

Harnessing chestnut-derived bioproducts to strengthen plant defense mechanisms

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Introduction

Optimizing plant-derived biomass is a crucial strategy for addressing climate change and promoting a circular economy. With approximately 200 billion tons of lignocellulosic material generated annually by the forestry and agricultural sectors, there is a massive opportunity to transform waste into value-added products. Residual wood, particularly from chestnut trees found in the Tuscan Apennines, is a rich source of bioactive compounds like tannins, which are known for their antimicrobial properties. This study evaluates the potential of both commercial chestnut-derived wood vinegar and laboratory-scale extracts as sustainable tools to help plants withstand biotic and abiotic stresses.



Fig. 1 Tuscan Apennine, marginal area

WOOD VINEGAR: Pyrolysis process by-product

Already marketed as a biostimulant and defence inducer, starting from chestnut waste

To replace traditional fertilisers, pesticides and herbicide



Fig. 2 Wood distillate produced by BioDea® (Arezzo, Italy)

Innovative «green extraction»: Infusion of chestnut woodchips



Fig. 3 Chestnut woodchips (A) were used to produced an acqueous extract both in a lab scale (B) and industrial prototype (C). A 3h infusion at 80-85 °C

Materials and methods

2 months pot experiment during July-August 2025

2 treatment, 1 x week

- Wood vinegar (WV) 1:300 and 1:600
- Woodchip extract (WCE) 1:300 and 1:600

Stress condition: NaCl 2 g/L during irrigation



N. tabacum



S. lycopersicum

Experimental setup

Physiological analysis

Gene expression analysis



Fresh/dry weight

Height

Internodes (tobacco)

Fruit (tomato)

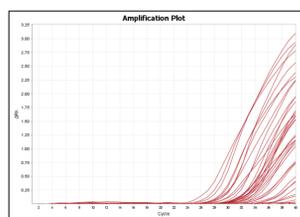
Chlorophyll a/b content

Stomatal conductance (gs_w) and photosystem II (PSII) activity



Target genes

- Auxin-responsive gene 1 (*ARF1*)
- Transport inhibitor response 1 (*TIR1*)
- Auxin efflux carrier *PIN1*
- Phenylalanine ammonia-lyase (*PAL*)
- Chalcone synthase (*CHS*)
- Pathogenesis-related proteins (*PR1*, *PR3* and *PR5*)
- Enolase (*ENO*)
- Thaumatococcus-like protein (*TLP*)
- Salt Overly Sensitive 1 (*SOS1*)
- Na⁺/H⁺ exchanger 1 (*NHX1*)



Conclusion

Chestnut-derived bioproducts significantly enhance plant growth and stress resilience, with efficacy being highly dosage-dependent. High concentrations of wood vinegar and a 1:300 dilution of WCE proved most effective. By modulating stress-related molecular pathways, these products serve as promising eco-friendly defense inducers

Results

The application of WV, WCE, and NB significantly mitigates the negative effects of salt stress in both tobacco and tomato plants. While salt stress generally reduces biomass and productivity, WV (particularly at 1:600 for tobacco and 1:300 for tomato) emerged as the most versatile treatment, enhancing height, root growth, and fruit restoration. WCE proved highly effective in boosting dry biomass across both species, whereas NB showed a specialized benefit, restoring tomato fruit count and tobacco height specifically under saline conditions. Overall, while physiological parameters like PSII remained stable, these biostimulants effectively counteracted salt-induced growth inhibition, with specific efficacy depending on the treatment type and plant species.

Bio-product treatments modulate gene expression based on salinity levels. Under salt stress, auxin-related genes (*ARF1*, *TIR1*) and abiotic stress markers (*ENO*, *NAC4*) are significantly upregulated. While secondary metabolism genes like *PAL* are generally suppressed by salinity, WV 1:600 selectively induces *TLP* expression. Overall, these treatments consistently activate specific stress-response pathways (*ENO*, *NAC4*) regardless of conditions, though most defense-related induction (like *PR5*) occurs only in non-stress environments

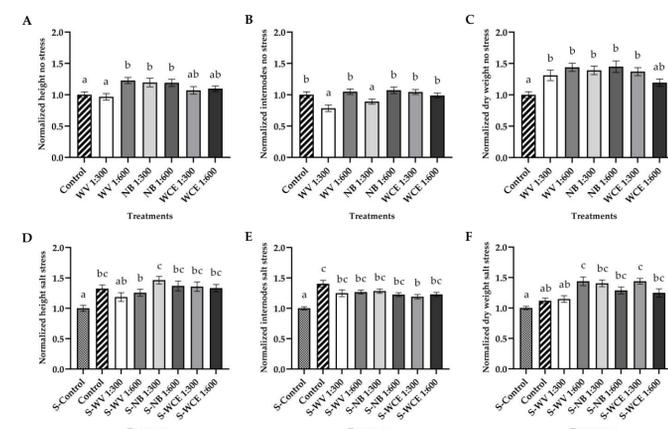


Figure 4. Height (A, D), internodes (B, E), dry weight of the aerial part (C, F), of treated *N. tabacum* plants under non-stress conditions (A-C) and salt stress condition (D-F). All values have been normalized by ratioing them to the corresponding control. Error bars represent the standard error of the mean (SEM). Letters indicate statistically significant differences between treatments assessed with Tukey's or Dunn's post hoc tests (p -value < 0.05)

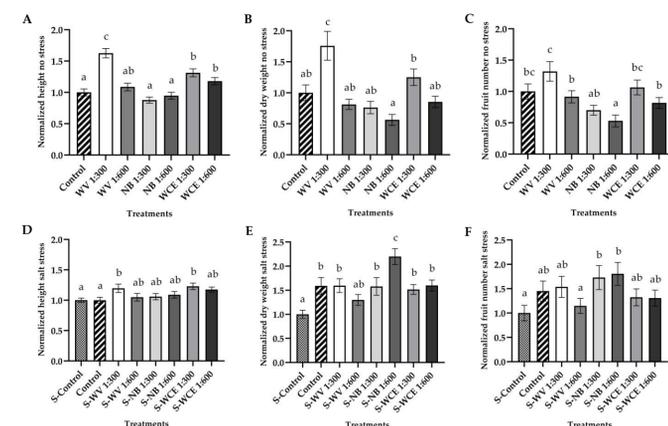


Figure 5. Height, dry weight, fruit number of treated *S. lycopersicum* plants under non-stress conditions (A-C) and salt stress condition (D-F). All values have been normalized by ratioing them to the corresponding control. Error bars represent the standard error of the mean (SEM). Letters indicate statistically significant differences between treatments assessed with Tukey's or Dunn's post hoc tests (p -value < 0.05)

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