

# Essential oils of basil cultivars selectively affect the activity of antioxidant enzymes in glial cells

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## Research Article

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**Essential oils of basil cultivars selectively affect the activity of  
antioxidant enzymes in glial cells**

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## Abstract

The qualitative and quantitative composition of essential oils (EO) of *Ocimum* species, cultivated in high altitude Armenian landscape was quite different and the main components of *O. basilicum* var. *purpureum*; *O. basilicum* var. *thyrsiflora* and *O. x citriodorum* oils belong to the class of oxygenated monoterpenes. Investigated EOs possess remarkable antioxidant activity. They inhibit the tyrosinase activity, the enzyme responsible not only for the melanin production, but also for various aging-related metabolic processes. Investigated EOs had no any significant effect on catalase at the protein levels, but alter its activity in neuroglial BV-2 different cell lines. Treatment of the neuroglial cell lines with the sub-cytotoxic concentrations of three mentioned EOs influence also the activity of acetyl-CoA oxidase type 1.

## Practical Applications

The microglial cells play a pivotal role as the neuroprotective agents against neuroinflammation. Different data included in the present article are stating that plant origin substances can play a role of regulators of enzymatic antioxidant capacity of cells. EOs extracted from the *Ocimum* different cultivars are able to trigger the activity of acetyl-CoA oxidase type 1 (or palmytoil-CoA oxidase type 1), which can serve as a basis of regulation of redox deviation in WT cells. So, it can be suggested them to be applied for the prevention of some processes, which can influence on the aging, as the process of ageing is commonly associated with mitochondrial dysfunction, oxidative stress caused by the increased level of free radical production, dysfunction of the microglia, high blood pressure and so on.

**Key words:** *Ocimum*; essential oil; oxygenated monoterpenes; microglia; palmytoil-CoA oxidase

## 1. Introduction

Plant species of Lamiaceae family are known for their application as spice and medicinal plants [1; 2]. The most common and widely used are aromatic plants of the *Ocimum* genus [3; 4]. They are rich source of essential oils (EO) and used to be applied since antiquity for different purposes, including for the prevention and treatment of various diseases [5; 6]. The recent studies state, that basil cultivars possess large spectrum of biological activity. Generally, the chemical composition and biological activity of basil may vary, depending on the ecological and soil conditions, cultivar, growing elevation, cultivation approaches and other parameters [7; 8]. Our previous studies state, that the chemical composition of EO extracted from the basil cultivars cultivated in high altitude of Armenian landscape varies from those, described in literature [7 - 11]. According to our recently published data *Ocimum basilicum* var. *purpureum* (purple basil) belongs to the methyl chavicol-rich chemotype (57.3%). The *O. basilicum* var. *thyrsiflora* (or thai basil) belongs to the linalool-rich chemotype (concentration of linalool was 68%). The *O. x citriodorum* produces two predominant components – citral (21%) and nerol (23%) and forms special chemotype. All of investigated basil cultivars possess expressed antimicrobial and antioxidant properties [3]. Focusing on these data, we aimed to reveal some mechanisms of influence of all three basil EO on the microglial cells, as recent-years research data state that these oils have anti-aging and neuroprotective action, and as it is supposed, against also some forms of neurodegenerations [12; 13]. The process of ageing is commonly associated with complex changes in the organism such as mitochondrial dysfunction, oxidative stress caused by the increased level of free radical production, dysfunction of the microglia, low levels of neurogenesis and so on [14; 15; 16; 17]. Sometimes aging is associated with different types of neurodegenerative disorders [18]. One of the mechanisms which underlie the neuroprotective

activity of any metabolite is the ability to inhibit the tyrosinase or tyrosine hydroxylase activity, which can prevent the high-blood pressure [19]. These enzymes catalyze the synthesis of dopamine in melanocytes which transports to the neuroglial and adrenal cells with blood flow, where a copper-dependant enzyme dopamine  $\beta$ -oxidase, in the presence of vitamin C, causes its hydroxylation to catecholamine transmitters (norepinephrine and epinephrine), which are able to increase the blood pressure [19; 20]. Some researchers mention, that various models of neurodegenerative disorders are useful for studying the neuroprotective activity of biologically active metabolites. The oxidative neuronal damage in the ageing brain is also associated with the decline in the production and functional activity of antioxidant enzymes [21].

So, we decided to evaluate the influence of the essential oils extracted from three basil cultivars (*O. basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora*, and *O. x citriodorum*), possessing remarkable antioxidant capacity on the activity of the main antioxidant enzymes in microglial BV-2 wild type (WT) and Acetyl-CoA oxidase deficient cell lines (*Acox1*<sup>-/-</sup>).

## 2. Materials and Methods

### *Plant material*

The basil cultivars (*O. basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora*, and *O. x citriodorum*) were grown in the Kotayk Region of Armenia as it was described previously (1600 m a.s.l.) [3]. Plant materials were collected during blossoming period (July–August, 2014) and were identified at the Institute of Botany, National Academy of Sciences of Armenia, Yerevan (Armenia) by Dr. M. Sargsyan. The samples of basil cultivars are also available at the Faculty of Biology, Yerevan State University, Yerevan, Armenia.

### *BV-2 microglia cell culture*

Murine microglial BV-2 cell lines (BV-2, Acyl-CoA oxidase type 1 (ACOX1) deficient mutants (*Acox1*<sup>-/-</sup>) and WT cells) were grown in a 5% CO<sub>2</sub> incubator at 37 °C in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) and 1% antibiotics (penicillin, streptomycin); culture medium was changed every 2 days. BV-2 cells were seeded on 96-well microplates at  $25 \times 10^4$  cells per well for viability assay, 6-well microplates at  $5 \times 10^5$  cells per well - for enzymatic activity.

### ***Essential oil extraction***

Essential oils were extracted from air dried plant material (aerial parts only) by hydro-distillation, using a Clevenger-type apparatus, and the process lasted 3 h as described before [3].

### ***Determination of essential oil chemical composition***

The gas chromatography (GC) mass selective (MS) analysis of the essential oils was performed using a Hewlett–Packard 5890 Series II gas chromatograph, fitted with a fused silica HP – 5MS capillary column (30 m  $\times$  0.25 mm, in thickness 0.25  $\mu$ m). Determination of chemical composition was carried out as described previously [3]. The oven temperature varied from 40–250 °C with the scanning rate of 3 °C/min. Helium (purity 5.6) was used as a carrier gas at a flow rate of 1 mL/min. The GC was equipped with Hewlett–Packard 5972 Series MS detector. The MS operating parameters were ionization voltage 70 eV and ion source temperature 250 °C. The diluted samples of essential oils (1/100, v/v in HPLC methanol) of 1  $\mu$ L had been injected manually. To avoid overloading the GC column, the essential oils were diluted 1:100 (v/v) in methanol. The identification of peaks was tentatively carried out based on library search using National Institute of Standards and Technology (NIST)-2013. Relative Retention Index (RRI) was calculated for HP-5MS column. For RRI calculation a mixture of homologues *n*-alkanes

(C9-C18) was used under the same chromatographic conditions as for analysis of the essential oils.

### ***3-(4,5-dimethyltrazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay***

Cells, plated in 96-wells plates, were treated for 24-72 h with different concentrations of EO (50 – 5.10<sup>-4</sup> µLmL<sup>-1</sup> in dimethylsulfoxide). Cells were incubated for 2 h with MTT dye, followed by the absorbance (Abs) measurement at the 570 nm with a microplate reader and the sub-cytotoxic concentration was selected for further investigations [22].

### ***Preparation of BV-2 cell lysate***

After treatment of BV-2 microglia cells with EO, cells were washed with Phosphate-buffered saline (PBS) and were lysed in 50 µL of radioimmunoprecipitation (RIPA) buffer: 50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.5% Na-deoxycholate, 0.1% sodium dodecylsulfate, 150 mM NaCl, 2 mM ethylenediamine tetraacetic acid (EDTA), 50 mM NaF. Cells were placed in ice for 30 min and the lysate was cleared by centrifugation at 20,000× g for 20 min (at -4°C). Protein content was assessed by a bicinchoninic acid assay [23]. The supernatant was stored at -80 °C until further use, if needed.

### ***Enzymatic activity measurement***

For catalase activity measurement, 10 µL of cell lysate was added to 190 µL of Tris-HCl buffer (pH 7.4) containing 20 mM H<sub>2</sub>O<sub>2</sub> and the decrease of the absorbance was monitored at 240 nm for 2 min [24]. The change in absorbance with time was proportional to the breakdown of H<sub>2</sub>O<sub>2</sub>. The catalase activity was expressed as units/mg of protein. ACOX1 activity measurement was performed, as described by Oaxaca-Castillo et al. (2007) [25] using fresh cell lysate. Total SOD activity was measured according to the Beauchamp and Fridovich (1971) [26].

### ***Data processing***

Experimental data were expressed as the mean  $\pm$  SD of all repetitions. Values were normalized to the control and are given as average of the repetitions. A statistical analysis was done with the Student-t test for calculating the probability values; and data were considered statistically different at a  $p$ -value of 0.05 or less.

### 3. Results

According to the data, obtained from the quantitative and qualitative analysis of the investigated essential oils more than 40 compounds were isolated and most of them identified for each essential oil sample. All dominant components were determined as oxygenated monoterpenes (linalool, methyl chavicol, nerol and geranial) (Table 1).

Regarding to our investigations, the sub-cytotoxic concentrations of essential oils were identified which was 0.5  $\mu$ L/mL for *O. basilicum* var. *purpureum* and *O. basilicum* var. *thyrsoflora* essential oils, but for the *O. x citriodorum* essential oil this parameter was higher twice (1  $\mu$ L/mL) for both microglial cell lines ( $p < 0.05$ ) (Fig 1 a-f). Further treatment of neuroglial cells was carried out with the mentioned concentrations of the investigated essential oils for excluding the toxic influence of investigated acting agent and considers only its activity on cellular enzymatic antioxidant system.

Under the treatment of BV-2 cells with the mentioned concentrations the viability of cells was not changed significantly approximately in all cases (Fig. 2) ( $p \leq 0.05$ ).

Data showed that treatment of WT cells during the 24 hour with *O. basilicum* var. *thyrsoflora* brings to the significant decreasing in the catalase activity (up to 95 %) ( $p < 0.005$ ). But in case of treatment with *O. basilicum* var. *purpureum* the tendency of the increasing was observed ( $p < 0.05$ ), meanwhile the future treatment of WT cells did not show any significant changes in catalase activity ( $p > 0.05$ ). Treatment of *Acox1*<sup>-/-</sup> cells with essential oils did not affect the activity of catalase (Fig. 3).



160 According to the Saih et al. (2017) [27], the suppression of ACOX1 activity in microglia cells and its  
161 consequence on the peroxisomal capacity in the  $\beta$ -oxidation of VLCFA may have a deleterious effect on  
162 brain functions and must be associated to neuroinflammation and neurodegeneration in peroxisomal  
163 leukodystrophies, including ACOX1 deficiency. 48-hour treatment of BV-2 WT microglia cells with *O.*  
164 *basilicum* var. *thyrsiflora* increases the palmytoil-CoA oxidase type 1 activity up to 90 % without  
165 significant changes in cell viability under the same conditions. The activity of this enzyme increases  
166 around 69 % under the influence of the treatment with *O. basilicum* var. *purpureum* EO during the same  
167 period, but the EO of *O. x citriodorum* had not any significant effect. The activity of ACOX1 decreased  
168 during the further treatment with all studied EOs, despite in control samples, where some increasing was  
169 observed (Fig. 2, Fig. 4). The treatment with *O. basilicum* var. *purpureum* brings to the significant  
170 changes in the SOD activity of BV-2 WT cells ( $p < 0.05$ ), but there were not any statistically significant  
171 data in other cases (Fig. 5).

#### 172 4. Discussion

173 As described above, the oxygenated monoterpenes are the main substances in the composition of  
174 EO of all three *Ocimum* cultivars. There is not any direct evidence, which will be able to state whether  
175 the described biological activity is due to these substances or not. But one thing is clear, that even small  
176 deviations in the chemical composition of any EO can alter their activity [3; 28]. So, the aim of this  
177 work was not only the indication of the activity of these EOs, but also to find a common mechanism or  
178 feature of influence of these substances which can be considered in their practical application in the  
179 future. There is a huge number of literature data stating that EOs generally express the antibacterial and  
180 antioxidant action [3; 28; 29]. Our previous investigations also showed that basil EOs possess expressed  
181 antiradical capacity: the highest activity possesses *O. basilicum* var. *thyrsiflora* EO with the  $IC_{50}$  value  
182 of 2.5  $\mu$ L/mL, which was equal to the standardized Grapefruit Seed Extract. The same activity for other

183 investigated oils was approximately 8-10 times lower [3]. This data seem to be unexpected, since  
184 usually the oils with higher phenolic content are the ones exhibiting higher radical scavenging abilities,  
185 but it is also known that usually substances possessing high antimicrobial activity express low or  
186 moderate antiradical activity in different tests, depending on their action mechanisms [30]. Our previous  
187 investigations showed the high antibacterial activity of *O. basilicum* var. *purpureum*, and *O. x*  
188 *citriodorum* [3]. Having all the mentioned, as well as data concerning tyrosinase activity inhibition  
189 capacities of investigated EO (the values for tyrosinase inhibitory activity of *O. basilicum* var.  
190 *thrysiflora*, *O. basilicum* var. *purpureum* and *O. x citriodorum* essential oils varied between the 12 and  
191 20 %) [28], we decided to check the influence of all three basil EO on the microglia cell lines. The MTT  
192 test expresses the mitochondrial function and viability of BV-2 cells [27] under the influence of  
193 investigated essential oils. Several possible mechanisms of antimicrobial activity are discussed, but  
194 information on possible mechanisms of antioxidant action in living systems is quite scarce. Our previous  
195 data were evaluated as a basis for the further investigations which are stating that the components of the  
196 investigated EOs may alter the activity of different enzymes in microglia cells without any visible effect  
197 on protein level (see above). We are observing some decreasing in catalase activity in most cases, which  
198 speaks about the fact that there is not any redox misbalance during the treatment of microglia cells with  
199 the investigated EOs. Interestingly, the 48-hour treatment is bringing to the activation of palmytoil-CoA  
200 oxidase type 1 in approximately all cases. This feature as well as our data stating that in the same  
201 conditions the tyrosinase activity inhibition can be achieved, could serve as a basis for the statement that  
202 the investigated EOs have neuroprotective action.

203 As the microglial cells are the resident macrophages of the central nervous system, they play a  
204 pivotal role as the neuroprotective agents against neuroinflammation. Different data included in the  
205 present article are stating that plant origin substances can play a role of regulators of enzymatic

206 antioxidant capacity of cells. EOs extracted from the *Ocimum* different cultivars are able to trigger the  
207 activity of acetyl-CoA oxidase type 1 (or palmytoil-CoA oxidase type 1), which can serve as a basis of  
208 regulation of redox deviation in WT cells. So, it can be suggested them to be applied for the prevention  
209 of some processes, which can influence on the aging, as the process of ageing is commonly associated  
210 with mitochondrial dysfunction, oxidative stress caused by the increased level of free radical production,  
211 dysfunction of the microglia, high blood pressure and so on.

## 212 **Declarations**

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221 **Conflicts of interest:** Not applicable

222 **Ethics approval:** Not applicable

223 **Availability of data and material:** Data and materials are available upon request

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312 [017-1582-7](https://doi.org/10.1007/s11094-017-1582-7)

313

| Class of compounds | Chemical component    | R R I <sup>a</sup> | <i>O. basilicum</i> var. <i>purpureum</i> , % | <i>O. basilicum</i> var. <i>thyrsiflora</i> , % | <i>O. x citriodorum</i> , % |
|--------------------|-----------------------|--------------------|---|---|-----------------------------|
| Monoterpene        | (Z)- $\beta$ -Ocimene | 1058               | -   | -   | 0.2                         |

|                               |                           |      |      |      |      |
|-------------------------------|---------------------------|------|------|------|------|
| hydrocarbones                 | $\gamma$ -Terpinene       | 1078 | -    | -    | 0.2  |
| Oxygenated<br>monoterpenes    | 1,8-cineole               | 1035 | 1.4  | 3.5  | -    |
|                               | Fenchone                  | 1089 | -    | -    | 0.3  |
|                               | Linalool                  | 1100 | 18.0 | 68.0 | 9.4  |
|                               | Camphor                   | 1146 | 1.3  | 1.4  | -    |
|                               | $\alpha$ -Terpineol       | 1181 | -    | -    | 0.6  |
|                               | Methyl chavicol           | 1203 | 57.3 | 20.0 | 9.4  |
|                               | Nerol                     | 1231 | -    | -    | 23.0 |
|                               | Neral                     | 1244 | -    | -    | 4.9  |
|                               | Geraniol                  | 1259 | -    | -    | 5.2  |
|                               | Geranial                  | 1274 | -    | -    | 15.8 |
|                               | Bornyl acetate            | 1291 | 0.1  | -    | -    |
| Aromatic<br>compounds         | Neryl acetate             | 1321 | -    | -    | 0.7  |
|                               | Methyl cinnamate          | 1338 | -    | -    | 0.5  |
| Sesquiterpene<br>hydrocarbons | $\beta$ -Elemene          | 1387 | 3.6  | 0.7  | 0.5  |
|                               | $\beta$ -Caryophyllene    | 1419 | 1.7  | -    | 7.8  |
|                               | $\beta$ -Copaene          | 1428 | 0.2  | -    | 0.6  |
|                               | $\alpha$ -(Z)-Bergamotene | 1433 | 4.3  | 1.3  | 3.5  |
|                               | $\alpha$ -humulene        | 1455 | 0.6  | 0.3  | 1.5  |
|                               | cis- $\beta$ -Farnesene   | 1472 | 0.3  | -    | 0.5  |
|                               | Germacrene-D              | 1482 | 0.7  | 0.2  | -    |
|                               | $\beta$ -Cubebene         | 1497 | -    | 0.8  | 2.3  |
|                               | $\alpha$ -Bulnesene       | 1502 | 1.4  | 0.7  | 0.5  |
|                               | $\alpha$ -Amorphene       | 1510 | 1.5  | 0.7  | -    |
|                               | $\delta$ -cadinene        | 1518 | -    | -    | 0.4  |



|                           |                            |      |     |     |     |
|---------------------------|----------------------------|------|-----|-----|-----|
|                           | Aromadendrene              | 1529 | 1.7 | 0.3 | -   |
|                           | iso-Caryophyllene<br>oxide | 1550 | 0.6 | -   | -   |
|                           | $\alpha$ -Bisabolene       | 1561 | -   | -   | 2.3 |
| Sesquiterpene alc<br>ohol | Spathulenol                | 1544 | 0.7 | -   | -   |

<sup>a</sup>for HP-5 capillary column

Table 1. Chemical composition of EOs of *O. basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora* and *O. x citriodorum*

**Fig.1** Effects of *O. basilicum* var. *purpureum* and *O. basilicum* var. *thyrsiflora* and *O. x citriodorum* essential oils on viability of BV-2 WT and ACOX1 deficient microglial cells (MTT assay; a-c and d-f, respectively). Cells were treated for 24 h with EO at different concentrations (100 to 0.001  $\mu$ L/mL). The significance is presented with the Student-t test:  $p < 0.05$  for BV-2 both cell lines.

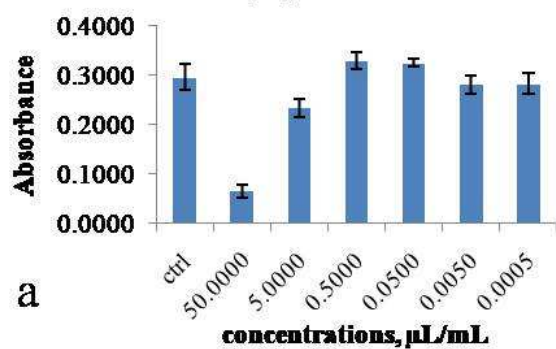
**Fig.2** *Ocimum* species EO influence dynamics on viability of BV-2 WT and Acox1 deficient microglia cells (MTT assay; A and B, respectively). The results were normalized to the control and the significance is presented with the Student-t test:  $p \leq 0.05$  for BV-2 both cell lines.

**Fig. 3** EOs influence dynamics on catalase activity of BV-2 WT and Acox1 deficient microglial cells (A and B, respectively). In case of *O. basilicum* var. *thyrsiflora*  $p < 0.005$ ;  $p > 0.05$  for \*, for \*\* and \*\*\*; in case of *O. basilicum* var. *purpureum*  $p < 0.05$  for \* for \*\* and \*\*\*  $p > 0.05$ ; in case of *O. x citriodorum*  $p > 0.05$  for all cases (for WT BV-2 cells); in case of *O. basilicum* var. *thyrsiflora*  $p < 0.05$  for \* and for \*\*; in case of *O. basilicum* var. *purpureum*  $p > 0.05$  for \* and ;  $p < 0.05$  for \*\*; in case of *O. x citriodorum*  $p > 0.05$  for all cases (for *Acox1*<sup>-/-</sup> BV-2).

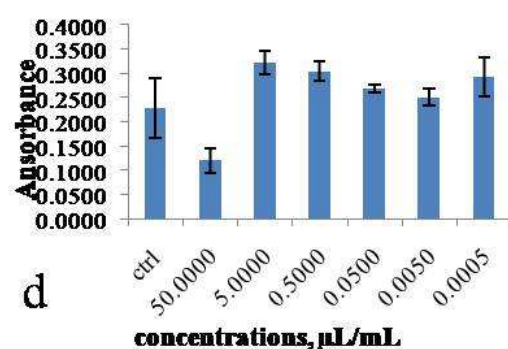
331 **Fig. 4** EOs influence dynamics on palmytoil-CoA oxidase type 1 activity of BV-2 WT cells,  $p < 0.05$ .  
332 **Fig. 5** EOs influence dynamics on SOD activity of BV-2 WT and Acox1 deficient microglial cells. In  
333 case of *O. basilicum* var. *thyrsiflora*  $p > 0.1$  for \*;  $p > 0.1$  \*\* and  $p < 0.05$  for \*\*\*; in case of *O.*  
334 *basilicum* var. *purpureum*  $p < 0.05$  for \*, for \*\* and \*\*\*, in case of *O. x citriodorum*  $p > 0.05$  for all  
335 cases (WT cells). In case of *O. basilicum* var. *thyrsiflora* and *O. basilicum* var. *purpureum*  $p > 0.05$  for  
336 \*;  $p < 0.05$  for \*\* and  $p = 0.1$  for \*\*\*; in case of *O. x citriodorum*  $p < 0.05$  for \*;  $p = 0.1$  for \*\* and  $p <$   
337  $0.05$  for \*\*\* (*Acox1*<sup>-/-</sup> cells).

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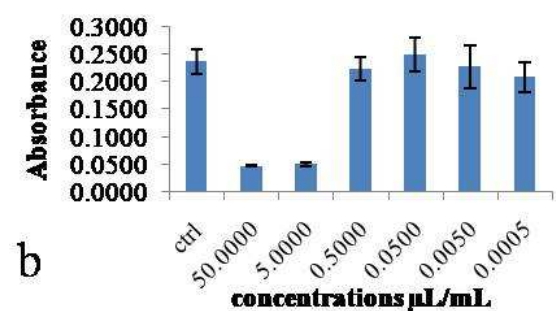
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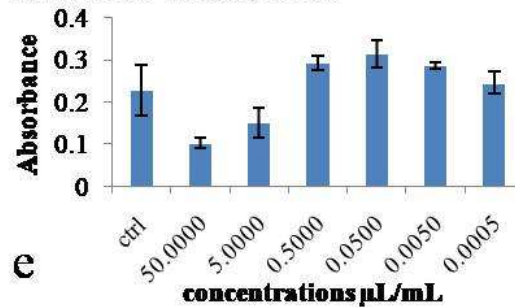
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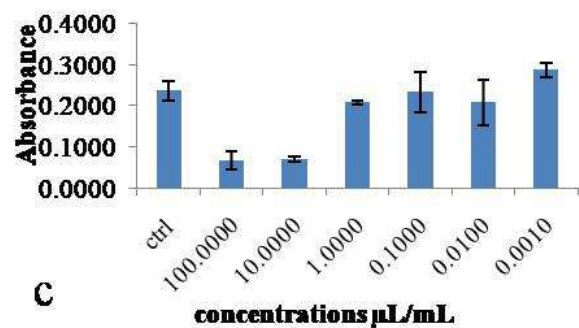
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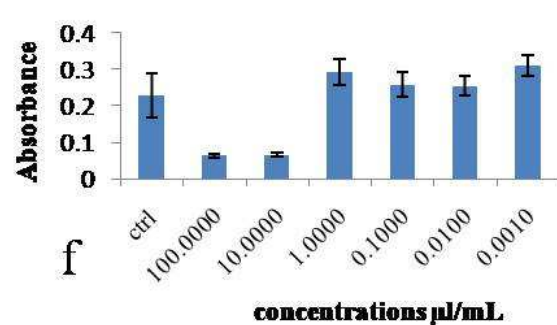
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***O. x citriodorum***



***O. x citriodorum***



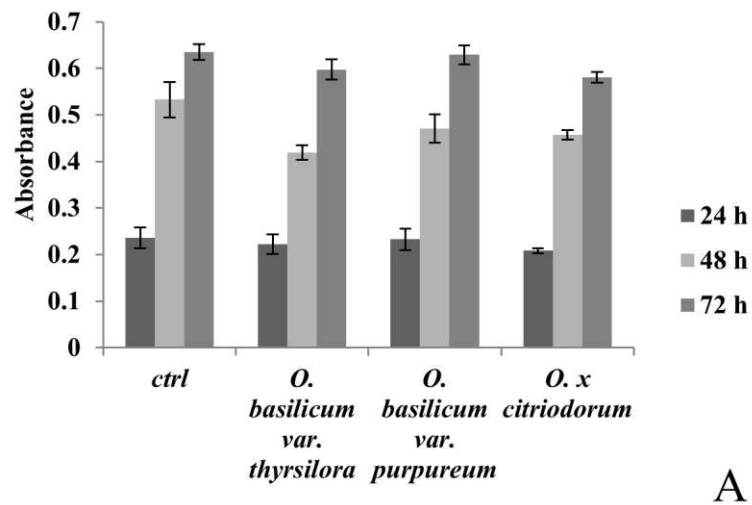
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340 Fig. 1

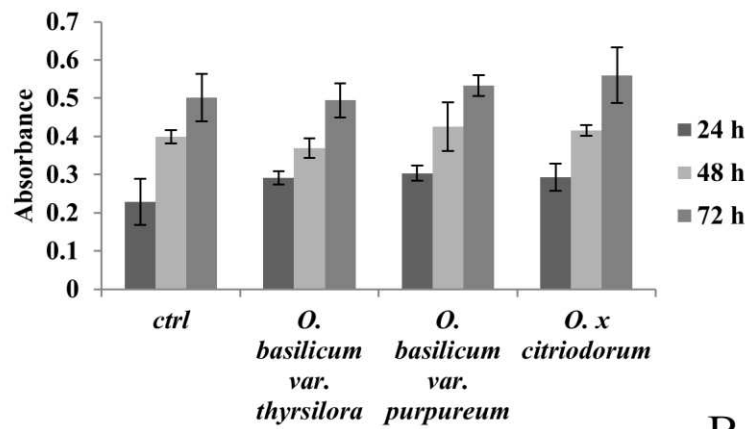
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A



B

Fig. 2

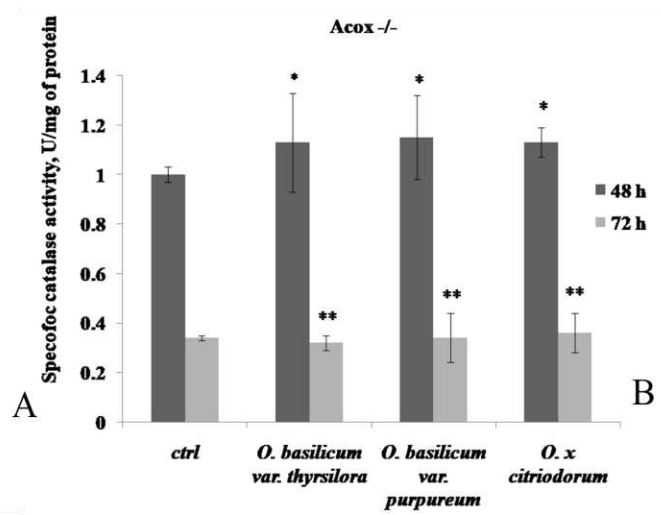
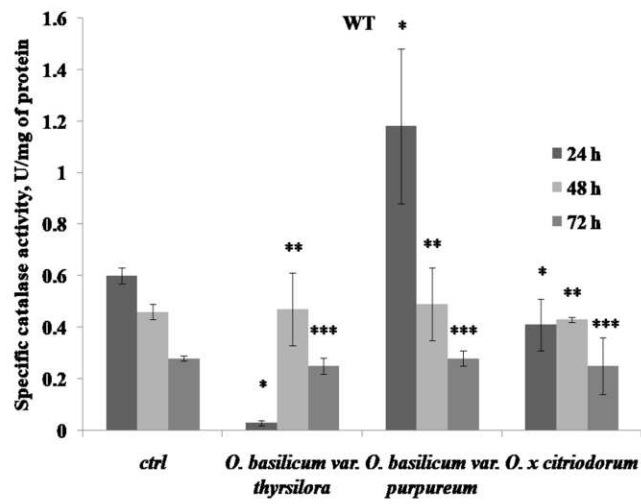


Fig. 3

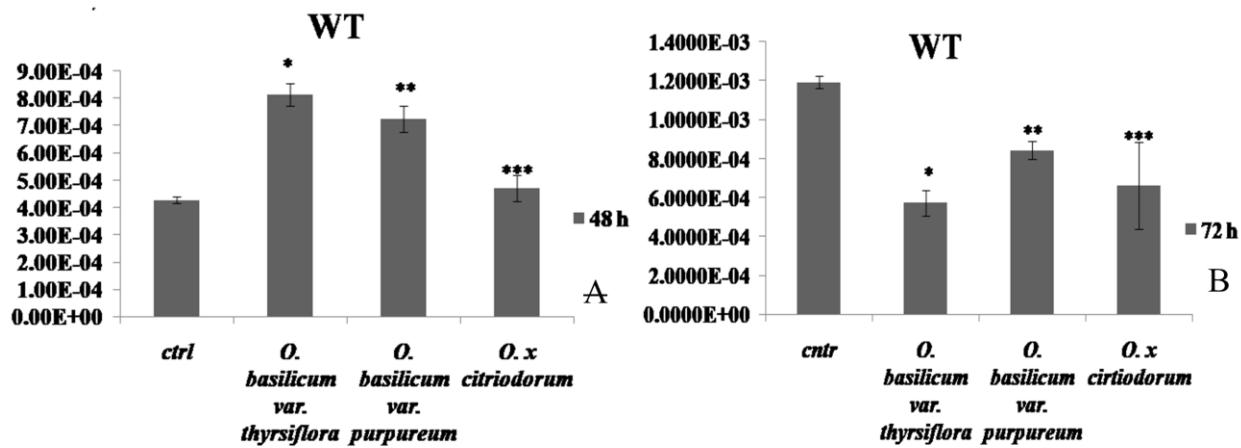


Fig. 4

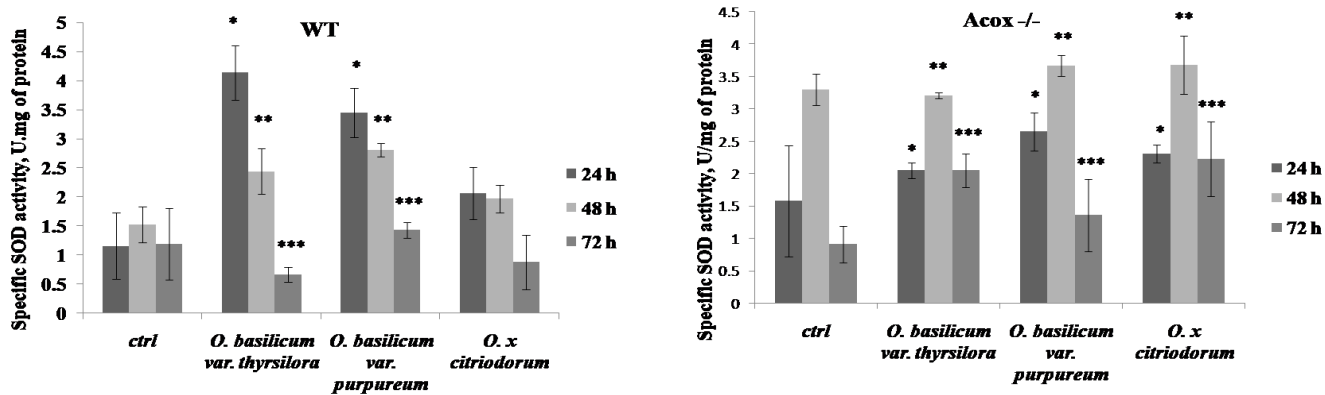
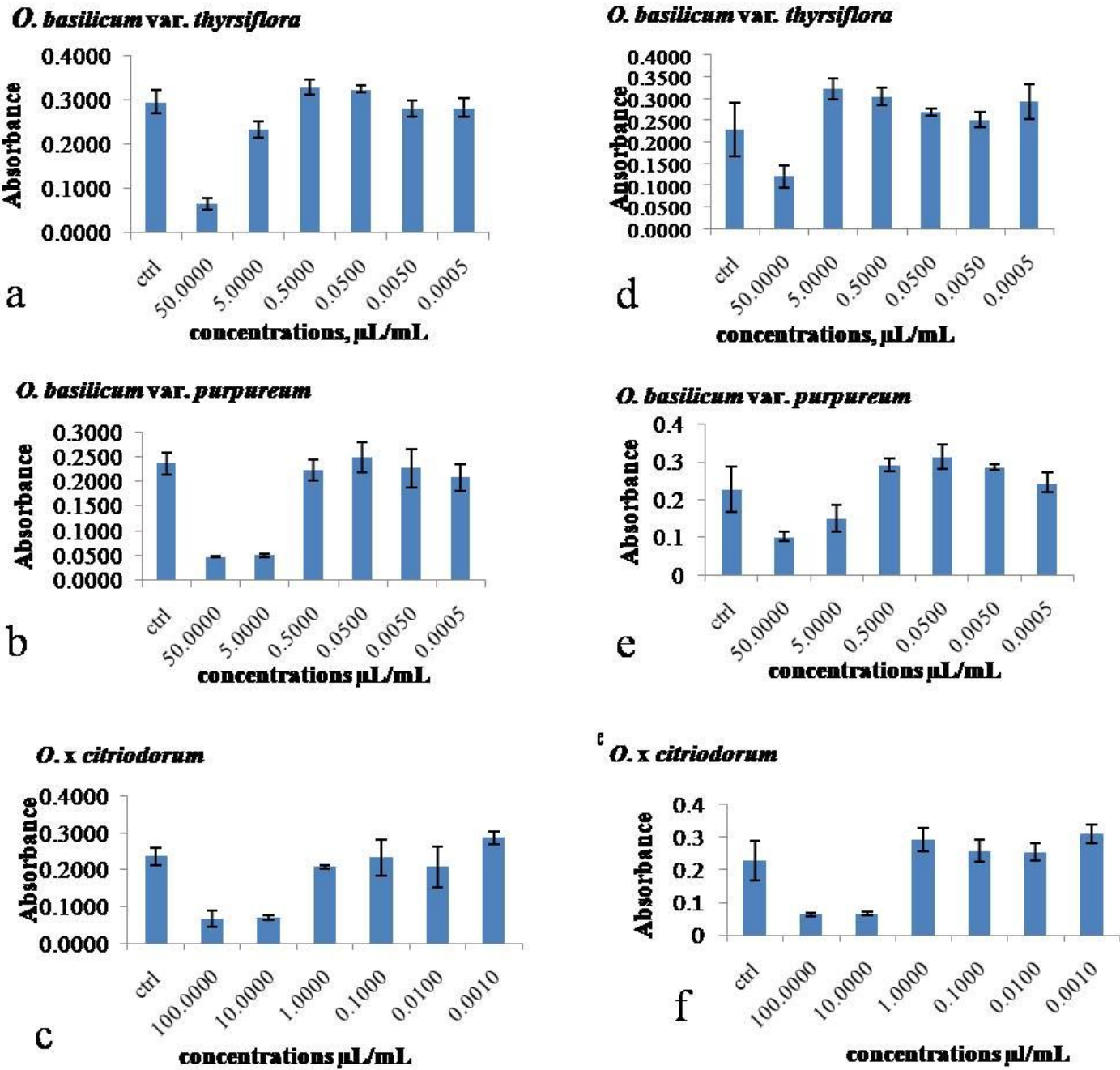


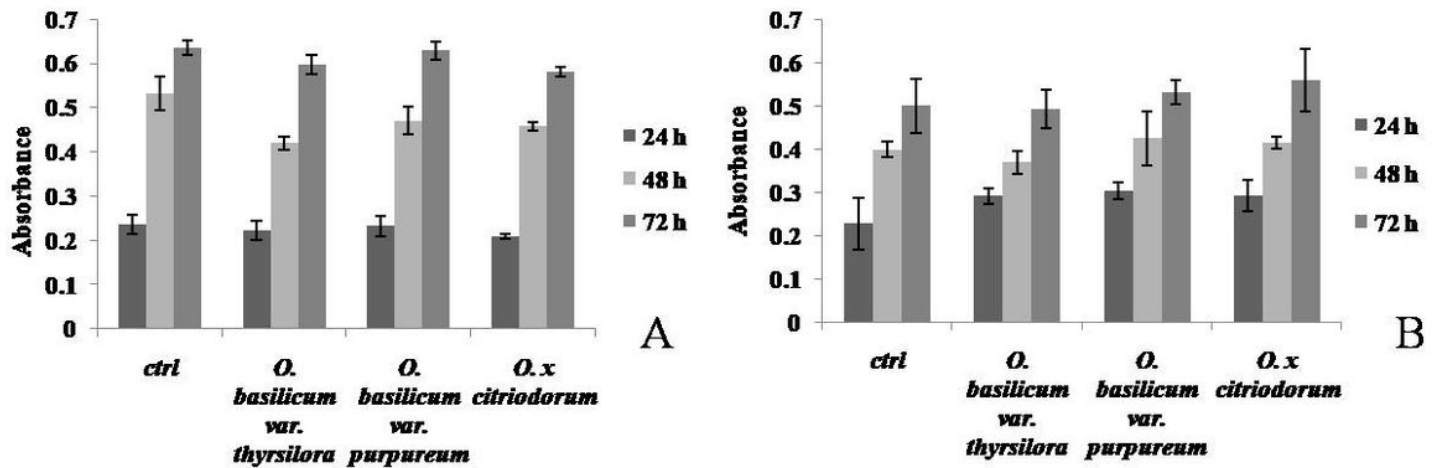
Fig. 5

# Figures



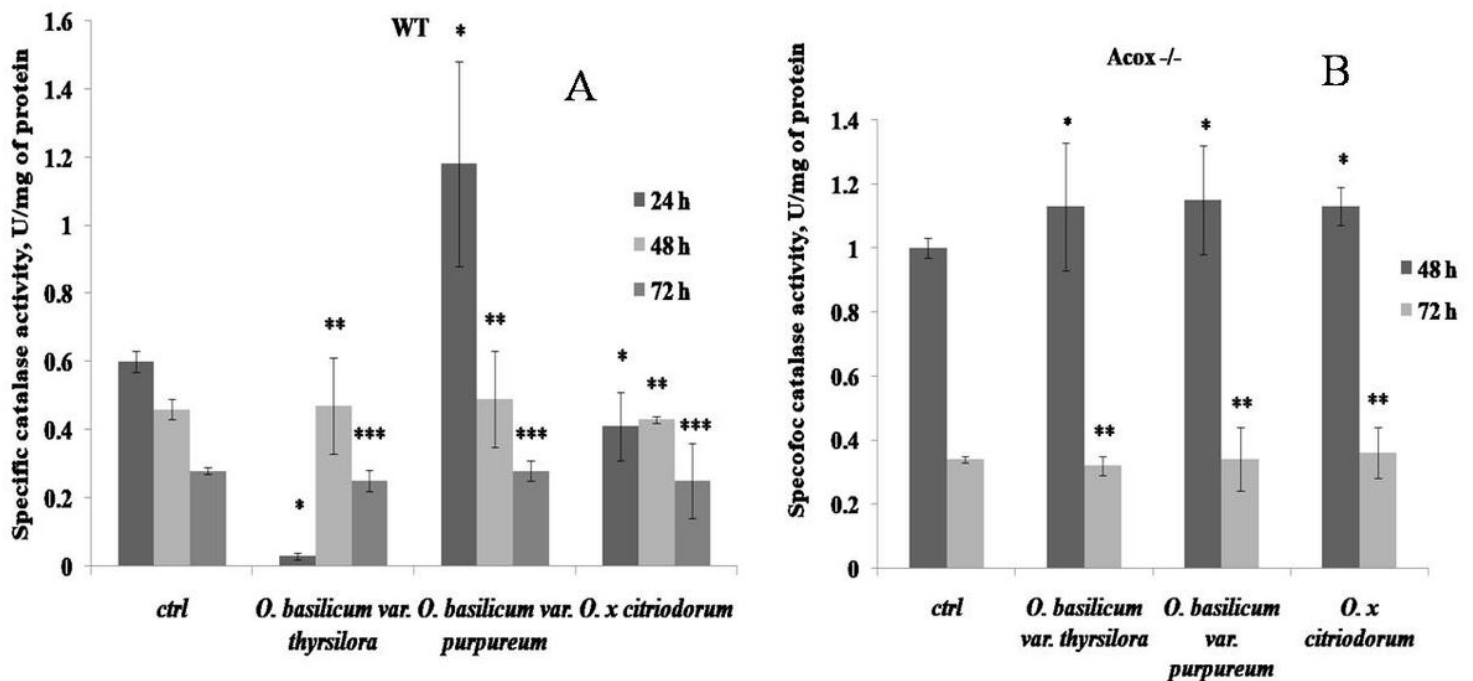
**Figure 1**

Effects of *O. basilicum* var. *purpureum* and *O. basilicum* var. *thyrsoiflora* and *O. x citriodorum* essential oils on viability of BV-2 WT and ACOX1 deficient microglial cells (MTT assay; a-c and d-f, respectively). Cells were treated for 24 h with EO at different concentrations (100 to 0.001  $\mu\text{L/mL}$ ). The significance is presented with the Student-t test:  $p < 0.05$  for BV-2 both cell lines.



**Figure 2**

Ocimum species EO influence dynamics on viability of BV-2 WT and Acox1 deficient microglia cells (MTT assay; A and B, respectively). The results were normalized to the control and the significance is presented with the Student-t test:  $p \leq 0.05$  for BV-2 both cell lines.



**Figure 3**

EOs influence dynamics on catalase activity of BV-2 WT and Acox1 deficient microglial cells (A and B, respectively). In case of *O. basilicum* var. *thyrslora*  $p < 0.005$ ;  $p \leq 0.05$  for \*, for \*\* and \*\*\*; in case of *O. basilicum* var. *purpureum*  $p < 0.05$  for \* for \*\* and \*\*\*  $p \leq 0.05$ ; in case of *O. x citriodorum*  $p \leq 0.05$  for all cases (for WT BV-2 cells); in case of *O. basilicum* var. *thyrslora*  $p < 0.05$  for \* and for \*\*; in case of *O.*



basilicum var. purpureum  $p \geq 0.05$  for \* and ;  $p < 0.05$  for \*\*; in case of *O. x citriodorum*  $p \geq 0.05$  for all cases (for Acox1-/- BV-2).

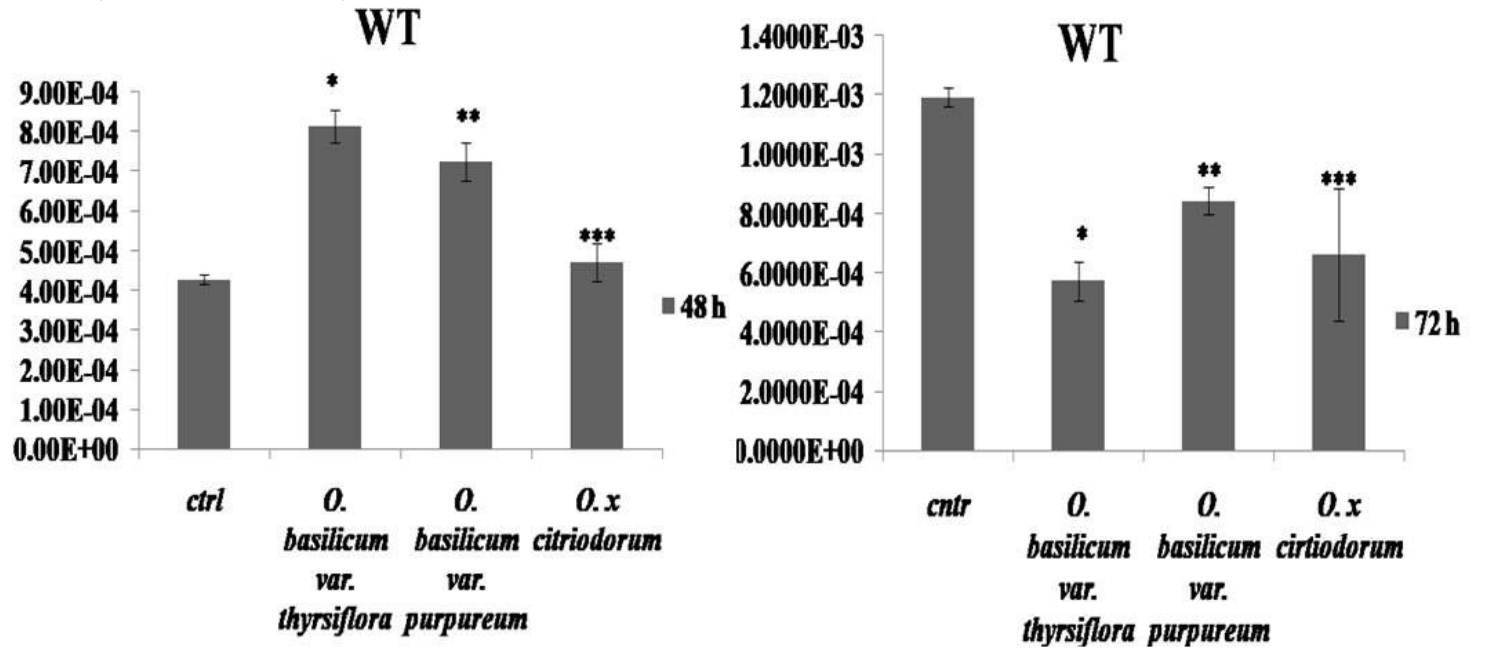


Figure 4

EOs influence dynamics on palmytoil-CoA oxidase type 1 activity of BV-2 WT cells,  $p < 0.05$ .

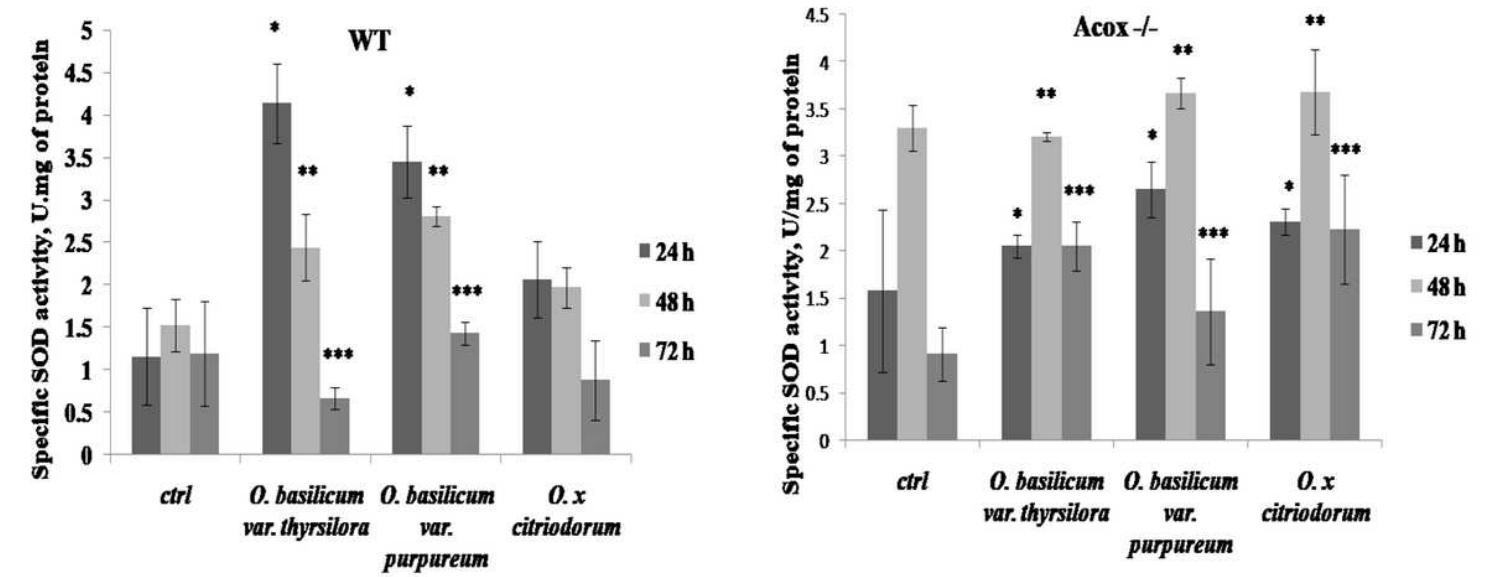


Figure 5

EOs influence dynamics on SOD activity of BV-2 WT and Acox1 deficient microglial cells. In case of *O. basilicum* var. *thrysiflora*  $p \geq 0.1$  for \*;  $p \geq 0.1$  \*\* and  $p < 0.05$  for \*\*\*; in case of *O. basilicum* var. *purpureum*  $p < 0.05$  for \*, for \*\* and \*\*\*; in case of *O. x citriodorum*  $p \geq 0.05$  for all cases (WT cells). In case of *O. basilicum* var. *thrysiflora* and *O. basilicum* var. *purpureum*  $p \geq 0.05$  for \*;  $p < 0.05$  for \*\* and  $p = 0.1$  for \*\*\*; in case of *O. x citriodorum*  $p \geq 0.05$  for \*;  $p = 0.1$  for \*\* and  $p < 0.05$  for \*\*\* (Acox1-/- cells).