ACCUMULATION OF ANTIOXIDANT METABOLITES IN *IN VITRO* PROPAGATED *ARNICA MONTANA* WITH YEAST EXTRACT AND SALICYLIC ACID

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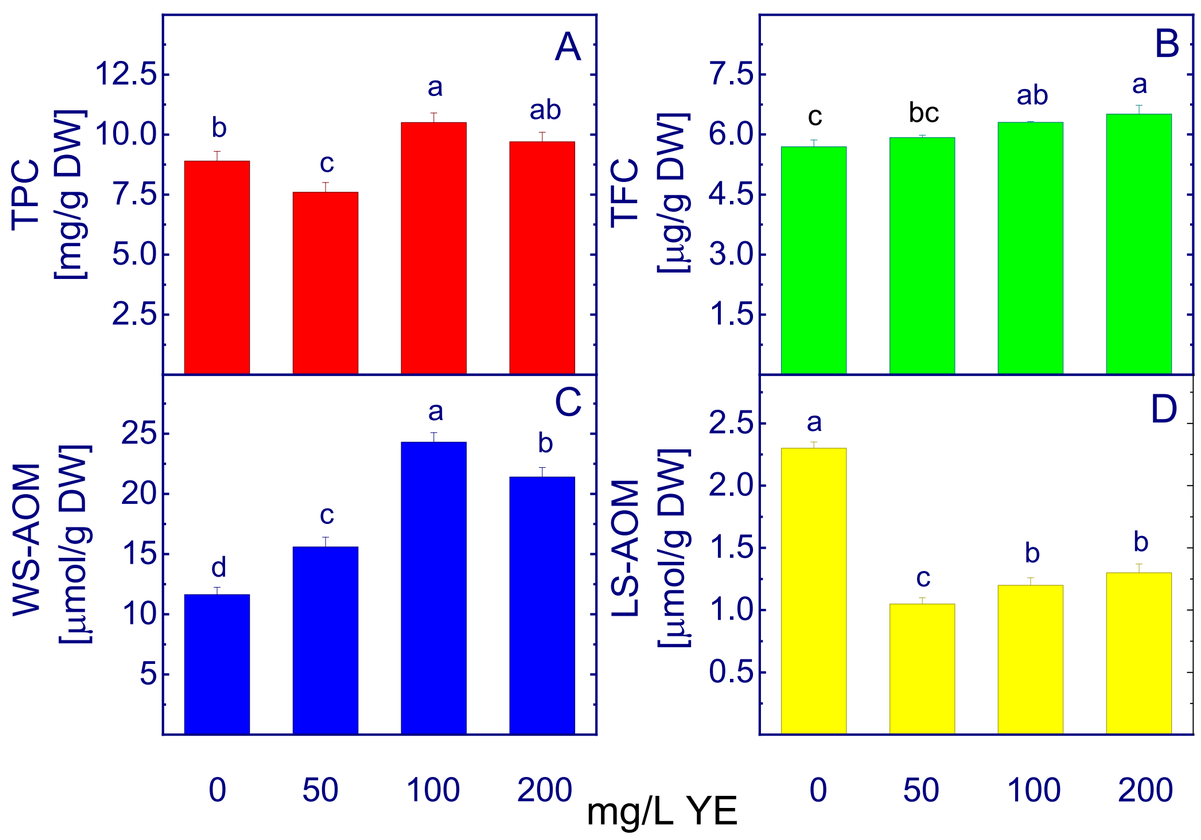
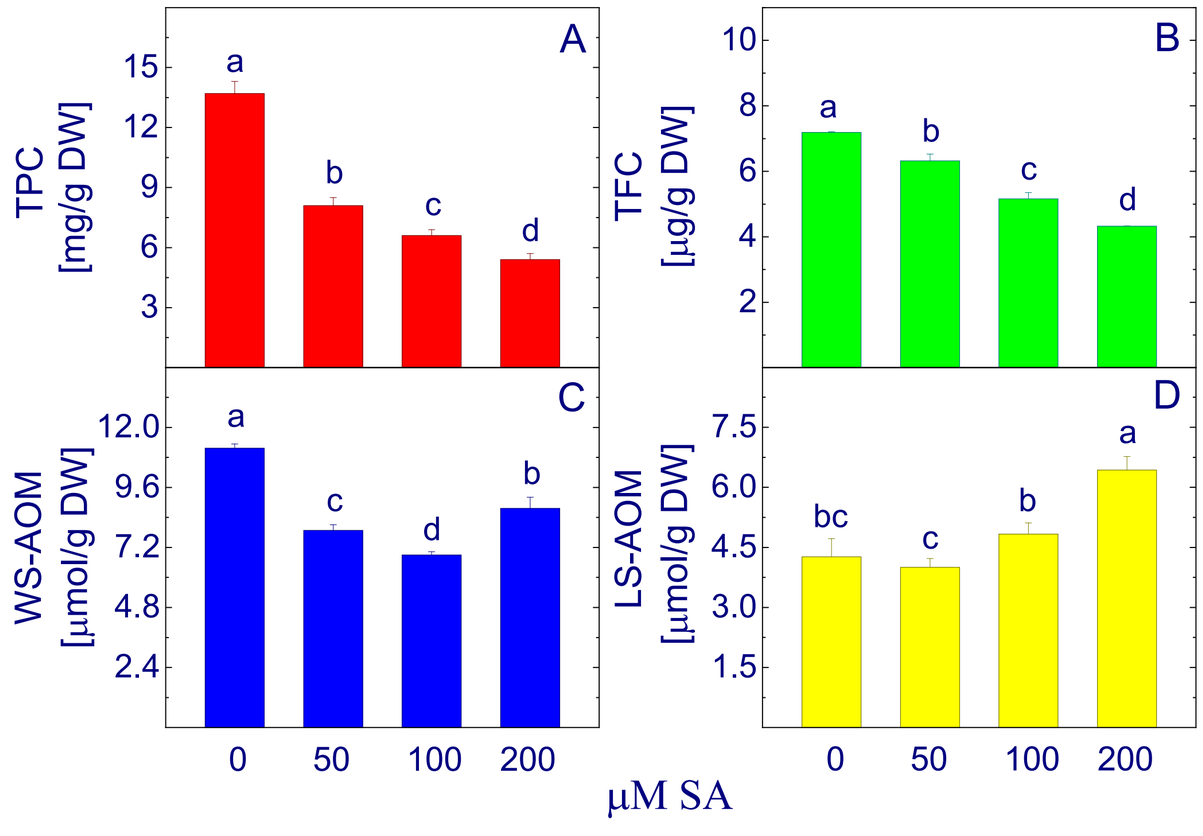
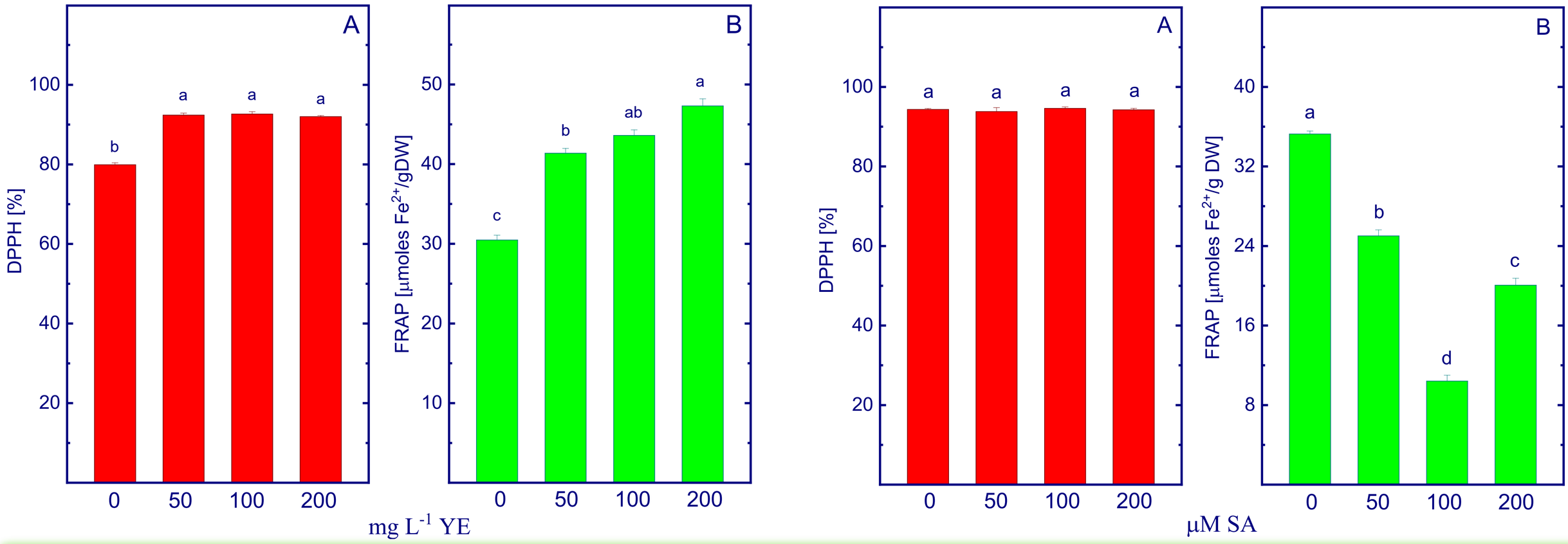
Introduction: *Arnica montana* (Asteraceae family) is an herbaceous medicinal plant native to Europe, serving as a source of raw materials rich in secondary metabolites. It has been extensively utilised in the pharmaceutical and cosmetic industries. Due to the manifold intensification of agriculture, overharvesting of plants, and high cattle density in arnica habitats, arnica populations have gradually diminished or disappeared entirely. To conserve *A. montana*, biotechnological approaches for propagation are being employed. However, there is very limited knowledge regarding the impact of treatment with abiotic and biotic elicitors during arnica *in vitro* propagation on the levels of secondary metabolites possessing antioxidant properties.

Material and Methods: Arnica montana plantlets were cultivated on MS nutrient medium supplemented with varying concentrations of yeast extract (50, 100, or 200 mg L^{-1} YE) or salicylic acid (50, 100, or 200 µM SA). Control shoots were grown on an MS nutrient medium supplemented with 0.5 mg L^{-1} BAP, 3% (w/v) sucrose, and solidified with 0.6% (w/v) agar without elicitor. Yeast extract was added to the medium mentioned above before autoclaving. The stock solution of SA was filter-sterilised through a 0.22 µm syringe Millipore filter and then aseptically added to the autoclaved MS medium containing 0.5 mg L^{-1} BAP.





Both the biotic YE and the abiotic SA elicitors have modulated the levels of metabolites with antioxidant potential against oxidative stress in different ways. The results indicated that YE applied at 100 mg L^{-1} significantly promotes total phenolic and flavonoid content, as well as water-soluble metabolites with antioxidant potential, compared to the untreated control plantlets. Conversely, the addition of salicylic acid to the MS culture medium resulted in a decrease in the accumulation of total phenols and flavonoids, along with water-soluble metabolites possessing antioxidant capacity. As the concentration of SA increases from 50 µM to 200 µM, the extent of decrease in these parameters intensified. Only the lipid-soluble metabolite content increased. *A. montana* plantlets micro-propagated with YE exhibited enhanced levels of total antioxidant capabilities measured by ferric reducing antioxidant power (FRAP method) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (DPPH method). When plantlets were treated with SA, the free radical scavenging activity remained unchanged, and the FRAP measured in the *A. montana* plantlets was significantly decreased compared with untreated control plants.



Conclusion: Overall, the results of this study revealed that the presence of biotic YE and abiotic SA elicitors in the MS nutrient medium alters the biosynthesis of antioxidant metabolites in *A. montana* plantlets, depending on the type and concentration of the elicitor. The results showed that yeast extract applied at 100 mg/L significantly increases the total phenolic content level, compared to the control untreated plantlets. Flavonoid content was the highest in samples treated with 200 mg L^{-1} of yeast extract. In the current study, salicylic acid is not found to be effective for phenolic compound production.

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