European Journal of Medicinal Plants
14(4): 1-11, 2016, Article no.EJMP.26121
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Elicitation of Biomass and Secondary Metabolite Production, Antioxidative and Antimicrobial Potential of Basil and Oregano Induced by BA and IBA Application

Erna Karalija[1*], Dolores Neimarlija[2], Jasmina Cakar[3] and Adisa Paric[1]

[1]Department of Biology, Faculty of Sciences, Laboratory for Research and Protection of Endemic Resources, University of Sarajevo, Zmaja od Bosne 35, 71000 Sarajevo, Bosnia and Herzegovina.
[2]Druga Gimnazija, Sarajevo, Bosnia and Herzegovina.
[3]Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina.

Authors' contributions

This work was carried out in collaboration between all authors. Authors EK and AP designed the study, performed part of the experimental analyses and the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DN and JC managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/26121
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Hatice Demiray, Ege University, Turkey.
(2) Mahipal S. Shekhawat, Biotechnology Laboratory, MGGAC, India.
Complete Peer review History: http://sciencedomain.org/review-history/14490

Short Research Article

Received 1st April 2016
Accepted 26th April 2016
Published 6th May 2016

ABSTRACT

Elicitation effects of indole-3-butyric acid (IBA) and benzyladenine (BA) on oregano and basil shoots were investigated through analysis of photosynthetic pigments content, biomass production, quantification of total phenols, flavonoids, flavanols and proanthocyanidins content. Also antioxidative and antimicrobial potential of extracts were assessed. Biomass production varied significantly among two species and treatments. Auxin induced decrease of chlorophyll levels. Accumulation of phenols was noticed in basil shoots when BA was applied in low concentrations, while in oregano high BA concentrations induced phenol accumulation. Flavonoids accumulation was noticed for 0.5 and 1.0 mg/L of BA as well for 2.0 and 4.0 mg/L BA in combination with 0.1

**Corresponding author: E-mail: erna.karalija@gmail.com;*

mg/L IBA in basil shoots. Addition of IBA induced accumulation of flavonoids in oregano shoots only when equal concentration of BA and IBA was applied. Flavanols content decreased by addition of IBA for basil shoots as well as for oregano. Small concentrations of auxins stimulated antioxidative response in oregano shoots. Elicitation of secondary metabolites resulted in elevation of antimicrobial potential of oregano adventive shoots when BA was applied.

Keywords: Basil; oregano; secondary metabolites; antioxidant; antimicrobial; Lamiaceae.

1. INTRODUCTION

Production of secondary metabolites in plant cell cultures, as a source of medicinal compounds, has been intensively studied [1]. A common problem harvesting cultivated plants is the qualitative and quantitative variability in plant responses to the environmental changes [2,3], which is avoided by use of *in vitro* plant culture. There are several strategies used to enhance the production of necessary phytochemicals in plant cultures (genetic engineering, selection of cell lines and use of elicitors) [1,4]. Plant growth regulators have been proven to be a useful tool in elicitation process in plants [5]. The concentration of plant growth regulators as well as the type affects the capacity of in vitro propagated plants through regulation of their cell division, differentiation and morphogenesis [6]. Accumulation of bioactive compounds can be affected through optimisation of culture conditions [7], as well as by variation of environmental parameters [8,9]. From all possible accumulating secondary metabolites, phenolic compound are very interesting for researchers regarding their high antioxidative potential, these metabolites are known to be widespread among members of Lamiaceae family [10].

In Lamiaceae family, mint family, secondary metabolites are usually synthetized in leaves [11], and a large number of family members are used in medicinal purposes [12-15]. Harvesting natural populations of mint family members can represent a problem since there is no consistency in metabolite production due to genetic and biochemical heterogeneity [16,17]. Basil (*Ocimum basilicum*) and oregano (*Origanum vulgare*) are representatives of this family that are widely used in culinary and pharmaceutical industry [14,15]. *Ocimum* genus is very rich in phenolic compounds and can be used in therapeutic purposes [18]. Parts of basil (roots, bark and leaves) are found to be cyanogenic [12]. Species of *Origanum* genus are widely used as a spice [19], with traditional use in many ways due to their bioactive properties against huge number of microorganisms [20]. *In vitro* techniques have been already employed for *in vitro* cultivation of Lamiaceae family in order to find new mutants [21] or to enhance metabolite production [22]. Plant growth regulators, such as cytokinins and auxins, are usually used in agricultural industry for growth or rhizogenesis stimulation [23-25], but stimulatory effect of cytokinins on growth parameters, as well as active constituent production and total carbohydrates content, has been also recorded [26-29].

The aim of this study was to investigate changes in phenylpropanoid metabolism, antioxidative and antimicrobial potential in *in vitro* basil and oregano shoot cultures under BA and IBA application. The study included analysis of photosynthetic pigments, multiplication index, fresh and dry mass, quantification of total phenols, total flavonoids, total flavanols and total proanthocyanidins, as well as analysis of antioxidative and antibacterial and antifungal potential.

2. MATERIALS AND METHODS

2.1 Plant Materials

In vitro germinated basil and oregano shoots were used for establishment of shoot culture. Shoots were cultivated on basal medium [30] with addition of BA (6-benzyladenine) in concentration of 0.1, 0.5, 1.0, 2.0 and 4.0 mg/L alone or in combination with 0.1 mg/L IBA (indole-3-butyric acid). For all media pH was adjusted to 5.8 then 0,8% agar was added, and media were autoclaved for 20 minutes under 1 bar pressure and 121℃. All cultures were kept under constant conditions of 16 h photoperiod (3 000 lux), 21℃ (±2℃) and 70% humidity (±5%).

2.2 Growth Parameters

Photosynthetic pigments. Quantification of pigments was done according to Porra [31] and Holm [32] in 80% acetone extracts. Pigments were expressed as mg of pigment per g of fresh

weight (FW). *Morphological parameters*: Multiplication/Rhizogenesis rate and index. Multiplication/Rhizogenesis rate was calculated as a percent of explants that gave rise to new shoots/roots, while multiplication/rhizogenesis index represents an average number of newly formed shoots/roots per explant. *Fresh and dry mass*. Fresh mass of shoots was recorded after removal of roots, then the shoots were dried in hot air oven at 60℃, until constant mass was obtained, to calculate dry weight.

2.3 Secondary Metabolite Analysis

Extraction of metabolites was done in 80% methanol by submerging macerated dried plant material in methanol over night at +4℃, and collecting the supernatant. Supernatant was evaporated and the dried extract was suspended in absolute ethanol (in final concentration of 1 mg/ml). *Total phenols*. Analysis of total phenols was done according to Wolfe et al. [33]. by Folin–Ciocalteu method using catechin as a standard. Phenols were expressed as a catechin equivalend per g of dry weight (mg CE/g DW). *Total flavonoids*. Two methods were used for determination of total flavonoids. First method was done according to Ordoñez et al. [34] using aquatic solutions of $AlCl_3$ and sodium acetate, and using catechin as a standard (mg CE/g DW), and second method employed methanol solutions of $AlCl_3$ and sodium acetate according to modified method of Popova [35], quercetin was used as a standard (mg QE/g DW). *Total flavanols*. Analysis of total flavanols was done according to Gadzovska et al. [36] using DMACA reagent (p-dimethyl amino cinnamaldehyde dissolved in HCl: CH3OH). Catechin was used as a standard (mg CE/g DW).

2.4 Antioxidative Potential

Antioxidative potential was assessed using ferric thiocyanate (FTC) method according to Larrauri et al. [37]. Mixture of extracts, ethanol, linoleic acid and phosphate buffer (pH 7.0) was placed in an oven at 40℃ and incubated for 96 h in the dark. Measurement of the extent of antioxidative activity was done by addition of 0.1 ml of reaction mixture to diluted ethanol (75%), ammonium thiocyanate and 0.02 M ferrous chloride in 3.5% hydrochloric acid. After three minutes the absorbance was measured at 500 nm. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used as positive control. Rate of inhibition of lipid peroxide oxidation was calculated against negative control (80% methanol) according to Elmastaş et al. [38].

2.5 Antimicrobial Potential

Antibacterial potential was tested against gram positive bacteria (*Staphylococcus aureus* subsp. *aureus* ATCC ® 6538™; *Enterococcus faecalis* ATCC ® 19433™ and *Bacillus subtilis* subsp. *spizizenii* ATCC ® 6633™) and gram negative bacteria (*Escherichia coli* ATCC ® 8739™ and *Salmonella enterica* subsp. *enterica serovar abony* NCTC 6017™). The bacterial cultures were incubated in liquid Mueller-Hinton medium (HiMedia; India) overnight, the colony concentration was determined comparing colour of suspensions to McFarland reagent. For antimicrobial testing discs impregnated by extracts [39], positive control (antibiotic) and negative control (80% methanol) were placed on Petri dishes containing Mueller-Hinton agar with 1 mL of standardized bacterial inoculum (comparing to McFarland reagent 10^6) distributed over the Mueller-Hinton agar. Inhibition zones were recorded after 24 h incubation and expressed as mm. *Antifungal potential* was tested for two fungi C*andida albicans ATCC(R)10321* and *Aspergillus brasiliensis* ATCC ® 16404™. Fungi were cultivated overnight in liquid Sabouard medium, concentration was adjusted using McFarland standard, nystatin represented positive control. Antifungal potential was tested by disc diffusion method [39] and expressed as mm of inhibition zones after 36 h incubation.

2.6 Statistical Analysis

All results represent mean value (±STDEV) of tree independent replications of randomized experiment. Analysis of variance was performed using ANOVA, Newman-Keuls Post hoc test at $p<0.05$ level. Statistical significance was indicated by assigning the letters to mean values.

3. RESULTS AND DISCUSSION

The effects of different concentrations of BA alone or in combination with IBA were examined.

3.1 Changes in Growth Parameters

All tested concentrations gave some effect on the photosynthetic pigments, morphological parameters and ration of fresh and dry mass.

Table 1. Changes in photosynthetic pigments in *Ocimum basilicum* and *Origanum vulgare* after addition of BA alone or in combination with IBA

Treatment	*Ocimum basilicum*				*Origanum vulgare*			
	Chl *a* mg/gFW	Chl *b* mg/gFW	Chl *a+b* mg/gFW	Car mg/gFW	Chl *a* mg/gFW	Chl *b* mg/gFW	Chl *a+b* mg/gFW	Car mg/gFW
noPGRs	0.65^{b} (±0.04)	0.18^{b} (±0.01)	0.27^{b} (±0.01)	0.17^{a} (±0.01)	0.72^{a} (± 0.03)	0.21^{a} (±0.01)	0.93^{a} (±0.04)	0.22^{a} (±0.01)
0.1 BA	0.29^{e} (±0.01)	0.09^{d} (±0.00)	0.12^{e} (±0.01)	0.08^{c} (±0.00)	0.65^{b} (±0.02)	0.19^{a} (±0.01)	0.84^{b} (±0.03)	0.19^{a} (±0.01)
0.5 BA	0.38^{d} (±0.01)	0.12^{c} (±0.01)	0.16^{d} (±0.00)	0.10^{b} (±0.00)	0.42^{c} (±0.01)	0.11^{b} (±0.00)	0.53^{c} (±0.01)	0.14^{bc} (±0.00)
1.0 BA	0.25^{e} (±0.02)	0.08^{d} (±0.01)	0.11^{e} (±0.01)	0.08^{c} (±0.00)	0.19^{e} (±0.00)	0.06^{c} (±0.00)	0.24^{e} (±0.00)	0.07^{d} (±0.00)
2.0 BA	0.71^{a} (±0.06)	0.20^{a} (±0.02)	0.30^{a} (±0.03)	0.18^{a} (±0.01)	0.35^{d} (±0.01)	0.10^{b} (±0.01)	0.45^{d} (±0.01)	0.12^{c} (±0.00)
4.0 BA	0.35^{d} (±0.02)	0.13^{c} (±0.00)	0.16^{d} (±0.01)	0.11^{b} (±0.00)	0.16^{e} (±0.00)	0.06^{c} (±0.00)	0.23^{e} (±0.01)	0.09^{cd} (±0.00)
0.1 BA + 0.1 IBA	0.35^{d} (±0.01)	0.12^{c} (±0.01)	0.16^{d} (±0.01)	0.11^{b} (±0.00)	0.43^{c} (±0.03)	0.13^{b} (±0.01)	0.56^{c} (±0.04)	0.16^{b} (±0.01)
0.5 BA + 0.1 IBA	0.26^{e} (±0.00)	0.09^{d} (±0.01)	0.12^{e} (±0.00)	0.08^{c} (±0.00)	0.38^{d} (±0.02)	0.12^{b} (±0.00)	0.50^{c} (±0.02)	0.14^{bc} (±0.01)
1.0 BA + 0.1 IBA	0.31^{d} (±0.00)	0.10^{d} (±0.00)	0.14^{e} (±0.00)	0.10^{b} (±0.00)	0.43^{c} (±0.01)	0.13^{b} (±0.01)	0.57^{c} (±0.02)	0.17^{b} (±0.00)
2.0 BA + 0.1 IBA	0.49^{c} (±0.01)	0.15^{c} (±0.01)	0.21^{c} (±0.01)	0.11^{b} (±0.01)	0.19^{e} (±0.01)	0.01^{d} (±0.00)	0.26^{e} (±0.02)	0.09^{cd} (±0.00)
4.0 BA + 0.1 IBA	0.39^{d} (±0.01)	0.13^{c} (±0.01)	0.17^{d} (±0.01)	0.11^{b} (±0.01)	0.17^{e} (±0.01)	0.01^{d} (±0.00)	0.24^{e} (±0.01)	0.09^{cd} (±0.00)

**treatments not shearing the same letter within one parameter differ significantly at p<0.05 level after Newman-Keuls test*

FW- fresh weight; Chl a – chlorophyll a; Chl b – chlorophyll b; Chl a+b – total chlorophylls; Car – carotenoids; BA – 6-benzyladenine; IBA – indole -3- butyric acid.

For *Ocimum basilicum* BA alone showed statistically significant stimulating effect on chlorophyll *a,* chlorophyll *b,* and total chlorophylls as well as carotenoids concentration, when applied in higher dosage (2.0 mg/L). Further elevation of BA concentration (4.0 mg/L) decreased chlorophyll content. Lower concentrations of BA (0.1, 0.5 and 1.0 mg/L) induced decrease in chlorophyll content when compared to control (no PGRs added). Stimulatory effect of BA on photosynthetic pigment production Stimulating effects of BA on photosynthetic pigments was noticed for foliar application of BA on some plants [40,41].

Decline of chlorophyll levels in senescing tissues, but also they enhance chlorophyll levels in developing tissues [42], as noticed in our study as well with differences between used concentrations of BA. Optimum concentration of BA for stimulation of chlorophyll production is 2.0 mg/L for *Ocimum basilicum*. The same concentrations of BA did not stimulate production of chlorophylls in *Origanum vulgare*, and all used BA concentration induced decrease in pigment content when compared to control (Table 1). When BA was applied in combination with IBA no stimulating effects were noticed, and a decrease in pigment concentration when compared to control was noticed (Table 1). Loss of chlorophylls induced by auxin application is recorded also for lettuce [43]. Decrease of chlorophyll when cytokinins and auxin were applied could be the result of ROS activation, mostly H_2O_2 [44]. Changes in antioxidant enzymes could cause leaf senescence differently in different plant species [45,46]. Plant growth regulators could regulate synthesis of antioxidant enzymes, which are in return included in plant growth regulators catabolism [47]. In our study effect of BA alone was species specific, with general remark that BA in combination with IBA did not show any stimulating effect on *O. basilicum* or *O. vulgare*. Different concentrations of BA stimulated shoot formation in *O. basilicum*, especially in high concentrations (2.0 and 4.0 mg/L). Addition of IBA induced rise of rhizogenesis rate up to 100% (Table 2).

Table 2. Changes in morphological parameters in *O. basilicum* and *O. vulgare* under BA and IBA application

PGR		*Ocimum basilicum*				*Origanum vulgare*			
BA (mg/L)	**IBA (mg/L)**	**MI**	**MR**	**RI**	**RR**	**MI**	**MR**	**RI**	**RR**
0	0	1.00^{d} (±0.00)	13.33^{f} (±5.77)	18.33^{a} (±0.58)	99.67^{a} (±0.58)	1.67^{e} (±0.58)	55.33^{c} (±5.03)	4.00^{c} (±0.00)	100.00^{a} (±0.00)
0.1	0	1.00^{d} (±0.00)	10.00^{f} (±0.00)	13.67^{b} (±0.58)	93.33^{b} (±5.77)	5.67^{bc} (±0.58)	82.00^{b} (±2.65)	6.33^{b} (±1.53)	100.00^{a} (±0.00)
0.5	0	1.67^{c} (±0.58)	23.33^{e} (±5.77)	15.67^{b} (±0.58)	100.00^{a} (±0.00)	6.33^{b} (±1.15)	98.33^{a} (±0.58)	13.67^{a} (±0.58)	100.00^{a} (±0.00)
1.0	0	1.00^{d} (±0.00)	13.33^{f} (±5.77)	13.67^{b} (±0.58)	100.00^{a} (±0.00)	4.33^{cd} (±0.58)	81.33^{b} (±1.53)	1.00^{d} (±0.00)	95.00^{a} (±4.58)
2.0	0	1.67^{c} (±0.58)	16.67^{f} (±5.77)	11.33^{c} (±0.58)	100.00^{a} (±0.00)	1.67^{e} (±0.58)	93.33^{a} (±8.96)	2.00^{d} (±1.00)	96.67^{a} (±5.77)
4.0	0	3.33^{a} (±0.58)	81.3^{3a} (±1.53)	12.33^{bc} (±0.58)	93.33^{b} (±5.77)	2.33^{de} (±0.58)	55.33^{c} (±5.03)	1.67^{d} (±0.58)	84.33^{b} (±4.04)
0.1	0.1	2.00^{b} (±0.00)	72.00^{b} (±2.65)	11.33^{c} (±1.53)	100.00^{a} (±0.00)	3.67^{d} (±0.58)	92.67^{a} (±10.12)	6.67^{b} (±2.08)	100.00^{a} (±0.00)
0.5	0.1	1.67^{c} (±0.58)	36.33^{d} (±5.51)	18.67^{a} (±0.58)	100.00^{a} (±0.00)	4.33^{cd} (±0.58)	92.67^{a} (±10.12)	3.00^{d} (±0.00)	98.67^{a} (±2.31)
1.0	0.1	1.33^{cd} (±0.58)	24.67^{e} (±4.51)	17.67^{ab} (±0.58)	100.00^{a} (±0.00)	8.00^{a} (±1.00)	98.67^{a} (±0.58)	4.33^{c} (±1.53)	99.33^{a} (±1.15)
2.0	0.1	2.00^{b} (±0.00)	59.67^{c} (±1.53)	12.67^{bc} (±0.58)	100.00^{a} (±0.00)	9.00^{a} (±1.00)	92.67^{a} (±10.12)	3.67^{cd} (±1.15)	96.00^{a} (±6.93)
4.0	0.1	2.33^{b} (±0.58)	89.00^{a} (±1.00)	16.67^{b} (±0.58a)	100.00^{a} (±0.00)	2.33^{de} (±0.58)	53.00^{c} (±3.00)	2.67^{d} (±0.58)	98.33^{a} (±2.08)

**treatments not shearing the same letter within one parameter differ significantly at p<0.05 level after Newman-Keuls test*

MI – Multiplication index; MR – Multiplication rate; RI – Rhizogenesis index; RR – Rhizogenesis rate

Origanum vulgare showed different response, and elevation in multiplication rate was noticed in combination of higher BA concentrations (2.0 mg/L BA) with low concentration of IBA (0.1 mg/L) with successful root formation (Table 2). Differences in plant response to BA alone and cytokinin/auxin ration in the media could be explained by differences in their genotype, as noticed by several authors [48-50]. Addition of auxins usually stimulates root formation, and can balance morphogenesis such as shoot and root formation [51]. Beside root induction on media containing PGRs, root induction was also noticed on media containing no PGRs. It is well known that MS hormone free media can induce root formation in Lamiaceae species [52].

Variation of water content and dry mass was evident for basil. Higher concentrations of BA induced water accumulation and resulted with lower dry mass content. Oregano showed more or less stabile water content up to 4.0 mg/L BA where decrease in dry mass was recorded. Application of IBA showed lower variation rate in dry mass content (Fig. 1) and higher yield of biomass. Simulative effect of auxins, in low concentrations, on biomass production has been reported for other plant species [53]. In our study IBA with BA stimulated higher rate of root formation with satisfactory shoot formation, while BA alone in moderate concentrations (1.0 mg/L) induced higher biomass production in *Origanum vulgare*. For *Ocimum basilicum* addition of auxin stimulated root formation, as well as biomass production (Fig. 1A). Induction of root formation in presence of auxin and cytokinin was recorded for other species also [54].

3.2 Changes in Secondary Metabolite Production

Phenol content in basil shoots showed variation depending upon treatment BA. Slightly higher phenol content was recorded when IBA was applied, but the elevation is still lower than phenol content in basil shoots on control treatment.

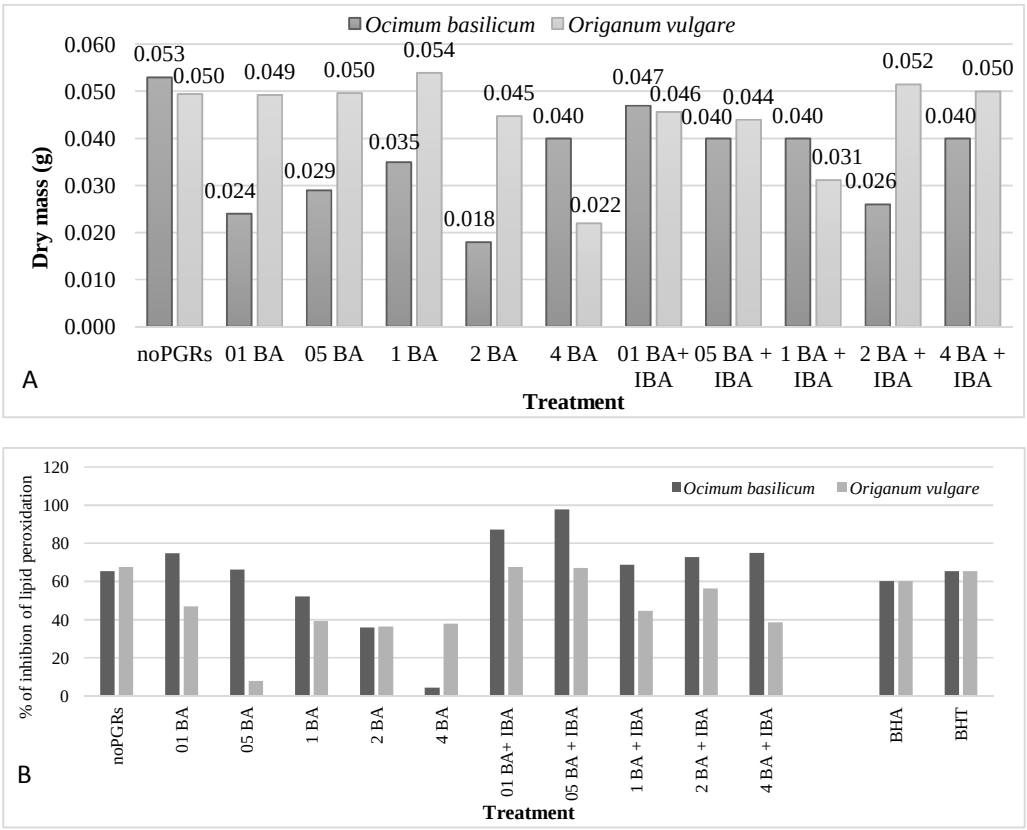


Fig. 1. Dry mass content (A) and lipid peroxidation (B) in *Ocimum basilicum* and *Origanum vulgare* shoots in relation to concentration of BA alone and in combination with IBA

noPGRs – no added growth regulators; 01BA – 0.1 mg/L BA; 05BA – 0.5 mg/L BA; 1BA – 1.0 mg/L BA; 2BA – 2.0 mg/L BA; 4BA – 4.0 mg/L BA; 01BA+ IBA – 0.1 mg/L BA + 0.1 mg/L IBA; 05BA + IBA – 0.5 mg/L BA+ 0.5mg/L IBA; 1BA + IBA – 1.0 mg/L BA+ 0.1 mg/L IBA; 2BA + IBA – 2.0 mg/L BA+ 0.1 mg/L IBA; 4BA + IBA – 4.0 mg/L BA+ 0.1 mg/L IBA

Application of 4.0 mg/L BA and 0.1 mg/L IBA showed significant increase of phenol content in oregano shoots (Table 3), elevation comparing to control was also noted for 2.0 mg/L BA; 4.0 mg/L BA; 2.0 mg/L BA + 0.1 mg/L IBA. Differences between the response of basil and oregano on BA treatment can be attributed to genetic differences between species. Flavonoid content in basil was elevated by addition of higher cytokinin dosage (2 and 4 mg/L) in combination with low concentration of IBA (0.1 mg/L). Equal ration of cytokinins and auxins induced elevation of flavonoid content in oregano (Table 3). Again genetic differences played their role in response to cytokinin dosage. Flavanols were decreased comparing to control for basil and oregano irrelevant to used treatment (Table 3). Elevation of phenol content can be achieved by application of plant growth regulators without any stress. Similar results were recorded previously [55]. Low concentrations of auxins and cytokinins have been recorded as degreasers of phenolic content, while higher concentrations of NAA and BA induce elevation of phenolic compounds [56].

3.3 Changes in Antioxidative and Antimicrobial Potential

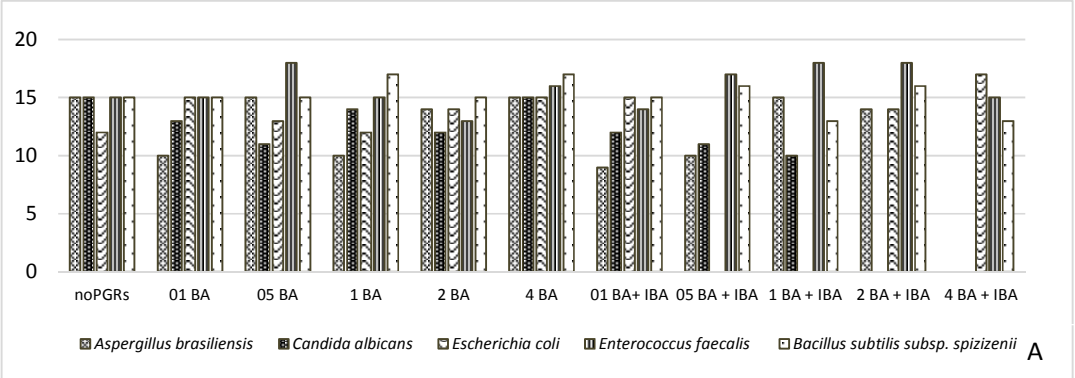
The highest antioxidative potential was recorded for treatments with 0.1 and 0.5 mg/L BA in combination with 0.1 IBA for both investigated species. High antioxidative potential of oregano inflorescence was previously recorded for wild plants [57]. Stimulatory effect of BA on antioxidative potential was recorded for *Campanula velebitica* [58], it was also recorded that auxins had no effect on antioxidative potential. In our case addition of small

concentration of auxin stimulated antioxidative potential, especially for oregano when compared with treatments containing the same concentrations of BA alone (Fig. 1B). Basil showed strong antimicrobial effect. Only two of tested bacteria were not affected (*Staphylococcus aureus* and *Salmonella abony*) (Fig. 2A). Oregano extracts showed week antimicrobial potential, only few treatments showed the antimicrobial effect, and only few bacteria were affected (Fig. 2B). Some BA treatments for basil showed better antimicrobial effect comparing to control antibiotic (Fig. 2C). Many studies showed that use of PGRs in in vitro culture can elevate antibacterial properties of plants by stimulating the production of compounds with intrinsic antibacterial properties [59,60].

Table 3. Secondary metabolite content in oregano and basil shoots after BA alone, and BA in combination with IBA treatment

PGR		*Ocimum basilicum*			*Origanum vulgare*		
BA (mg/L)	**IBA (mg/L)**	**Phenols (mg/g DW)**	**Flavonoids (mg/g DW)**	**Flavanols (mg/g DW)**	**Phenols (mg/g DW)**	**Flavonoids (mg/g DW)**	**Flavanols (mg/g DW)**
0	0	10.25^{b} (±1.48)	42.32^{d} (±1.89)	10.49^{a} (±0.30)	114.14^{c} (±4.95)	11.08^{e} (±0.69)	8.49^{a} (±0.44)
0.1	0	16.10^{a} (±1.21)	24.62^{f} (±2.82)	2.04^{de} (±0.20)	24.02^{g} (±0.21)	23.55^{c} (±0.63)	2.84^{b} (±0.07)
0.5	0	3.28^{e} (±0.88)	51.21^{c} (±6.32)	3.58^{c} (±0.25)	57.63^{e} (±1.06)	33.81^{b} (±2.97)	2.73^{b} (±0.24)
1.0	0	7.51^{c} (±1.07)	68.59^{b} (±5.69)	3.53^{c} (±0.12)	65.77^{d} (±3.16)	24.62^{c} (±2.89)	2.37^{b} (±0.03)
2.0	0	5.11^{e} (±0.81)	42.21^{d} (±2.39)	4.38^{c} (±0.17)	147.83^{b} (±12.43)	16.75^{c} (±2.90)	2.23^{b} (±0.02)
4.0	0	4.45^{e} (±0.12)	28.80^{f} (±1.90)	2.06^{de} (±0.16)	105.67^{c} (±12.98)	11.84^{e} (±1.20)	0.95^{cd} (±0.04)
0.1	0.1	5.63^{e} (±0.13)	39.64^{e} (±8.06)	2.52^{de} (±0.13)	69.48^{d} (±8.39)	57.43^{a} (±2.67)	2.92^{b} (±0.23)
0.5	0.1	6.33^{dc} (±0.64)	23.64^{f} (±2.76)	1.52^{e} (±0.34)	16.42^{h} (±0.84)	7.14^{f} (±1.68)	1.08^{c} (±0.10)
1.0	0.1	6.96^{c} (±0.80)	44.75^{d} (±5.35)	2.19^{de} (±0.53)	39.46^{f} (±3.92)	28.34^{b} (±2.34)	2.36^{bc} (±0.22)
2.0	0.1	5.72^{ed} (±0.36)	67.84^{b} (±6.47)	8.02^{b} (±0.38)	113.91^{c} (±4.41)	5.04^{f} (±10.12)	0.93^{cd} (±0.08)
4.0	0.1	5.53^{e} (±0.44)	74.37^{a} (±6.43)	1.8^{e} (±0.09)	616.04^{a} (±1.96)	13.18^{de} (±2.05)	1.77^{c} (±0.14)

**treatments not shearing the same letter within one parameter differ significantly at p<0.05 level after Newman-Keuls test DW – dry weight*



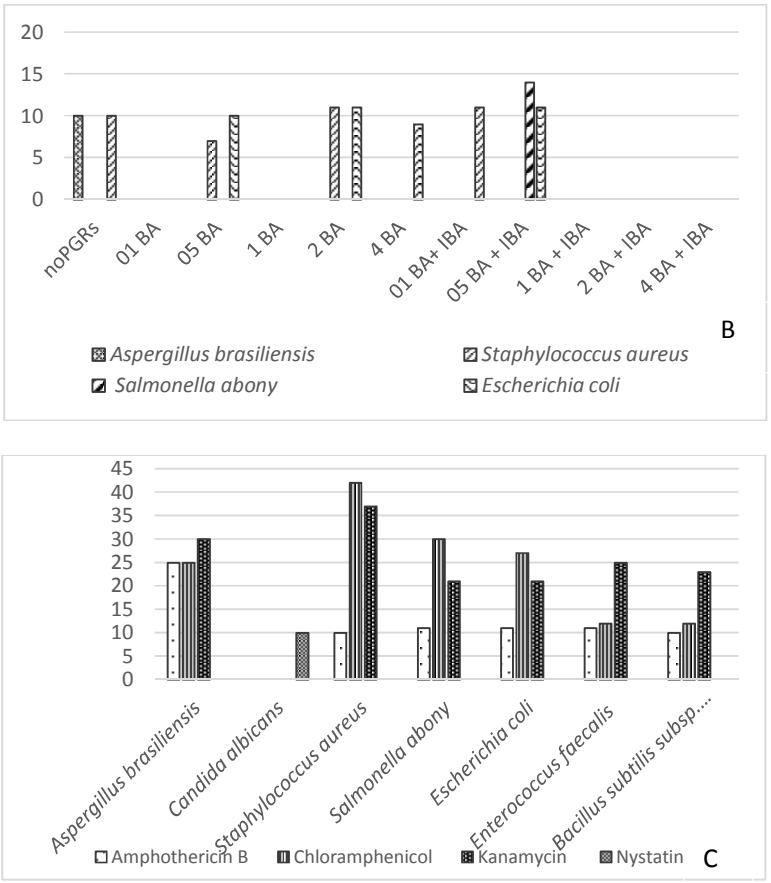


Fig. 2. Inhibition zones for extracts of *Ocimum basilicum* (A), *Origanum vulgare* (B) and standards (C)

noPGRs – no added growth regulators; 01BA – 0.1 mg/L BA; 05BA – 0.5 mg/L BA; 1BA – 1.0 mg/L BA; 2BA – 2.0 mg/L BA; 4BA – 4.0 mg/L BA; 01BA+ IBA – 0.1 mg/L BA + 0.1 mg/L IBA; 05BA + IBA – 0.5 mg/L BA+ 0.5mg/L IBA; 1BA + IBA – 1.0 mg/L BA+ 0.1 mg/L IBA; 2BA + IBA – 2.0 mg/L BA+ 0.1 mg/L IBA; 4BA + IBA – 4.0 mg/L BA+ 0.1 mg/L IBA

4. CONCLUSION

Stimulatory effects of BA, on metabolite production, were species specific. Synergistic effect of IBA and BA depended upon their individual concentration as well as their ration. Accumulation of metabolites was detected on some treatments, which is individual for each species tested. Antimicrobial potential of oregano shoots was elevated and in some cases excided the antimicrobial potential of used standards.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Verpoorte R, Contin A, Memelink J. Biotechnology for the production of plant secondary metabolites. Phytochem Rev. 2002;1:13-252.
2. Croteau R, Kutchan TM, Lewis NG. Natural products (secondary metabolites). Biochem Mol Biol Plant. 2000;24:1250-1319.
3. Harrewijn P. Natural terpenoids as messengers: A multidisciplinary study of their production, biological functions, and

practical applications. Springer Science & Business Media, Heidelberg; 2001.
4. Vanisree M, Tsay HS. Plant cell cultures: Production of biologically important secondary metabolites from medicinal plants of Taiwan. Medicinal Plant biotechnology from basic research to industrial application WILEY-VCH Verlag GmbH & Co KGaA, Weinheim; 2007.
5. Byun SY, Pedersen H, Chin CK. Two-phase culture for the enhanced production of benzophenanthridine alkaloids in cell suspensions of *Eschscholtzia californica*. Phytochemistry. 1990;29:3135-3139.
6. Phillips D. Apical dominance. Ann Rev Plant Physiol. 1975;26:341-367.
7. Mulabagal V, Tsay HS. Plant cell cultures – an alternative and efficient source for the production of biologically important secondary metabolites. IJASER. 2004; 2:29-48.
8. Kirakosyan A, Kautman P, Warber S, Zick S, Aaronson K, Bolling S, Change SC. Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. Physiol Plant. 2004; 121:182-186.
9. Arencibia AD, Bernal A, Yang L, Cortegaza L, Carmona ER, Pérez A, Hua CJ, Li YR, Zayas CM, Santana I. New role of phenylpropanoid compounds during sugarcane micropropagation in temporary immersion bioreactors (TIBs). Plant Sci. 2008;175:487-496.
10. Chang SS, Ostric-Matijasevic B, Heish O AL, Haung CL. Natural antioxidant from rosemary and sage. J Food Sci. 1977;42:1102–1076.
11. Turner GW, Gershenzon J, Croteau RB. Distribution of peltate glandular trichomes on developing leaves of peppermint. Plant Physiol. 2000;124:655-664.
12. Sahoo Y, Pattnaik SK, Chand PK. In vitro clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation. In Vitro Cell Devel Biol Plant. 1997;33: 293–296.
13. Phippen WB, Simon JE. Anthocyanins in basil. J Agric Food Chem. 1998;46:1734-1738.
14. Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. Regulation of essential oil production in plants. Plant Growth Regul. 2001;34:3-21.
15. Ram D, Ram M, Singh R. Optimization of water and nitrogen application to menthol mint (*Mentha arvensis* L.) through sugarcane trash mulch in a sandy loam soil of semi-arid subtropical climate. Bioresour Technol. 2006;97:886-893.
16. Shetty K. Biotechnology to harmess the benefits of dietary phenols: Focus on lamiaceae. Asia Pacific J Clin Nutr. 1997;6:162-171.
17. Vieira RF, Grayer RJ, Paton A, Simon JE. Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. Biochem Syst Ecol Kidlington. 2001;29:287-304.
18. Prakash P, Neelu G. Therapeutic uses of Ocimum sanctum linn (tulsi) with a note on eugenol and its pharmacological Actions: A short review. Indian J Physiol Pharmacol. 2005;49:125-131.
19. Simon JE. Essential oils and culinary herbs. Timber Press, Portland; 1990.
20. Kokkini S. Taxonomy, diversity and distribution of origanum species. In: Padulosi, S. (ed.), Ciheam, Valenzano, Oregano proceedings of the International Plant Genetic Resources Institute (IPGRI); International Workshop on Oregano, 8 – 12 May, 1996. Bari, Italy.
21. Kumari N, Saradhi PP. regeneration of plants from callus culture of *Origanum vulgare* L. Plant Cell Rep. 1992;11:476-479.
22. Svoboda KP, Finch RP, Cariou E, Deans SG. Production of volatile oils in tissue culture of *Origanum vulgare* L. and *Tanacetum vulgare*. Acta Hort. 1995; 390:147-152.
23. Briant RE. An analysis of the effects of gibberellic acid on tomato leaf growth. J Exp Bot. 1974;25:764-771.
24. Hassan EA, El-Quesni FM. Application of growth regulators in agriculture a cytokinin-induced new morphogenetic phenomena in carnation (*Dianthus caryophyllus* L). Bull Fac Agric Cairo Univ. 1989;40:187-196.
25. Shudok K. Chemistry of phyenylurea cytokinins. In Cytokinins: Chemistry, activity and function; 1994.
26. Hassanein MA. Effect of some growth regulators and potassium feritilizers on growth, yield and essential oil production of geranium plants (*Pelargonium graveolens* L). M. Sc. Thesis, Fac. Agric, Cairo University; 1985.
27. Menesi FA, Nofal EMS, El-Mahrouk EM. Effect of some growth regulators on *Calendula officinalis.* L Egypt J Applied Sci. 1991;6:1-15.

28. Mazrou MM, Afify MM, El-Kholy SA, Morsy GA. Physiological studies on *Ocimum basilicum* plant. I. Influence of kinetin application on the growth and essential oil content. Menofiya J Agric Res. 1994; 19:421-434.
29. Eid RA Abou-Leila BH. Response of croton plants to gibberellic acid, benzyl adenine and ascorbic acid application. World J Agric Sci. 2006;2:174-179.
30. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant. 1962;15:473-497.
31. Porra R, Thompson W. Kriedemann P. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption. Int J Biochem Biophys Mol Biol. 1989;975:384-394.
32. Holm G. Chlorophyll mutations in barley. Acta Agricul Scand. 1954;4, 457-471.
33. Wolfe K, Wu X, Liu R. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51:609-614.
34. Ordoñez A, Gomez J, Vattuone M, Isla M. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem. 2006;97:452-458.
35. Popova M, Bankova VS, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini AG, Marcazzan GL, Bogdanov S. Validated methods for the quantification of biologically active constituents of poplar type propolis. Phytochem Anal. 2004; 15:235-240.
36. Gadzovska S, Maury S, Delaunay A, Spasenoski M, Joseph C, Hagège D. Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphtodianthrones. Plant Cell Tissue Organ Cult. 2007;89:1-13.
37. Larrauri JA, Rupereze P, Borroto B, Saura-Calixto F. Mango peels with high antioxidant activity. Eur Food Res Technol. 1997;205:39-42.
38. Elmastaş M, Gülçin Đ, Beydemir Ş, Küfrevioğlu ÖĐ, Aboul-Enein HY. A study on the *in vitro* antioxidant activity of juniper (*Juniperus communis* L.) seeds extracts. Anal Lett. 2006;39:47-65.
39. Bauer A, Kirby W, Sherris J, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;36:493-496.
40. Sardoei AS. Plant growth regulators effects on the growth and photosynthetic pigments on three indoor ornamental plants. J Exp Bot. 2014;4:311-318.
41. Rahbarian P, Sardoei AS, Imani AF. Stimulatory Effect of benzyladenine and gibberellic acid on growth and photosynthetic pigments of (*Spathiphyllum wallisii* Regel) plants. IJABBR. 2014;2:230-237.
42. Fletcher AR, McCullagh D. Cytokinin-Induced chlorophyll formation in cucumber cotyledons. Planta. 1971;101:88-90.
43. Aharoni N. Interrelationship between ethylene and growth regulators in the senescence of lettuce leaf discs. J Plant Grow Regul. 1989;8:309-317.
44. Hung KT, Kao CH. Hydrogen peroxide is necessary for abscisic acid-induced senescence of rice leaves. J Plant Physiol. 2004;161:1347-1357.
45. Kar M, Mishra D. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol. 1976;57: 315-319.
46. Prochazkova D, Sairam RK, Srivastava GC Singh DV. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Sci. 2001;161:765-771.
47. Szechyńska-Hebda M, Skrzypek E, Dąbrowska G, Biesaga-Kościelniak J, Filek M, Wędzony M. The role of oxidative stress induced by growth regulators in the regeneration process of wheat. Acta Physiol Plant. 2007;29:327-337.
48. Khann HK, Raina SK. Genotype x culture media interaction effects on regeneration response of three indica rice cultivars. Plant Cell Tissue Organ Cult. 1998;52:145-153.
49. Singh NK, Sehgal C. Micropropagation of "Holy basil" (*Ocimum sanctum* L.) from young inflorescens of mature plants. Plant Growth Regul. 1999;29:161-166.
50. Sharzad A, Siddiqui SA. *In vitro* organogenesis in *Ocimum sanctum* L. – A multipurpose heb. Phytomorphology. 2000; 50:27-35.
51. Rout GR, Samantaray S, Das P. *In vitro* manipulation and propagation of medicinal plants. Biotechnol Adv. 2000;18:91-120.
52. Dode LB, Bobrowski VL, Bolacel Braga EJ, Seixas FK, Schuch MW. *In vitro* propagation of *Ocimum basilicum* L.

(Lamiaceae). Acta Sci Biol Sci. 2003; 25:435-437.
53. Khan ZD, Tabassum Z. Effect of IAA, GA3 and adenosine on the growth of isolated epicotyl segments of *Zea mays* seedlings. Biologia. 1982;28:91-100.
54. Vajsadová H. Factors effecting seed germination and seedling growth of terrestrial ochrids cultured *in vitro*. Acta Biol Crac Ser Bot. 2006;48:109-113.
55. Kim HJ, Chen F, Wang X, Rajapakase NC. Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). J Agric Food Chem. 2006; 54:2327-3701.
56. North JJ, Ndakidemi PA, Laubscher CP. Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micropropagation of *Strelitzia reginae*. IJPS. 2012;7:638-646.
57. López V, Akerreta S, Casanova E, García-Mina JM, Cavero RJ, Calvo MI. *In vitro* antioxidant and anti-rhizopus activities of Lamiaceae herbal extracts. Plant Foods Hum Nutr. 2007;62:151-155.
58. Stamenković V, Jelenčić B, Tkalec M. Propagation and antioxidative enzymes of *Campanula velebitica* Borbas grown *in vitro*. Period Biol. 2012;114:77-82.
59. Pitta-Alvarez SI, Medina-Bolivar F, Alvarez MA, Scambatto AA, Marconi PL. *In vitro* shoot culture and antimicrobial activity of *Berberis buxifolia* Lam. *In vitro* Cell Dev Biol Plant. 2008;44:502-507.
60. Gibbons S. Anti-staphylococcal plant natural products. Nat Prod Rep. 2004; 21:263-277.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14490