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# DYNAMICS AND FUNCTION OF THE REDOX REGULATORY NETWORK OF PLANTS UNDER STRESS

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Environmental changes have profound impacts on biological systems, triggering a cascade of enzymatic and chemical reactions, molecular rearrangements, and alterations in molecular interactions. In the face of environmental stressors, plants employ various protective mechanisms to maintain cellular homeostasis. Reactive oxygen species (ROS), generated during metabolic processes, serve as signaling molecules regulating plant metabolic pathways. To counteract the detrimental effects of ROS accumulation, plants possess robust ROS-scavenging mechanisms. These encompass enzymatic antioxidants such as superoxide dismutase, catalase, monodehydroascorbate, peroxiredoxins, alongside non-enzymatic antioxidants like ascorbic acid, α-tocopherols, glutathione, proline, phenolic substances, and carotenoids. However, under stress conditions and toxin exposure, ROS generation escalates within plant cells, particularly in organelles like chloroplasts, peroxisomes, and mitochondria, leading to oxidative stress and cellular damage.

Studies employing gas chromatography-mass selective analyses revealed significant concentrations of methyl chavicol and menthol in essential oils extracted from select plant species. *In vitro* studies utilizing PAM-fluorometric data demonstrated optimal treatment periods for *Arabidopsis thaliana* leaf disks, aiding in elucidating potential allelochemical action mechanisms. Redox gel electrophoresis coupled with immune analysis facilitated the assessment of the redox status of key proteins *in vivo*, notably chloroplast peroxiredoxin (2-CysPrx), pivotal in photosynthetic regulation. Investigation into the redox status of 2-CysPrx under essential oil treatments unveiled menthol-induced oxidation and subsequent dimerization of the enzyme, suggesting a disruption in photosynthetic processes.

Non-protein thiols (NPT), including glutathione (GSH), play vital roles in plant stress tolerance. Deviations in NPT concentrations serve as indicators of cellular redox status. Notably, treatment with menthol resulted in a statistically significant decrease in NPT levels, corroborating findings of altered peroxiredoxin redox states and implying perturbations in cellular homeostasis.

Moreover, NIR KLAS 100 data highlighted allelochemical influences on the photosynthetic apparatus of *A. thaliana* wild-type and mutant plants, further elucidating the impacts of these compounds on plant physiological processes.

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**Introduction.** Usage of synthetic herbicides in agriculture brought to weed resistance development. The main reason for this evolution is the long-term use of herbicides with one target site in plants. Inappropriate adjustment of herbicides to weed species, application of these substances at the irrelevant developmental stage and in unsuitable weather conditions, leaded to the accumulation of active compounds in the soil and acceleration of the evolution of resistant biotypes [1]. Most of weed species are resistant to acetyl CoA carboxylase, photosystem II and, acetolactate synthase (ALS) inhibitors (A, B and C1 groups of herbicides). Formation of herbicide-resistant weeds demands new solutions to cope with this continuously growing problem. As literature state, the presently applied synthetic herbicides are not approved for use in organic agriculture [2]. So the need of creation of new classes of herbicides with new mechanisms of action and less harmless to environment is still actual. Natural compounds represent a large field for the discovery of new environmentally friendly herbicides, the so-called "bioherbicides". Some members of the large group of plant secondary metabolites (so called allelopathins) take part in allelopathic interactions between the plants. Allelopathins are plant secondary metabolism products (terpenes, tannins, lignin, and so on), which released into the environment by plant different organs [1–8]. Allelopathic compounds affect germination and growth of neighboring plants by disruption of various metabolic pathways and physiological functions, including photosynthesis, respiration, signaling, water, redox processes and hormonal balance. The main action mechanism is the inhibition of different enzyme activity [9].

Some of the allelochemicals have to be modified into the active form by microorganisms or by specific environmental conditions (pH, moisture, temperature, light, oxygen etc. Mode of action of some allelochemicals is very similar to synthetic herbicides [10]. This described features have allowed them to be considered for possible use in weed management. Allelochemicals are highly attractive as new classes of herbicides due to a variety of advantages: their chemical structure is more environmentally friendly, decreased environmental half-life, which prevent their accumulation in soil and eventual influence on non-target organisms.

Allelochemicals, unlike synthetic herbicides, have a multi-site effect in plants without a high degree of specificity. This property precludes their use as a selective herbicide or completely prevents their use in weed control. Allelopathins, on the other hand, have a dose-dependent impact. This opens up the possibility of finding molecules with selectivity. Allelochemical resistance is higher in monocotyledonous plants than in dicotyledonous plants. As a result, using a chemical as a potential herbicide is viable, but limited to the growth of specific crops with specific weed compositions [1].

Exploration of some species' allelopathic potential allows for the introduction of alternate weed management approaches, such as the use of allelopathic plant extracts as foliar sprays. This strategy not only lowers herbicide application costs, but it also enhances crop yield. Phytotoxic water extracts from sorghum (*Sorghum bicolor* L.) and sunflower (*Helianthus annuus* L.) herbage are two well-known examples of natural bioherbicides that can be employed to protect plants without reducing yield. There are a few more allelochemicals that have agricultural applications. The ability of plant extracts to selectively kill weeds without affecting crop productivity is most likely due to changes in plant physiological stages and subsequent plant competition. Purified allelopathins with potential herbicide use are disclosed. Herbicides based on modified allelopathins that have already been approved for use are also included [11]. Essential oils (EO) have recently sparked renewed interest as allelopathins with bioherbicide potential. Some of them have already been developed and introduced into organic agriculture with great success. They damage the cuticle, causing desiccation or burning of immature tissues. GreenMatch EX, a commercially marketed bioherbicide including lemongrass oils, or InterceptorTM, a bioherbicide containing 10% pine (*Pinus sylvestris* L.) oil, are two examples [1]. Monoterpenes, sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones make up EO, which are complex combinations of monoterpenes, sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones. Monoterpenes (C10) and sesquiterpenes (C11) are the most common terpenoids found in volatile EO (C15). There are numerous examples of EOs being used as herbicides [1, 12]. Allelochemicals, a class of compounds sometimes known as biocommunicators, appear to be a promising challenge for merging conventional agricultural techniques with novel pest management tactics. Allelopathic interactions are engaged in nearly every element of plant growth in both natural and agricultural settings, as they can act as stimulants and suppressants.

The ancient knowledge of well-known poisonous effects of water extracts of a number of allelopathic plants provides a foundation for developing a revolutionary weed management strategy. The development of bioherbicides based on allelochemicals opens up the option of utilizing natural substances in plant protection and demonstrates the ability to deal with developed herbicide resistance. Mints generate EO, which have been shown to influence plant growth [13].

So, we decided to investigate the influence of EO and its main component menthol on the *Arabidopsis thaliana* model plant on a molecular level to investigate some mechanisms behind this allelopathic effect and to see if the EO distilled from mint (*Mentha arvensis*) widely growing in high altitude Armenian landscape has phytotoxic effect. The inquiry objects were *A. thaliana* wild type and 2-cysPRXAB peroxiredoxine-deficient mutant line (Δ2CP) [14], as the peroxiredoxine class of enzymes plays an important role in regulation of the oxygenic environment of the chloroplast [15]. 2-Cysteine peroxiredoxins (2-CPs) constitute a ubiquitous group of peroxidases that reduce cell-toxic alkyl hydroperoxides to their corresponding alcohols. In the mutant lines a 2-CP deficiency developed during early leaf and plant development and eventually the protein accumulated to wild-type levels. In young mutants with reduced amounts of 2-CPs, photosynthesis was impaired, the light-harvesting protein complex associated with photosystem II, chloroplast ATP synthase, and ribulose-1,5-bisphosphate carboxylase/oxygenase were decreased [14]. These investigations will make it possible to evaluate the allelopathic influence of natural metabolites.

**Material and Methods.** *Mentha arvensis* (minth) and *Ocimum basilicum* var. *purpureum* (basil) plant material was harvested from the Kotayk Region of Armenia at 1650 *m* a. s. l. during the blossoming period (August 2020). The plant samples were identified at the Institute of Botany, NAS of RA.

*A. thaliana* wild type and 2-cysPRXAB peroxiredoxine-deficient mutant line (Δ2CP) [14] were kindly provided by prof. K.-J Dietz (Department of Biochemistry and Physiology of Plants, Faculty of Biology, University of Bielefeld, Germany) and used as investigation objects.

***Essential oil Extraction.*** As an EO source the aerial parts of *M. arvensis* plants, cultivated in high altitude Armenian landscape (Kotayk Region, Armenia, 1600 *m* a. s. l.) were used. EO were extracted from air dried plant material (by hydro-distillation, using a Clevenger-type apparatus and lasted 3 *h*. The distilled EO had been dehydrated with anhydrous sodium sulphate and stored at 4°C in dark airtight bottles until further analysis [16, 17].

***Determination of Essential Oil Chemical Composition.*** The gas chromate-graphy mass selective analysis of the EO 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 $\mu m$) as described [17, 18].

**Experimental Design.** The 4–5 week old *A. thaliana* 6–10 *mm* leaf disks were treated with different concentrations (1.0–0.07 *mM*) of *M. arvensis* and *O. basilicum* var. *purpureum* EO and their main components methyl chavicol and menthol, respectively. The concentrations of EO were recalculated based on the concentrations of the main components. The maximum quantum yield ($F_v/F_m$) of photosystem II of *A. thaliana* leaf disks was determined by Mini-PAM-II photo-synthesis yield analyzer (chlorophyll fluorometer,Walz, Germany). The photosyn-thetic parameters like the redox response of ferredoxin, photosystem II, photosystem I and plastocyanin with the NIR KLAS 100 (Walz, Germany) were determined.

Redox state of proteins *in vivo* the redox gel electrophoresis followed by immune analysis by extraction and labeling with maleimid was applied [19]. Protein content was measured according to the Bradford assay [20]. Non-protein thiols (NPT) content determination was carried out using Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid (DTNB)) [21].

***Chemicals, Reagents, and Statistical Analysis.*** All applied chemicals and reagents were purchased from "Sigma-Aldrich Co. Ltd" (Germany). All data presented represent averaged results of 3 independent biological replicates. Experimental data were expressed as the mean ± SD of all repetitions. Statistical analysis was done with the Student's *t*-test for calculating the probability values.

**Results and Discussion.** Biological systems are affected by different environ-mental changes, which cause various onenzymatic and enzyme-catalyzed chemical reactions, structural and molecular rearrangements, and changes of molecular interactions. Under these conditions, the functioning of the homeostasis systems maintaining is important for survival and fitness. Understanding of the molecular mechanisms allowing formation of response to adverse environmental factors, including the influence of different allelochemicals, is one of the issues that will help to rulethese relationships between the species and consider them in weed mana-gement. Maintenance of cellular homeostasis in plants under the influence of various external factors is provided by a number of protective systems. Normally, ROS are generated by metabolic activity of the plants and act as signaling molecules for regulating plant metabolic pathways. To combat the harmful effect of increased ROS accumulation, plants are equipped with effective ROS-scavenging mechanisms. Plants have evolved two types of scavenging tools; enzymes (superoxide dismutase, catalase, monodehydroascorbate, peroxiredoxines and others) and antioxidant molecules like ascorbic acid, α-tocopherols, glutathione, proline, phenolic substanses and carotenoids. However, under environmental stresses, as well as the influence of different toxins, generation of ROS increases in various compartments of plant cell such as chloroplasts, peroxisomes and mitochondria. Higher accumulation of ROS leads to oxidative stress in plant, causing damage to the cell membranes and biomolecules [22].

*$EC_{50}$ values of O. basilicum var. purpureum EO, methyl chavicol, M. arvensis EO, and menthol*

| $EC_{50}$ values, *mM* | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ocimum basiicum* var. *purpureum* EO | | | | | | | |
| 0-hour treatment | | 3-hour treatment | | 6-hour treatment | | 24-hour treatment | |
| 0.946±0.3 | 1.024±0.5 | 0.38±0.09 | 0.57±0.1 | 0.35±0.1 | 0.37±0.08 | 0.28±0.05 | 0.25±0.08 |
| Methyl chavicol | | | | | | | |
| 3.5±1.4 | 2.97±1.3 | 0.59±0.1 | 0.59±0.08 | 0.58±0.08 | 0.63±0.07 | 0.55±0.06 | 0.56±0.05 |
| *Mentha arvensis* EO | | | | | | | |
| 1.164±0.9 | 2.38±1.8 | 0.6±0.04 | 0.6±0.08 | 0.6±0.07 | 0.66±0.09 | 0.54±0.08 | 0.4±0.07 |
| Menthol | | | | | | | |
| 1.69±0.3 | 5.16±3.2 | 0.59±0.06 | 0.61±0.12 | 0.55±0.06 | 0.58±0.06 | 0.56±0.07 | 0.6±0.03 |

According to the previously done gas chromatography mass selective analyses concentration of methyl chavicol in *O. basilicum* var. *purpureum* EO cultivated in high altitude Armenian flora was 57.3%, and concentration of menthol in *M. arvensis* EO reached 69% [17, 18]. PAM-fluorometric data ($F_v/F_m$) showed that the optimal treatment period of *A. thaliana* leaf disks was 3-hour treatment (see Table). EC50 concentration data were used to further treatment of investigated plants in order to reveal some action mechanisms of influence of potential allelopchemicals.

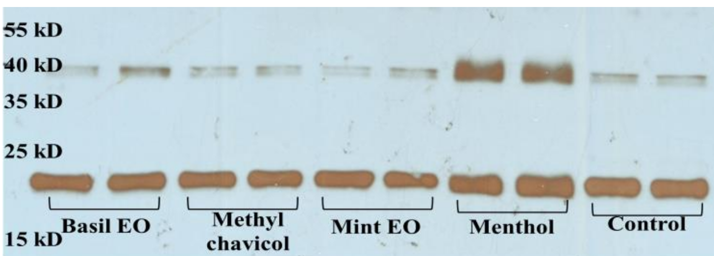


Fig 1. Immune analysis of peroxiredoxins of *A. thaliana* WT plants chloroplasts, under the treatment of basil EO, methyl chavicol, minth EO and menthol.

In order to reveal the redox state of proteins of interest *in vivo* the redox gel electrophoresis followed by immune analysis by extraction and labeling was applied. Chloroplast peroxiredoxin (2-CysPrx) has decisive role in regulating of photosynthesis [22]. So, it has been investigated the redox status of this enzyme in *A. thaliana Wt* (wild type) under the treatment of essential oils and their main components.

The results of investigations showed that the treatment with menthol is bringing to the oxidation of 2-CysPrx, which leaded to the dimerization of some amount (around 48%) of the enzyme (Fig. 1).

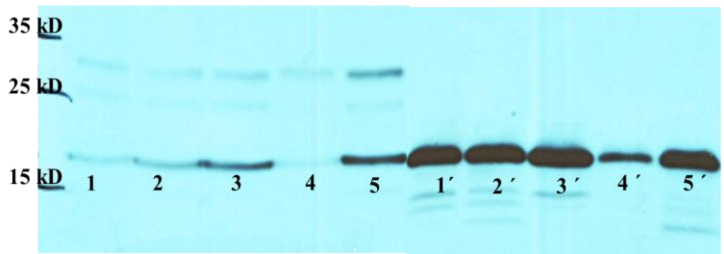


Fig. 2. Immune analysis of mitochondrial peroxiredoxins (PrxIIF) of *A. thaliana* WT, treated with $EC_{50}$ concentrations of investigated allelochemicals: 1, 2, 3, 4 – treatment with basil EO, methyl chavicol, mint EO, menthol, respectively; 5 – control (untreated plant). PrxIIF were labeled with maleimid. 1´, 2´, 3´, 4´ – are not labeled, but treated samples; 5´ – control (labeled, but untreated plant).

Data, represented in Fig. 2, show strong influence of menthol on PrxIIF. NPT such as glutathione play a central role in plant tolerance to different stressors, including herbicides [24]. So the deviation in the concentration of these substances can be informative for understanding the redox status of plant tissue.

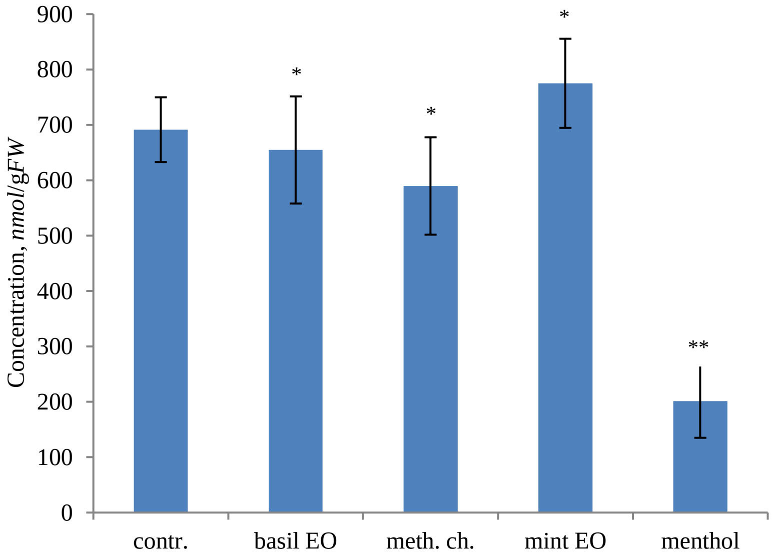


Fig. 3. Content of NPT in *A. thaliana* WT under the treatment of basil EO, methyl chavicol (meth. ch.), mint EO and menthol (* $p > 0.05$; ** $p < 0.05$).

According to our investigations only in case of the treatment with 0.5 *mM* concentration of menthol it has been reported the statistically significant decreasing (up to 71%) of the NPT quantity in plant tissue (Fig. 3). This data confirming the above mentioned results obtained from the immune analysis of peroxiredoxins that menthol can bring to the deviation in plant cellular homeostasis.

NIR KLAS 100 data also showed some influence of investigated allelochemicals on photosynthetic apparatus of *A. thaliana* WT and mutant plants.

**Conclusion.** In conclusion, the intricate interplay between environmental stressors, allelochemicals, and plant cellular responses underscores the profound impacts of environmental changes on biological systems. Understanding the molecular mechanisms enabling responses to adverse factors is pivotal for comprehending interspecies relationships and devising effective weed management strategies.

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Ն. Ժ. ՍԱՀԱԿՅԱՆ

# ՍԹՐԵՍԱՅԻՆ ՊԱՅՄԱՆՆԵՐՈՒՄ ԲՈՒՅՍԵՐԻ ՎԵՐՕՔՍ ԿԱՐԳԱՎՈՐՈՂ ՀԱՄԱԿԱՐԳԻ ԴԻՆԱՄԻԿԱՆ ԵՎ ԳՈՐԾԱՌՈՒՅԹԸ

Շրջակա միջավայրի փոփոխությունները մեծ ազդեցություն ունեն կենսաբանական համակարգերի վրա՝ առաջացնելով ֆերմենտային և քիմիական ռեակցիաների կասկադ, մոլեկուլային վերադասավորումներ և մոլեկուլային փոխազդեցությունների փոփոխություններ: Շրջակա միջավայրի սթրեսների դեմ պայքարի համար բույսերը օգտագործում են տարբեր պաշտպանիչ մեխանիզմներ՝ պահպանելով բջջային հոմեոստազը: Թթվածնի ակտիվ տեսակները (ԹԱՁ), որոնք առաջանում են նյութափոխանակության գործընթացների ժամանակ, ծառայում են որպես բույսերի նյութափոխանակության ուղիները կարգավորող ազդանշանային մոլեկուլներ: ԹԱՁ-ի կուտակման վնասակար հետևանքներին հակազդելու համար բույսերն ունեն այդ նյութերի չեզոքացման հզոր մեխանիզմներ: Դրանք ներառում են ֆերմենտային հակաօքսիդանտներ, ինչպիսիք են սուպերօքսիդ դիսմուտազը, կատալազը, մոնոդեհիդրոասկորբատը, պերօքսիրեդոքսինները, ինչպես նաև ոչ ֆերմենտային հակաօքսիդանտները, ինչպիսիք են ասկորբինաթթուն, α-տոկոֆերոլները, գլուտաթիոնը, պրոլինը, ֆենոլային նյութերը և կարոտինոիդները: Այնուամենայնիվ, սթրեսային պայմաններում և տոքսինների ազդեցությամբ ԹԱՁ-ի առաջացումը աճում է բույսերի բջիջներում, մասնավորապե այնպիսի օրգանոիդներում, ինչպիսիք են քլորոպլաստները, պերօքսիսոմները և միտոքոնդրիումները, ինչը հանգեցնում է օքսիդային սթրեսի:

Գազային քրոմատոգրաֆիկ ուսումնասիրությունները ցույց տվեցին մեթիլ խավիկոլի և մենթոլի զգալի կոնցենտրացիաներ բույսերի ընտրված տեսակներից ստացվող եթերայուղերում: PAM-ֆլյուորաչափական *in vitro* ուսումնասիրությունները ցույց են տվել *Arabidopsis thaliana* տերևային սկավառակների մշակման օպտիմալ կոնցենտրացիաները: Գել-էլեկտրոֆորեզը՝ զուգակցված իմունային վերլուծության հետ, նպաստեց առանցքային սպիտակուցների, հատկապես քլորոպլաստային պերօքսիրեդոքսինի (2-CysPrx) վերօքս կարգավիճակի գնահատմանը *in vivo*, որն առանցքային նշանակություն ունի ֆոտոսինթեզի կարգավորման մեջ: Եթերայուղերով մշակման ժամանակ 2-CysPrx-ի օքսիդացման վերօքս կարգավիճակի ուսումնասիրությունը բացահայտեց մենթոլով մակածվա օքսիդացումը և ֆերմենտի հետագա դիմերիզացումը, ինչը ենթադրում է ֆոտոսինթետիկ գործընթացների խախտում:

Ոչ սպիտակուցային թիոլները, ներառյալ գլուտաթիոնը, կենսական դեր են խաղում բույսերի սթրեսների նկատման կայունության գործընթացում: Թիոլների կոնցենտրացիաների շեղումները ծառայում են որպես բջջային

վերօքս կարգավիճակի ցուցանիշ: Հատկանշական է, որ մենթոլով մշակումը հանգեցրեց թիոլների մակարդակների վիճակագրորեն զգալի նվազման, հաստատելով պերօքսիրեդոքսինի դերը բջջային հոմեոստազի խանգարումներում: Ավելին, NIR KLAS 100-ի տվյալները ընդգծեցին ալելոքիմիական ազդեցությունները *A. thaliana* վայրի տիպի և մուտանտ բույսերի ֆոտոսինթետիկ ապարատի վրա:

Н. Ж. СААКЯН

# ДИНАМИКА И ФУНКЦИЯ ОКИСЛИТЕЛЬНО-ВОССТАНОВИТЕЛЬНОЙ РЕГУЛЯТОРНОЙ СЕТИ РАСТЕНИЙ В УСЛОВИЯХ СТРЕССА

Изменения окружающей среды оказывают глубокое воздействие на биологические системы, запуская каскад ферментативных и химических реакций, молекулярных перестроек и изменений в молекулярных взаимодействиях. Перед лицом стрессовых факторов окружающей среды растения используют различные защитные механизмы для поддержания клеточного гомеостаза. Активные формы кислорода (АФК), образующиеся в ходе метаболических процессов, служат сигнальными молекулами, регулирующими метаболические пути растений. Чтобы противодействовать пагубному эффекту накопления АФК, растения обладают надежными механизмами их удаления. К ним относятся ферментативные антиоксиданты, такие как супероксиддисмутаза, каталаза, монодегидроаскорбат, пероксиредоксины, а также неферментативные антиоксиданты, такие как аскорбиновая кислота, α-токоферолы, глутатион, пролин, фенольные вещества и каротиноиды. Однако в условиях стресса и воздействия токсинов генерация АФК возрастает в клетках растений, особенно в таких органеллах, как хлоропласты, пероксисомы и митохондрии, что приводит к окислительному стрессу и повреждению клеток.

Исследования с использованием газовой хроматографии и масс-селективного анализа выявили значительные концентрации метилхавикола и ментола в эфирных маслах, экстрагированных из некоторых видов растений. Исследования *in vitro* с использованием данных PAM-флуорометрии продемонстрировали оптимальные периоды обработки дисков листьев *Arabidopsis thaliana*, что способствовало выяснению потенциальных механизмов аллелохимического действия. Гель-электрофорез в сочетании с иммунным анализом облегчил оценку окислительно-восстановительного статуса белков *in vivo*, в частности пероксиредоксина хлоропластов (2-CysPrx), играющего ключевую роль в регуляции фотосинтеза. Исследование окислительно-восстановительного статуса 2-CysPrx при обработке эфирными маслами выявило индуцированное ментолом окисление и последующую димеризацию фермента, что указывает на нарушение фотосинтетических процессов.

Небелковые тиолы (НТБ), включая глутатион, играют жизненно важную роль в устойчивости растений к стрессу. Отклонения концентраций НТБ служат индикаторами окислительно-восстановительного статуса клеток. Примечательно, что обработка ментолом привела к статистически значимому снижению уровней НТБ, что подтверждает данные об изменении окислительно-восстановительных состояний пероксиредоксина и предполагает нарушения клеточного гомеостаза. Более того, данные NIR KLAS 100 выявили аллелохимические влияния на фотосинтетический аппарат растений *A. thaliana* дикого типа и мутантных растений, что дополнительно проясняет влияние этих соединений на физиологические процессы растений.