

PhD. Thesis Title

DEVELOPMENT OF NOVEL FERTILIZATION STRATEGIES FOR IMPROVING THE QUALITY AND YIELD OF GRAPEVINE GENOTYPES UNDER THE CURRENT CLIMATE CHANGE CONDITIONS OF THE ȘTEFĂNEȘTI-ARGEȘ VITICULTURAL AREA

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Abstract

In the context of growing concerns regarding the impact of agriculture on the environment and human health, modern viticulture requires sustainable solutions and innovative fertilization technologies. The doctoral research conducted in the Ștefănești–Argeș viticultural area focused on the development of a biostimulant extract obtained through nanotechnology and the valorization of viticultural waste, with the aim of improving grapevine productivity, grape quality, and wood maturation. Phytochemical, physiological, and biometric analyses, as well as assessments of crop quality, were carried out within an interdisciplinary collaboration involving several research institutes and specialized laboratories.

The fundamental research problem addressed in this study is the identification of environmentally friendly and effective alternatives to synthetic fertilizers and chemical products commonly used in viticulture, in the context of environmental degradation, climate change, and the increasing pressure to achieve high yields. The study investigates the valorization of viticultural waste through the production of bioactive extracts and phytosynthesized nanoparticles capable of enhancing productivity, improving grape quality, and increasing plant tolerance to pathogens. Another important issue concerns the maintenance of the physiological balance of grapevines by stimulating photosynthesis, wood maturation, and the accumulation of reserve compounds necessary for plant resilience. The research also highlights the need to develop sustainable technologies that reduce environmental impact and promote the reuse of viticultural waste in agriculture.

The main research hypothesis was that hydroalcoholic extracts obtained from viticultural waste and phytosynthesized silver nanoparticles could represent effective and environmentally friendly alternatives to synthetic chemical products used in viticulture. It was hypothesized that these extracts contain bioactive compounds capable of stimulating grapevine physiological processes, enhancing productivity and grape quality, and promoting wood maturation. A further

hypothesis was that silver nanoparticles produced through phytosynthesis could exhibit antifungal and antioxidant effects, reducing the incidence of pathogens such as *Uncinula necator* and *Plasmopara viticola*. Additionally, it was assumed that the valorization of viticultural waste could contribute to the development of sustainable technologies and to the reduction of agriculture's negative environmental impact.

CHAPTER I. IMPORTANCE AND POTENTIAL OF GRAPEVINE CULTIVATION

Grapevine cultivation represents one of the most important agricultural activities worldwide. It makes a significant contribution to both the economy and food supply through the production of grapes and wine. Vineyards generate large quantities of plant waste each year as a result of pruning operations and the winemaking process. These by-products can be effectively valorized due to their rich content of bioactive compounds.

Viticultural waste includes seeds, skins, leaves, canes, and pomace generated during grape processing. These materials contain polyphenols, flavonoids, antioxidants, and other compounds of high biological value. The extracted compounds are widely used in the food, cosmetic, and pharmaceutical industries. Their valorization contributes to reducing environmental impact and supports the development of a circular economy.

Nanotechnology offers the possibility of producing nanoparticles using extracts derived from viticultural waste. These nanoparticles exhibit significant antimicrobial and antioxidant properties. Their application in agriculture may reduce the use of synthetic pesticides and chemical fertilizers. Furthermore, the use of environmentally friendly synthesis methods supports the development of sustainable agriculture.

Viticultural waste can be utilized for the production of biostimulants, antifungal extracts, and eco-friendly materials. Extracts rich in stilbenes and polyphenols have demonstrated antimicrobial and antioxidant effects. Some by-products are also used in the manufacture of composite materials and innovative industrial products. These applications promote the high-value utilization of viticultural resources while contributing to pollution reduction.

CHAPTER II. NATURAL EXPERIMENTAL FRAMEWORK

The National Institute of Research and Development for Biotechnology in Horticulture Ștefănești is located in the Ștefănești–Argeș viticultural region, within the Subcarpathian area of Muntenia. The territory comprises plateaus, slopes, and the floodplain of the Argeș River, all of which provide favorable conditions for grapevine cultivation. The viticultural area extends across the territories of Ștefănești and Topoloveni, as well as the communes of Călinești and Leordeni. The topography and geographical location contribute to the development of a climate highly favorable to viticulture.

The soils within the research area of the National Institute of Research and Development for Biotechnology in Horticulture Ștefănești are diverse and well developed in depth. They belong

to several soil classes, including Protisols, Luvisols, and Anthrosols. The main pedogenetic processes are carbonate leaching and the downward migration of clay particles within the soil profile. These soil characteristics play a significant role in determining soil fertility and the development of vineyard plantations.

Climatic factors, particularly temperature and humidity, play a crucial role in grapevine growth and development. Climatic analyses revealed an increase in maximum temperatures during the period 2021–2023 compared with the long-term average. Precipitation patterns and atmospheric humidity remained within favorable limits for viticultural production. Monitoring of environmental conditions in both greenhouse and field environments highlighted differences in temperature and humidity between the two cultivation systems.

CHAPTER III. DEVELOPMENT OF NEW ALTERNATIVES FOR VITICULTURE – MATERIAL RESOURCES USED AS SUBSTITUTES FOR SYNTHETIC PRODUCTS

The use of chemical fertilizers and pesticides contributes to increased vineyard productivity; however, it may also have adverse effects on the environment and human health. Excessive application of these substances leads to soil and water pollution, as well as the degradation of biodiversity. Soil compaction and deterioration of soil structure are additional negative consequences associated with the intensive use of synthetic products. For this reason, considerable efforts are being directed toward the development of environmentally friendly and sustainable alternatives for viticulture.

Natural extracts represent an eco-friendly alternative for disease control and the stimulation of grapevine growth and development. These extracts contain bioactive compounds with antifungal, antimicrobial, and biostimulatory properties. Numerous studies have demonstrated the effectiveness of plant extracts, algal extracts, and biopreparations in reducing pathogen incidence. Their application contributes to decreasing pesticide use while promoting environmental protection.

Nanotechnology offers modern solutions for crop protection and growth enhancement in agriculture. Nanoparticles may exert antimicrobial, antifungal, and biostimulatory effects on plants. Research has demonstrated the effectiveness of silver, zinc, and chitosan nanoparticles in controlling grapevine pathogens. The use of nanomaterials has the potential to reduce pesticide consumption and improve the productivity of agricultural crops.

CHAPTER IV. RESEARCH AIM AND OBJECTIVES. MATERIALS AND METHODS

The aim of this research was to develop innovative fertilizers and alternative fertilization technologies for grapevines, with beneficial effects on productivity, grape quality, and wood maturation. To achieve these objectives, natural extracts derived from viticultural waste were obtained and characterized using both conventional and microwave-assisted extraction methods. The study focused on the biochemical characterization of the extracts, the evaluation of their

physiological and biometric effects on grapevine plants, and the assessment of crop productivity and quality. Furthermore, the research highlighted the potential of viticultural waste valorization and the use of silver nanoparticles as environmentally friendly alternatives to synthetic chemical products.

This chapter describes the utilization of viticultural waste for the production of natural extracts and the development of eco-friendly fertilization solutions for viticulture. Plant material, consisting of grapevine canes and leaves, was collected, dried, and processed to obtain extracts using both conventional and microwave-assisted extraction techniques. The resulting extracts were biochemically characterized and subsequently used for the phytosynthesis of silver nanoparticles applied to grapevine plants. The effects of hydroalcoholic extracts and their silver nanoparticle-enhanced formulations on plant growth, productivity, grape quality, and wood maturation were evaluated throughout the study.

CHAPTER V. CHARACTERIZATION OF THE DEVELOPED SOLUTIONS

The determination of total phenolic content revealed significant differences among the extracts obtained from viticultural waste, depending on the type of plant material used. The highest polyphenol concentrations were recorded in extracts derived from the canes of the Fetească neagră 6 Șt. and Fetească regală 72 Șt. genotypes, confirming their high antioxidant potential. In contrast, the extract obtained from grapevine leaves exhibited the lowest phenolic compound content. Analysis of stum from the Fetească regală 72 Șt. genotype showed that the application of grapevine leaf extract enriched with silver nanoparticles resulted in a significant reduction in total polyphenol content compared with the control treatment.

The antioxidant capacity of the extracts obtained from viticultural waste varied according to the plant material employed. The highest antioxidant activity was recorded in extracts derived from the canes of the Fetească neagră 6 Șt. and Fetească regală 72 Șt. genotypes, compared with extracts obtained from grapevine leaves. The Fetească neagră 6 Șt. cane extract exhibited the greatest DPPH radical scavenging activity, demonstrating superior antioxidant potential. In the stum obtained from the Fetească regală 72 Șt. genotype, the application of leaf extract containing silver nanoparticles significantly increased antioxidant activity compared with the control treatment.

HPLC analysis of phenolic compounds revealed a high concentration of bioactive substances in the extracts obtained from viticultural waste. Hydroalcoholic extracts contained higher concentrations of phenolic compounds than their silver nanoparticle-enhanced counterparts, suggesting the involvement of these compounds in the phytosynthesis process. Numerous important phenolic compounds were identified in leaf and cane extracts, including resveratrol, catechin, epicatechin, rutin, quercetin, and luteolin, all known for their antioxidant and antifungal properties. The highest concentrations of resveratrol and catechin were recorded in extracts obtained from the canes of the Fetească neagră 6 Șt. and Fetească regală 72 Șt. genotypes, highlighting their considerable potential as sources of bioactive compounds.

HPLC analyses performed on grape stum demonstrated that the application of extracts and nanoparticles influenced its phenolic composition. Treatment V2 was characterized by a high

tannic acid content, whereas treatment V3 showed elevated concentrations of epicatechin and hyperoside. A reduction in phenolic compounds was also observed in treatments containing silver nanoparticles, confirming their consumption during the phytosynthesis process. Analysis of pomace and fern extracts likewise revealed high concentrations of phenolic compounds and flavonoids, including epicatechin, syringic acid, rutin, and quercetin, which contribute to the strong antioxidant potential of the extracts and play an important role in nanoparticle stabilization.

The characterization of the phytosynthesized nanomaterials confirmed the successful synthesis of silver nanoparticles using natural extracts obtained from grape pomace and ferns. UV–Vis analyses revealed characteristic absorption bands of silver nanoparticles within the 300–700 nm range, confirming both nanoparticle formation and long-term stability. X-ray diffraction (XRD) studies demonstrated the presence of crystalline silver with a cubic structure, identifying diffraction peaks corresponding to the (111), (220), and (311) crystal planes, thus confirming the formation of crystalline metallic nanoparticles. In the case of the fern extract, diffraction patterns indicated the presence of both metallic silver and silver oxide.

Transmission electron microscopy (TEM) analyses revealed predominantly spherical and hemispherical nanoparticles, together with a limited number of triangular and ellipsoidal forms. The particle size distribution was relatively uniform, and the TEM images confirmed the small dimensions and good dispersion of the phytosynthesized nanomaterials. The results demonstrated that grape pomace and fern extracts played a crucial role both in the reduction of metal ions and in the stabilization of the synthesized nanoparticles. These findings confirm the effectiveness of environmentally friendly phytosynthesis methods and highlight the potential of plant waste as a resource for the production of nanomaterials with applications in agriculture and plant protection.

The results obtained revealed significant differences between fern extract and phytosynthesized silver nanoparticles regarding their antimicrobial activity against the pathogen *Plasmopara viticola*. Laboratory tests showed that the fern extract did not exhibit significant antimicrobial activity, producing results similar to those of the negative control. In contrast, the treatment containing phytosynthesized silver nanoparticles demonstrated a pronounced inhibitory effect on pathogen development, producing an inhibition zone approximately half the size of that observed for the commercial fungicide used as the positive control. These findings confirm the considerable potential of silver nanoparticles for the control of grapevine phytopathogens.

CHAPTER VI. RESULTS OF THE APPLICATION OF THE PROPOSED SOLUTIONS

Observations made following the application of extracts derived from viticultural waste and phytosynthesized nanoparticles revealed a significant antifungal effect against the pathogen *Uncinula necator* in both grapevine genotypes analyzed. In the Fetească neagră 6 Șt. genotype, repeated applications of the extracts and the organic product significantly reduced disease incidence, and following the final treatment, treated plants no longer exhibited symptoms of powdery mildew. In contrast, the number of infected leaves in the control treatment continued to increase progressively until the entire leaf canopy was affected. Furthermore, both disease severity

and disease index were significantly lower in the treated variants compared with the negative control, with the protective effect being maintained throughout the experimental period.

In the Fetească regală 72 Șt. genotype, all extracts obtained from viticultural waste significantly reduced the number of leaves affected by powdery mildew compared with the control treatment. Certain experimental variants, particularly V1, V3, and V5, maintained a constant number of infected leaves throughout the study period, demonstrating a strong capacity for pathogen control. Disease severity and the percentage of leaf area covered by powdery mildew were significantly reduced in all treated variants, especially during July and August. While disease progression stabilized or ceased by the end of August in the treated variants, infection continued to increase in the negative control until October.

The experimental methodology consisted of monitoring disease incidence (F%), disease severity (I%), and disease index (Ga%) of powdery mildew on leaves treated foliarly with hydroalcoholic extracts, silver nanoparticle-enhanced formulations, and the organic product Growspore LF. Evaluations were conducted periodically over several growing months by comparing the experimental treatments with the negative control. The data were statistically analyzed using ANOVA and Tukey's test to identify significant differences among treatments. The study confirms the effectiveness of plant extracts and phytosynthesized nanoparticles as environmentally friendly alternatives for the control of powdery mildew in grapevine cultivation.

Vineyard trials highlighted the physiological and biochemical effects of extracts obtained from viticultural waste and phytosynthesized nanoparticles on the Fetească neagră 6 Șt. and Fetească regală 72 Șt. genotypes. Observations regarding pollen grain viability showed that treatments containing silver nanoparticles, particularly V2, V4, and V6, significantly improved both pollen viability and pollen grain size compared with the control treatment. The most pronounced effects were recorded in the Fetească regală 72 Șt. genotype, where pollen viability increased by more than 8% in treatments involving cane extracts and silver nanoparticles. In contrast, some experimental treatments and the organic product Growspore LF reduced the total number of pollen grains.

Leaf area assessments revealed genotype-dependent responses. In the Fetească neagră 6 Șt. genotype, all experimental treatments reduced leaf area compared with the control, both during inflorescence emergence and at the onset of veraison. Conversely, in the Fetească regală 72 Șt. genotype, most treatments stimulated leaf blade development, resulting in larger leaf areas than those observed in the negative control. These observations indicate distinct responses of the two genotypes to the application of extracts and phytosynthesized nanoparticles.

Analyses of chlorophyll and carotenoid pigment content demonstrated significant treatment effects on plant photosynthetic activity. During the flowering stage, most extracts reduced chlorophyll a and chlorophyll b contents in the Fetească neagră 6 Șt. genotype, indicating an inhibitory effect on the photosynthetic apparatus. In contrast, in the Fetească regală 72 Șt. genotype, treatments containing silver nanoparticles, particularly V2 and V4, significantly

increased chlorophyll a, chlorophyll b, and carotenoid pigment levels. During berry development and ripening, treatment effects varied according to genotype and extract type, with both increases and decreases in photosynthetic pigments being observed. The most favorable effects were associated with silver nanoparticle-enhanced treatments, which stimulated the accumulation of photosynthetic pigments and positively influenced the chlorophyll a/b ratio.

Measurements of stomatal length and ostiole opening demonstrated that the extracts and nanoparticles influenced physiological processes involved in gas exchange and transpiration regulation. In the Fetească neagră 6 Șt. genotype, increases in stomatal length were observed particularly in treatments V1 and V3, while ostiole opening showed significant fluctuations throughout the growing season. In the Fetească regală 72 Șt. genotype, treatments V4 and V6 increased ostiole opening in June, whereas reductions in this parameter were observed in August. These physiological changes suggest that the applied treatments influenced plant adaptation to environmental conditions and the functioning of the stomatal apparatus.

The application of extracts derived from viticultural waste and phytosynthesized nanoparticles significantly influenced the biochemical composition of grape stum and the degree of wood maturation. Regarding the sugar content of stum obtained from the Fetească regală 72 Șt. genotype, all experimental treatments resulted in significant increases compared with the control. The greatest increases were recorded for the organic product Growspore LF and treatments V5 and V6, demonstrating that the extracts and nanoparticles can contribute to carbohydrate accumulation and consequently improve grape quality and the alcoholic potential of wine. Simultaneously, all treatments reduced stum acidity compared with the negative control, with the largest decreases observed in treatments V3 and V4. These biochemical changes indicate a favorable influence on the balance between sugar and acidity, two essential parameters of wine quality.

Analyses of the mineral composition of grape stum revealed increases in calcium and potassium ion concentrations in most experimental treatments compared with the control. Calcium ion concentrations increased particularly following the application of treatments V1, V3, V4, V5, and V6, an important aspect for grape resistance to pathogens and for the physiological stability of berries. Potassium concentrations were higher in all treated variants, suggesting stimulation of metabolic processes and carbohydrate accumulation. In the case of nitrates, only treatments V2 and V6 exhibited increased concentrations, whereas the remaining treatments recorded lower values than the negative control. Furthermore, all experimental treatments maintained an acidic pH of the stum, favorable for fermentation processes and wine stability.

The assessment of wood maturation demonstrated that the applied extracts affected cane maturation differently depending on the genotype and the cane section analyzed. In the Fetească neagră 6 Șt. genotype, certain experimental treatments reduced pith diameter and modified wood diameter; however, the pith-to-wood ratio remained below one in all treatments, indicating adequate wood maturation. In the Fetească regală 72 Șt. genotype, the effects on cane maturation were less pronounced, with only slight reductions in pith diameter and pith-to-wood ratio observed

in certain treatments. Overall, the applied treatments did not negatively affect maturation processes, and the pith-to-wood ratio values confirmed satisfactory cane lignification.

Measurements of water content in woody tissue showed that the treatments influenced cane maturation particularly in the Fetească regală 72 Șt. genotype. Treatments V1, V3, and V6 reduced both free water and total water content in the canes, indicating a higher degree of maturation and greater winter hardiness. At the same time, dry matter content increased significantly in treatments V1, V3, and V5 compared with the control. These observations demonstrate that extracts obtained from viticultural waste and phytosynthesized nanoparticles can contribute to improved wood maturation and enhanced physiological resilience of grapevine plants.

Biochemical analyses of woody tissue investigated the influence of extracts obtained from viticultural waste and the organic product Growspore LF on grapevine cane maturation through the determination of starch, soluble sugar, and total sugar contents. In the Fetească neagră 6 Șt. genotype, significant differences were observed only in the silver nanoparticle-enhanced treatments V4 and V6, where increases in starch and total sugar contents were recorded compared with the control treatment. These increases indicate a greater accumulation of reserve substances within the canes and suggest a positive effect of silver nanoparticles on wood maturation. In contrast, soluble sugar content did not differ significantly among the experimental treatments.

In the Fetească regală 72 Șt. genotype, the application of extracts and the organic product Growspore LF did not significantly influence starch, soluble sugar, or total sugar contents, with recorded values remaining close to those of the negative control. These observations suggest that the physiological response of the maturation process differs according to genotype. The study confirms the importance of reserve carbohydrate accumulation in cane maturation and demonstrates that treatments containing silver nanoparticles produced the most favorable effects on the biochemical composition of woody tissue in the Fetească neagră 6 Șt. genotype.

CHAPTER VII. GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

The doctoral thesis entitled “Development of New Fertilization Strategies for Improving the Quality and Yield of Grapevine Genotypes under the Current Climate Change Conditions of the Ștefănești–Argeș Viticultural Area” primarily aimed to develop innovative fertilizers and establish sustainable alternative technological sequences capable of improving grapevine productivity, grape quality, and wood maturation, in accordance with current requirements for reducing chemical inputs and adapting to climate change.

The proposed specific objectives generated numerous relevant findings, reflecting both the complexity of the physiological and biochemical processes investigated and the considerable potential of the synthesized extracts and nanomaterials for application in viticulture.

Conclusions in Relation to the Specific Objectives

The conducted research demonstrated that extracts obtained from viticultural waste can be effectively utilized as alternative fertilizers, exhibiting high bioactive potential and differentiated effects depending on the type of plant material, genotype, and application sequence (phenological stage, number of applications, intervals between applications, and application method).

Phytochemical and chromatographic analyses confirmed the existence of a complex phenolic profile in the extracts, highlighting their capacity to positively influence grapevine secondary metabolism and contribute to enhanced antioxidant activity in grapes.

The development and application of the alternative fertilization sequence resulted in favorable physiological and biochemical responses, demonstrating that these approaches may represent viable solutions for optimizing viticultural technologies under climate stress conditions.

Productivity assessments revealed that the experimental treatments supported the productive potential of the studied genotypes without inducing major imbalances between vegetative and reproductive growth.

Determinations related to wood maturation showed that the application of the extracts did not negatively affect lignification processes or the accumulation of reserve compounds, contributing to the maintenance of an optimal physiological condition of the plants.

The physiological and biometric parameters analyzed revealed a differentiated impact of the treatments on vegetative vigor and leaf apparatus functionality, emphasizing the need to adapt fertilization strategies to the specific characteristics of each genotype.

The evaluation of grape quality confirmed the positive influence of the treatments on the technological and biochemical parameters of the harvest, with direct implications for the oenological value of the raw material.

The phytopathological assessments demonstrated the effectiveness of the extracts in reducing pathogen pressure, highlighting their potential integration into environmentally friendly grapevine protection strategies.

Production, Characterization, and Application of Fertilizing Extracts

The extracts obtained from viticultural waste exhibited considerable variability in phytochemical composition, determined by the type of plant material used (leaves versus canes), genotype, and enrichment method. Cane-derived extracts demonstrated superior bioactive potential compared with leaf-derived extracts, particularly regarding polyphenol content and antioxidant activity, confirming the value of viticultural waste as a raw material for alternative fertilizers.

Phytochemical and Chromatographic Analysis (TPC, DPPH, HPLC)

Total polyphenol content varied significantly depending on the type of viticultural waste and treatment variant. The highest values were associated with cane extracts, whereas leaf extracts exhibited lower concentrations. The application of hydroalcoholic leaf extract enriched with silver nanoparticles resulted in a reduction of the total polyphenol content of grape stem compared with the negative control.

Antioxidant capacity determined using the DPPH assay revealed higher values for cane extracts than for leaf extracts. The leaf extract enriched with silver nanoparticles stimulated the antioxidant activity of grape stem in the Fetească regală 72 Şt. genotype, whereas no relevant differences from the control were observed in the remaining variants.

HPLC analyses revealed a complex phenolic profile for the investigated extracts, with a greater number of compounds identified in cane extracts than in leaf extracts. Resveratrol, catechins, and epicatechins were identified as the predominant phenolic compounds, confirming the high bioactive potential of the hydroalcoholic extracts. Enrichment with silver nanoparticles induced quantitative changes in phenolic composition, reflected by reductions in the concentrations of certain compounds without affecting the overall diversity of the chemical profile.

Development of Alternative Fertilization Strategies

The application of the experimental fertilization strategies generated distinct physiological and biochemical responses that were dependent on genotype and phenological stage. Some experimental variants exhibited stimulatory effects on photosynthetic and reproductive metabolism, whereas others induced temporary inhibitory responses without compromising overall plant development.

Productivity Assessment (CFA, CFR, Cluster Weight)

Fertility coefficients indicated an improvement in productive potential in most experimental variants compared with the negative control. Average cluster weight and the weight of 100 berries were significantly influenced by the treatments, with variants V2, V3, V6, and the organic product Growspore LF showing particularly favorable effects on biomass accumulation.

Wood Maturation (Pith-to-Wood Ratio, Water Content, Carbohydrates)

Anatomical and biochemical analyses demonstrated that the application of the extracts did not negatively affect wood maturation, as the pith-to-wood ratio remained below one in all experimental variants. In the Fetească regală 72 Şt. genotype, some extracts promoted a reduction in free water content and an increase in dry matter content, indicating improved maturation of woody tissues.

Physiological and Biometric Determinations (Stomata, Leaf Area, Shoots)

The applied treatments induced moderate changes in ostiole opening and stomatal length, with genotype-specific responses. Leaf area was slightly reduced in Fetească neagră 6 Şt. but increased in Fetească regală 72 Şt. , while shoot growth highlighted a differentiated impact of the experimental variants on vegetative vigor.

Grape and Stem Quality (Chemical Determinations and HPLC Analysis)

Extracts derived from viticultural waste contributed to increased sugar content and reduced acidity in grape stem, improving the technological characteristics of the raw material intended for

winemaking. HPLC analyses demonstrated enrichment of the grape polyphenolic profile and enhanced antioxidant activity, confirming the positive impact of the treatments on harvest quality.

Control of the Phytopathogen *Uncinula necator*

All experimental variants demonstrated effectiveness in reducing the incidence, severity, and disease index of powdery mildew compared with the negative control. Extracts obtained from viticultural waste limited pathogen progression throughout the growing season, confirming their potential use as environmentally friendly alternatives in grapevine disease management.

Final Conclusions

In conclusion, the research conducted within this doctoral thesis supports the hypothesis that extracts obtained from viticultural waste can be successfully integrated into alternative grapevine fertilization and protection technologies, contributing to the sustainability of viticultural systems and the high-value utilization of agro-industrial by-products.

Future Perspectives

Future research will focus on testing and optimizing the developed extracts and fertilization strategies across a broader range of grapevine genotypes, as well as evaluating their application under both controlled conditions in protected environments and in open-field cultivation systems. These studies will aim to assess physiological and productive responses under diverse environmental conditions.

In addition, long-term investigations will be conducted to evaluate grape and stem quality, plant biochemical parameters, and the physiological mechanisms involved. The ultimate objective is to develop sustainable, efficient, and climate-resilient viticultural strategies adapted to current and future environmental challenges.

CHAPTER VIII. ORIGINAL CONTRIBUTIONS

Theoretical and Scientific Contributions

- Establishment of the relationship between mineral nutrition, physiological parameters, and grape quality under the specific pedoclimatic conditions of the Ștefănești–Argeș viticultural area, in the context of current climate change.
- Demonstration of the role of viticultural waste-derived extracts enriched with silver nanoparticles in stimulating physiological processes, activating plant defense mechanisms, and enhancing the accumulation of bioactive compounds relevant to grape quality.
- Integration and correlation of biochemical, physiological, biometric, and productivity analyses, leading to a comprehensive and holistic understanding of the effects of innovative fertilization strategies on grapevine performance.

Methodological Contributions

- Development and validation of innovative fertilization strategies combining conventional fertilizers, organic products, and biopreparations derived from viticultural waste.
- Implementation and comparison of modern methods for obtaining and characterizing plant extracts, including conventional extraction and microwave-assisted extraction, complemented by advanced analytical techniques such as HPLC, UV–Vis spectroscopy, XRD, and TEM.
- Integration within a unified experimental framework of multidimensional analyses, including: biochemical analyses (polyphenol content and antioxidant capacity); physiological assessments (stomatal characteristics, leaf area, and shoot growth); biometric and productivity evaluations (pollen viability, fertility coefficients, cluster weight, and berry weight); phytopathological assessments (disease severity caused by *Uncinula necator*); grape stum quality analyses (sugar content, acidity, and pH).

Applied and Practical Contributions

- Proposal of viable and sustainable alternatives to conventional fertilization through the use of innovative biopreparations and organic products, with demonstrated benefits for grapevine productivity and grape quality.
- Valorization of viticultural waste within a circular economy framework by transforming these by-products into biopreparations with high agronomic value and reduced environmental impact.
- Formulation of practical recommendations for grape growers aimed at enhancing grapevine resilience to climatic stresses, maintaining production quality, and adapting vineyard management practices to evolving market requirements.
- Development of a reproducible and transferable experimental model that can be applied to other viticultural regions with similar environmental conditions, thereby providing the thesis with broad scientific and practical relevance.

REFERENCES

- Gabaston, J.; Cantos-Villar, E.; Biais, B.; Waffo-Teguo, P.; Renouf, E.; Corio-Costet, M.-F.; Richard, T.; Mérillon, J.-M. Stilbenes from *Vitis vinifera* L. Waste: A Sustainable Tool for Controlling *Plasmopara viticola*. *J. Agric. Food Chem.* 2017, 65, 2711–2718.
- Aliaño-González, M. J.; Richard, T.; Cantos-Villar, E. Grapevine Cane Extracts: Raw Plant Material, Extraction Methods, Quantification, and Applications. *Biomolecules* 2020, 10(8), 1195.
- Baroi, A. M.; Popitiu, M.; Fierascu, I.; Sărdărescu, I.-D.; Fierascu, R. C. Grapevine Wastes: A Rich Source of Antioxidants and Other Biologically Active Compounds. *Antioxidants* 2022, 11(2), 393.
- Goufo, P.; Singh, R. K.; Cortez, I. A Reference List of Phenolic Compounds (Including Stilbenes) in Grapevine (*Vitis vinifera* L.) Roots, Woods, Canes, Stems, and Leaves. *Antioxidants* 2020, 9, 398.
- Richard, T.; El Khawand, T.; Taillis, D.; Da Costa, G.; Pedrot, E.; Cluzet, S.; Decendit, A.; Valls Fonayet, J. Chemical Process to Improve Natural Grapevine-Cane Extract Effectivity against Powdery Mildew and Grey Mould. *OENO One* 2021, 55, 81–91.
- Rashad, Y.M.; El-Sharkawy, H.H.A.; Belal, B.E.A.; Abdel Razik, E.S.; Galilah, D.A. Silica Nanoparticles as a Probable Anti-Oomycete Compound Against Downy Mildew, and Yield and Quality Enhancer in Grapevines. *Front. Plant Sci.* 2021, 12, 763365.
- Elmer, W.; White, J. C. The Future of Nanotechnology in Plant Pathology. *Annu. Rev. Phytopathol.* 2018, 56, 111–133.
- Gogos, A.; Knauer, K.; Bucheli, T. D. Nanomaterials in Plant Protection and Fertilization: Current State, Foreseen Applications, and Research Priorities. *J. Agric. Food Chem.* 2012, 60(39), 9781–9792.
- Fernandes, F.; Ramalhosa, E.; Pires, P.; Verdial, J.; Valentão, P.; Andrade, P.; Bento, A.; Pereira, J. A. *Vitis vinifera* Leaves Towards Bioactivity. *Ind. Crops Prod.* 2013, 43, 434–440.
- Călinescu, M.; Ungureanu, C.; Marin, F.; Militaru, M.; Soare, L.C.; Fierăscu, R.; Fierăscu, I. Antifungal Activities of Vegetal Extract Obtained from *Dryopteris filix-mas* (L.) Fern. *Fruit Grow. Res.* 2019, 35, 65–71.
- Fierascu, I. C.; Fierascu, I.; Baroi, A. M.; Ungureanu, C.; Ortan, A.; Avramescu, S. M.; Dinu-Parvu, C. E. Phytosynthesis of Biologically Active Silver Nanoparticles Using *Echinacea purpurea* L. Extracts. *Materials* 2022, 15(20), 7327.

Avellan, A.; Yun, J.; Morais, B. P.; Clement, E. T.; Rodrigues, S. M.; Lowry, G. V. Critical Review: Role of Inorganic Nanoparticle Properties on Their Foliar Uptake and In Planta Translocation. *Environ. Sci. Technol.* 2021, 55, 13417–13431.

Liu, R.; Lal, R. Potentials of Engineered Nanoparticles as Fertilizers for Increasing Agronomic Yields. *Sci. Total Environ.* 2015, 514, 131–139.

Jackson, R.S. *Wine Science: Principles and Applications*. Academic Press, 2008.

Dejeu, L. *Viticultură, Vol. 1 (Bazele biologice)*. Editura București, 2006.