Abstract

Growing restrictions on the use of synthetic herbicides intensify the search for sustainable plant-derived alternatives. In this study, by combining biochemical, cytological, and molecular approaches, we assessed the phytotoxic effects, selectivity, and mechanism of action of essential oil from rhizomes of sweet flag (*Acorus calamus* L., SEO). Oil composition analysis revealed the dominance of phenylpropanoids, mainly β-asarone, along with other phenylpropanoids and sesquiterpenes. To ensure comparable stress intensity, species-specific half-inhibitory concentrations (IC_{50}) for root growth were established: for two Fabaceae (*Vicia faba*: 0.03%; *Lupinus luteus*: 0.025%) and two Brassicaceae (*Brassica napus*: 0.01%; *Arabidopsis thaliana*: 0.005%). A 24-h exposure to emulsified SEO reduced fresh and dry root biomass, impaired membrane integrity, increased electrolyte leakage, and decreased cell viability due to elevated reactive oxygen species (ROS) levels. Despite activation of both enzymatic and non-enzymatic antioxidant responses, lipid peroxidation was detected, and metabolic changes were revealed by isothermal calorimetry and analysis of proteins, sugars, and fatty acids. The general response pattern indicated higher resistance in Fabaceae, whereas Brassicaceae showed weaker metabolic adjustment and stronger damage. Since root growth depends on meristematic proliferation, we next analyzed the impact of SEO on meristem activity. ROS accumulated mainly in organelles, causing membrane damage, inhibition of hexokinase (HXK), and activation of the MAPK (p44/42) pathway, transmitting stress signals to the nucleus. They also induced DNA breaks and replication stress. As a consequence, chromatin remodeling, DNA hypermethylation, loss of active transcription marks, reduced RNA synthesis, nucleolar vacuolization, and stress-related epigenetic foci were observed. Cells accumulated in G1 phase, while the number of replicating cells (EdU-incorporating) declined and S phase was prolonged, particularly during replication of condensed heterochromatin. These effects were accompanied by activation of repair mechanisms, confirmed by γ-H2AX signals and specific histone modifications. Despite genotoxic stress, SEO did not induce extensive DNA fragmentation or switch to endocycling. However, it reduced the number of mitotic cells, and in dividing cells caused metaphase arrest and chromosome aberrations, associated with excessive stabilization of cytoskeletal fibers, impairing spindle and phragmoplast reorganization. SEO also disrupted epigenetic regulation of mitosis through interference with control mechanisms involving Cdc2 kinase and PP1/2A phosphatases. Comparative analyses showed greater chromatin plasticity and more efficient repair mechanisms in Fabaceae, whereas Brassicaceae exhibited stronger transcriptional repression and replication stress. Altogether, the results demonstrate that SEO acts through a complex, multitarget mechanism involving simultaneous disruption of membranes, metabolism, signaling cascades, chromatin organization, and cytoskeletal dynamics. This systemic mode of action explains the clear interfamily selectivity and reduces the risk of resistance evolution. Integration of phytotoxic selectivity with multilayered cellular disruption makes SEO a promising candidate for bioherbicide development in sustainable agriculture.