growth regulators and repeated subculturing of *Rosmarinus officinalis* cultures

Data presented in table (3) show the effect of plant growth regulators on callus dry weight (g) during three successive subcultures. During the first subculture, the highest significant dry weight (0.232g) was obtained when callus culture was allowed to grow on culture MS medium supplemented with 1.5 mg/L NAA + 1.0 mg/L BAP then (0.215g) was obtained when callus culture using MS supplemented with 2.0 mg/L NAA + 1.0 mg/L BAP. The results obtained have also revealed that the lowest significant dry weight (0.160g) was obtained using on hormone-free MS medium. At the end of the second subculture, data in table (3) showed that the highest significant dry

weight (0.343g) was obtained when callus was cultured on MS medium supplemented with 2.0 mg/L NAA + 1.0 mg/L BAP then (0.237g) was obtained in case of using 4.0 mg/L NAA + 1.0 mg/L BAP while the lowest significant dry weight (0.081g) was detected when callus culture was carried out on hormone-free MS medium. Results of the third subculture (table 3) showed that the highest significant dry weight (0.278g) was obtained when callus allowed to grow on MS medium supplemented with 2.0 mg/L NAA + 1.0 mg/L BAP then (0.163g) was obtained when callus culture was on MS medium supplemented with 3. 1.0 mg/L NAA + 2.0 mg/L BAP while the lowest significant dry weight (0.098g) was detected when callus culture was incubated on hormone-free MS medium.

More or less similar results were previously observed by several authors. For example, (**Leelavathi and Kupp, 2013)** on *Rosmarinus officinalis* was noticed be the best medium for the induction and growth of whitish green compact callus was obtained when cultured the apical bud explants on MS + BAP (8.88 μM) + IAA (5.70 μM). **Dong et al., (2012),** reported that BAP 0.5 mg/L, NAA 0.5 mg/L, and 50 g/L sucrose was proved to be the optimal medium for the production of calli. On other hand **(Youssef, 1997)** had tested the effect of various plant growth regulators (NAA, 2,4-D alone or in combination with BAP or kin at 10.0 mg/L for each one on fresh weight of Ceratonia siliqua callus. He revealed that 10.0 mg/L 2,4-D plus 10.0 mg/L BAP resulted in the heaviest weight of callus, while the other tested treatments produced the lowest weights

Table 3. Means of callus dry weight of *Rosmarinus officinalis* explants as affected by different growth regulator and different light condition after first, second, third and fourth subcultures at 25±1 ºC

Treatments (MS +)	Callus dry weight (g)		
	1st subculture	**2nd subculture**	**3rd subculture**
	Mean ± SE	Mean ± SE	Mean ± SE
MS (hormone-free)	0.160±0.011 c	0.081±0.007 d	0.098±0.009 c
1.5 mg/L NAA + 1.0 mg/L BAP	0.232±0.009 a	0.17±0.051 bc	0.019±0.009 b
2.0 mg/L NAA + 1.0 mg/L BAP	0.215±0.009 ab	0.343±0.023 a	0.278±0.006 a
4.0 mg/L NAA + 1.0 mg/L BAP	0.183±0.015 bc	0.237±0.009 b	0.021±0.015 b
1.0 mg/L NAA + 2.0 mg/L BAP	0.198±0.0144 ab	0.16±0.007 c	0.163±0.014 b
P value	**	***	***
F ratio	5.757	34.905	14.440

Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0. 001 (Note: each Colum of means compared according to different treatments showed in first Colum)

3.5. Secondary metabolites production

3.5.1. Effect of light quality and microwave on callus growth (fresh and dry weight)

Data presented in table (4) showed the effect of elicitation treatments (including light quality and microwave irradiation) on callus growth in terms of fresh and dry weights. As for fresh weight, the presented data indicated no significant variations in fresh weight in response to the different tested elicitation treatments. data showed that the highest fresh weight (2.49 g) was obtained when callus culture was when callus cultures irradiated with 200 watts of microwave for 60 sec. while the lowest fresh weight (1.617 g) was detected when callus culture was incubated under yellow light.

As regards to the dry biomass, data showed that the highest significant dry weight (0.177 g) was obtained when callus culture was allowed to irradiated with 200 watts of microwave for 60 sec. The results obtained have also revealed that the lowest significant dry weight (0.11 g) was obtained when callus culture was detected when callus culture was incubated under red light. The results obtained have also revealed that the power of microwave irradiation and exposure time also effected on the callus growth. When callus cultures irradiated with 200 or 400 watts of microwave, the callus fresh weight increased with increasing exposure time from 30 to 60 seconds also the callus fresh weight increased with increasing exposure irradiation douse from 200 to 400 watts. The results obtained may agree and confirm the results obtained previously by **(Abd El-Kadder *et al*, 2014)** on *Dillenia indica* who noticed that the highest significant fresh weights (5.96 and 5.58 g) were obtained when callus culture was irradiated with ultraviolet at 30 watts for one hour, and callus incubated under green light, respectively while the lowest significant fresh weight (2.318 g) was detected when callus culture was incubated under full darkness. **Moeller**

(1992) mentioned that microwave are electromagnetic waves that cause a clear effect on cells and tissues growth of several plant species. On *Satureja bachtiarica*, **Vishki *et al.*, (2012)** disclosed that a significant decrease in fresh and dry weight was observed in comparison with control. **Park *et al.*, (2003),** studied the Production of camptothecin (CPT) from callus cultures of *Camptotheca acuminata* Decne was affected by light and culture conditions. The highest cell growth was obtained in dark and green light condition, respectively.

Table 4. Means of callus fresh weight and dry weight (g) of callus culture of *Rosmarinus officinalis* as affected by Microwave and light quality.

Elicitor	Treatment	Fresh weight (g).	Dry weight (g).
		Means ± SE	Means ± SE
Light quality	3000Lux	2.467±0.203	0.173±0.0186[ab]
	Red light	1.933±0.296	0.11±0.0153[d]
	Green light	1.777±0.167	0.113±0.0145[d]
	Yellow light	1.617±0.0726	0.12±0.01[cd]
	Blue light	2.1±0.306	0.133±0.0145[bcd]
microwave	100 watts for 30 sec	2.067±0.176	0.157±0.0145[bc]
	100 watts for 60 sec	2.197±0.297	0.17±0.0115[ab]
	200 watts for 30 sec	2.323±0.148	0.17±0.0115[ab]
	200 watts for 60 sec	2.49±0.127	0.177±0.00882[a]
		NS	---
P value		1.94	4.174
F ratio		0.116	**

Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, (Note: each Colum of means compared according to different treatments showed in first Colum)

4. Effect of light quality and microwave on antioxidant activity

Data presented in table (5) showed the effect of elicitation treatments (including light quality and microwave irradiation) on antioxidant activity. The presented data indicated that significant variations in antioxidant activity in response to the different tested elicitation treatments. the highest significant antioxidant activity (314.326%) was detected when callus culture was incubated under green light, whereas the lowest one (98.512%) was recorded when callus culture was incubated at 3000 Lux. Light is an important factor affecting growth, organogenesis and the formation of plant products including both primary and secondary metabolites. The stimulatory effect of light on the formation of compounds, including flavonoid and anthocyanins has been shown in plants **(Krewzaler and Hahlbrock, 1976; Zhong *et al.*,1991)**. Certain wavelengths of lights have been reported to increase antioxidative actions against abiotic challenges. In broad bean leaves, for instance, significant increase of catalase activity under red light contributed to scavenging of hydrogen peroxide generated by *Botrytis cinerea* infection **(Islam *et al.*, 2011)**. Moreover, **(Ahmed, 2010)** reported that callus cultures of *Cleome droserifolia* exposed to microwave at 65 watts for 60 sec or ultraviolet irradiation for 24 hrs gave the highest value of flavonoids compared with control and other treatments.

Table 5. Antioxidant activity (Inhibition %) of *Rosmarinus officinalis in vitro* callus culture as affected by elicitation treatments (Microwave and light quality).

Elicitor	Treatment	Inhibition %
		Means ± SE
Light quality	3000Lux	98.512±1.493[e]
	Red light	206.58±10.249[b]
	Green light	314.326±18.157[a]
	Yellow light	132.021±10.273[cd]
	Blue light	127.563±14.432[cde]
	100 watts for 30 sec	125.126±9.267[cde]

microwave	100 watts for 60 sec	117.452±1.25^{de}
	200 watts for 30 sec	147.272±4.636^{c}
	200 watts for 60 sec	125.76±4.378^{cde}
P value		***
F ratio		45.471

Columns with similar letters are not significantly different according to Fisher LSD. NS=non-significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0. 001 (Note: each Colum of means compared according to different treatments showed in first Colum).

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