PHYTOPATHOGENIC FUNGI CONTROL THROUGH THE USE OF EXTRACTS DERIVED FROM GRAPEVINE WASTES

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In this study grapevine wastes were used as significant sources of bioactive compounds with antifungal properties. So, hydroalcoholic extracts were from Fetească regală 72 Șt. and Fetească neagră 6 Șt. canes, and grapevine leaves, which have been characterized for total polyphenol content and antioxidant activity, and evaluated for their antifungal effect against the Alternaria solani and Fusarium oxysporum pathogens. The extracts were obtained using the classical method, and a part of these were enhanced with silver nanoparticles. The leaf extract (at a concentration of 15%) inhibited 100% the growth of phytopathogenic fungi in the laboratory conditions.

Keywords: *Alternaria solani*, *Fusarium oxysporum*, cane, leaves, polyphenols

1. Introduction

Viticulture significantly influences the efficient development of sectors specialized in obtaining grapevine genotypes. The pruning process is crucial for managing the growth and development of grapevines, constituting the main source of grapevine waste generation. Because viticulture is widespread globally, it contributes significantly to the accumulation of biomass waste, such as shoots, stems, and leaves [1]. Agricultural waste can be efficiently transformed into sustainable resources, thereby avoiding negative environmental impacts and reducing costs [2]. Practicing viticulture comes with notable benefits, but, at the same time, represents a challenge in managing significant quantities of grapevine waste. After grapevine pruning in months like October and March, significant amounts of canes and stems result, representing residues [3].

However, it is important to emphasize that these residues can be viewed from a wider perspective, as a resource rich in bioactive compounds.

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These compounds have the potential for various applications in fields such as medicine, cosmetics, and the food industry, contributing to waste reduction and more efficient utilization of grapevine waste [4]. Additionally, waste is generated at every stage of the wine production process [5]. Therefore, waste can be divided into two distinct categories based on their origin: those resulting from grape collection (solid waste) and those originating from wine production stages (liquid waste) [6]. The category of solid waste mainly includes grape bunches (7.5% of total solid waste generated from the winery), grape pomace (45%), grape seeds (6%) [7], and wine lees [8]. Grape pomace represents approximately 20-25% of the initial weight of grapes and is a solid residue obtained from the fermentation and pressing processes of grapes [9,10]. Additionally, wine lees constitute about 5% of the total weight of grapes, being rich in ethanol, tartaric acid, phenolic compounds, and yeast [11]. Grape peels are usually removed before the fermentation stage to avoid excessive astringency in wine. This represent a rich source of phenolic compounds, especially tannins, flavan-3-ols, hydroxycinnamic acids, flavonols, monomeric and oligomeric stilbenes, as well as lignocellulosic compounds (hemicellulose, cellulose, and lignin), most with antioxidant properties [12]. Antioxidants, phenolic compounds, phytosterols, fibres, proteins, carbohydrates, minerals, and even vitamin E, especially lipids and melatonin are found in grape seeds [13]. On the other hand, grape peels and pulp are a rich source of fibers, phenolic acids, flavonols, and anthocyanins [14]. However, the wine industry generates a significant amount of waste and by-products in a short period, representing approximately 30% of the initial weight of grapes [13]. The storage of these wastes can cause economic and environmental problems due to their organic matter content, low pH, salinity, and the presence of heavy metals [14].

Fruit and vegetable peels resulting from the food industry can be a source of antimicrobial compounds [15], and grapevine waste along with grape pomace can also be used as antimicrobial and antioxidant agents [16]. For example, products derived from grapevine shoots have antimicrobial effects against the yeasts *Brettanomyces bruxellensis* and *Zygosaccharomyces bailli* [17], which are responsible for microbial spoilage in red wine [18] and, respectively, food spoilage [19]. Also, grapevine stems can be used to inhibit pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*, and *Escherichia coli* [20].

Species within the genus *Fusarium* are globally widespread pathogenic fungi known for toxin production. These toxins can cause health issues associated with cellular damage, cancer risks, and adverse effects on the growth and development of both animals and humans [21]. Another genus that includes a wide range of species is *Alternaria*, one of the most widespread fungal groups found in various contexts, contain saprophytic, endophytic, and parasitic species that can affect a variety of crops. The small-sized spores of this pathogen are omnipresent,

contributing to the degradation of food quality, leading to a decrease in its nutritional value through the generation of toxic metabolites. This situation has a negative impact on the economic value of food products [22]. *Alternaria solani* and *Fusarium oxysporum* cause substantial losses in tomato production [23, 24].
This study examines the conventional technique for extracting bioactive compounds from vineyard waste (stems and leaves) and how to improve them by adding silver nanoparticles to obtain extracts with potential antifungal properties against the pathogens *A. solani* and *F. oxysporum*.

2. Material and methods

The study was conducted within the National Research and Development Institute for Biotechnology in Horticulture, Stefanesti - Argeș, Romania. In December, during the endo-dormancy phase, grapevine canes resulting from agrotechnical work were collected from the Fetească neagră 6 Șt. and Fetească regală 72 Șt. clones, located in the field. Subsequently, in January, leaves from twenty-one different grapevines genotypes located in a protected environment (greenhouse) were gathered following their natural physiological falling process.

The resulting waste was transported to the laboratory to undergo a complete dehydration process. The waste collection stage is an essential part of responsible resource management and minimizing environmental impact. To extract phytochemical substances from the canes waste (Fetească neagră 6Șt., Fetească regală 72 Șt.) and leaves, the classic solid-liquid extraction method was used. The ratio between solid material and solvent was 1:5 (w/v), with 96% ethyl alcohol used as the solvent. The total polyphenolic content (TPC) of the extracts was determined using the colorimetric method (Folin-Ciocâlteu reagent), while the antioxidant capacity of the extracts was assessed using the DPPH radical scavenging method. The quantity of hydroalcoholic extract, obtained from waste using the solid-liquid method, was divided into two parts: one remained as pure hydroalcoholic extract, while the other part was enhanced with silver nanoparticles. The hydroalcoholic extracts and those enhanced with silver nanoparticles were evaluated for their *in vitro* antifungal effect against the phytopathogenic fungi *F. oxysporum* and *A. solani*, cultivated on potato-dextrose agar (PDA) medium.

Classic Solid-Liquid Extraction

For this type of extraction, a Nitech-POL-EKO-APARATURA drying oven was used. Thus, for each type of waste (canes from Fetească neagră 6 Șt., Fetească regală 72 Șt., and leaves), three variants/samples were prepared with different concentrations of 96% ethyl alcohol (supplied by Chimreactiv, Bucharest, Romania) in a ratio of 1:5 (w/v), according to the established experimental parameters (Table 1).

Table 1

Parameters established for classical extraction.

No.	Sample name	Relative humidity (%)*	Water-to-alcohol ratio (%)	Plant-to-solvent ratio	Extraction time (h)	Temperature (°C)
1	C_Fn	21.13	0:100	1:5	3	75
2	C_Fr	19.47				
3	F	21.43				
* The relative humidity of the plant material was determined after grinding.						

Abbreviations for Table 1: C_Fn-extract from Fetească neagră 6 St. canes; C_Fr-extract from Fetească regală 72 St. stems; F-extract from leaves.

After the obtaining process, the hydroalcoholic extracts were filtered and stored in tightly sealed containers at a temperature of +4 °C.

Determining the Total Polyphenol Content (TPC)

The Folin-Ciocâlteu spectrophotometric method accurately allowed the determination of the total polyphenol concentration in the studied samples, providing information about their chemical composition and antioxidant potential. This method is recognized for its accuracy in measuring polyphenol content and is widely used in organic chemistry laboratories. Different volumes were taken from the experimental variants, which were subsequently diluted. Then, a volume of 200 µL was taken from each dilution, to which 1 mL of the Folin-Ciocâlteu reagent was added. After approximately 3-8 min, 400 µL of 7.5% Na_2CO_3 solution (supplied by Chimreactiv, Bucharest, Romania) was added. The samples were kept in the dark at room temperature for 60 min and then subjected to spectrophotometric analysis at a wavelength of 765 nm.

The results obtained from the analysis were expressed in g/g dry matter (DM) based on the calibration curve constructed for different concentrations of the standard, specifically for 5 points of concentrations ranging from 10 to 50 µg/mL gallic acid.

Antioxidant Capacity Determination (DPPH)

The antioxidant capacity of hydroalcoholic extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. This technique is employed to measure how efficiently a sample can neutralize free radicals, providing information about its antioxidant properties. The ratio used between the analyzed samples and the DPPH reagent was 1:1 (v/v). From each extract sample, 1000 µL was pipetted into Eppendorf tubes. Then, 1000 µL of DPPH solution was added to them. The samples were placed on a shaker for 30 min and subsequently kept in the dark for an additional 30 min. Additionally, a control solution was prepared identically to the method described above, with the only difference being the addition of 1000 µL of hydroalcoholic mixture (ethyl alcohol:distilled water, in a 1:1 (v/v) ratio). The absorbance of the samples was read at a wavelength of 517

nm using the UV-Vis SPECOR PLUS 210 spectrophotometer. The antioxidant activity of the samples is directly proportional to the percentage reduction of the DPPH radical, so a high reduction percentage indicates strong antioxidant activity. This percentage was calculated according to the formula:

$$AA\% = \frac{\mathbf{A517nm\ (M) - A517nm\ (P)}}{\mathbf{A517nm(M)}} \boldsymbol{x100}$$

Where:

A517nm (M) = represents the absorbance read at a wavelength of 517 nm for the control sample.

A517nm (P) = represents the absorbance value of the analyzed sample.

Antifungal Activity

To obtain pure colonies, the two studied phytopathogens (*A. solani* and *F. oxysporum*) were isolated on PDA medium. The process involved taking a small mycelial fragment with a diameter of about 5 mm from plant material (tomatoes) and placing it in the center of a Petri dish, which was then kept at room temperature for a period of 7 days, allowing the colonies to reach the necessary maturity for replication and purification.

Hydroalcoholic extracts and those enhanced with silver nanoparticles were incorporated into the culture medium (warm) at different concentrations and then distributed in Petri dishes. After cooling and solidification, mycelial fragments of *A. solani* and *F. oxysporum* were transferred to the culture medium. Four different concentrations were used for each type of extract: 0.5%, 2%, 9%, and 15%. These concentrations were selected to determine the optimal variant for controlling the studied pathogens. To obtain relevant data, observations were recorded at five distinct time intervals: 3, 5, 7, 10, and 14 days. This approach aimed at obtaining precise information about how these concentrations influence the growth of fungi.

The colony diameter was measured in two directions, and the average value was recorded. The percentage of mycelial growth inhibition was calculated using Vincent's formula [25].

$$\mathbf{I\%} = \frac{\mathbf{C - T}}{\mathbf{C}} \boldsymbol{x100}$$

Where:

I = inhibition percentage;

C = colony diameter (control);

T = diameter of the colony under treatment.

3. Results and Discussions

Polyphenol Analysis

The total polyphenol content showed variability depending on the type of hydroalcoholic extract used. Thus, the highest concentration of polyphenols was recorded in the hydroalcoholic extract obtained from Fetească neagră 6 Șt. grapevines, with a value of 21.5825 mg/g gallic acid equivalent (GAE). It was followed by the extract from Fetească regală 72 Șt. grapevines, which had a concentration of 20.4079 mg/g GAE. The lowest value was recorded in the hydroalcoholic extract obtained from leaves (11.1698 mg/g GAE) (Table 2). Therefore, the polyphenol content of the extract from the leaves was lower by 48% and 54.43%, respectively, than that obtained from the canes.

Table 2

Total polyphenol content of extracts from grapvine canes and leaves waste.

Total phenolic content							
No.	Sample	A765 nm			Standard Deviation	Mean	g/g dry matter (DM)
1.	F	0.233	0.232	0.230	0.0000374	0.232	11.1698
2.	C_FN	0.451	0.450	0.450	0.0004714	0.450	21.5825
3.	C_FR	0.426	0.426	0.425	0.0004714	0.426	20.4079

Abbreviations for Table 2: C_FN-Fetească neagră 6 Șt. canes extract; C_FR-Fetească regală 72 Șt. canes extract; F-leaves extract.

Antioxidant Activity Evaluation Using the DPPH Method.

To assess the antioxidant capacity of the extracts, the stable free radical DPPH was used. The data presented in table 3 indicate that the highest antioxidant capacity was recorded in the hydroalcoholic extract obtained from Fetească neagră 6 Șt. grapevine canes, showing an inhibition percentage of 28.75±1.93% DPPH, followed by the hydroalcoholic extract from Fetească regală 72 Șt. canes, with an inhibition percentage of 42.03±0.78% DPPH. Meanwhile, the hydroalcoholic extract obtained from leaves exhibited a lower inhibition value, specifically 28.75±1.93% DPPH (Table 3).

Table 3

Antioxidant potential of extracts from grapevine waste canes and leaves.

No.	Sample	Extracts by classical method
		% DPPH Inhibition
1.	F	28.75±1.93
2.	C_FN	47.68±1.48
3.	C_FR	42.03±0.78

Abbreviations for Table 3: C_FN – extract from canes of Fetească neagră 6 Șt.; C_FR – extract from canes of Fetească regală 72 Șt.; F – extract from leaves.

Dynamic growth of mycelial hyphae on culture medium

The mycelial dynamics were rapid, with radial development from the inoculation point. The average colony diameter for *A. solani* was 2.4/2.6 cm at 3 days and 8/8 cm at 10 days, while the phytopathogenic agent *F. oxysporum* exhibited a much faster growth, with the colony diameter averaging 3.4/3.5 cm at 3 days and 8/8 cm at 7 days post-inoculation (Fig. 1).

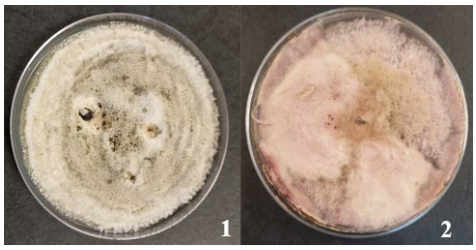

Fig. 1 *A. solani* (1) & *F. oxysporum* (2) on PDA medium (control)

Antifungal Activity of Hydroalcoholic Extracts and Those Enhanced with Silver Nanoparticles.

In order to establish a notable inhibitory percentage on the dynamics of the two pathogens under study, four concentrations of each hydroalcoholic extract were used (hydroalcoholic extract from leaves; hydroalcoholic extract from canes Fetească neagră 6 Șt. and Fetească regală 72 Șt.): 0.5%; 2%; 9%, and 15%.

Fig. 2 illustrates the action of the three hydroalcoholic extracts obtained through the classical extraction method on the mycelial hyphae of the pathogenic fungus *A. solani*.

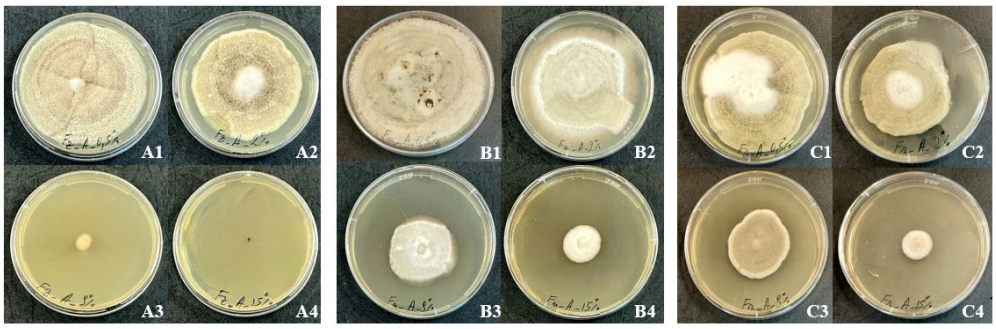

Fig. 2. Growth of the pathogenic fungus *A. solani* on PDA medium amended with hydroalcoholic extracts. (A1= leaf grapevine Fz_A_0,5%; A2 = leaf grapevine Fz_A_2%; A3 = leaf grapevine Fz_A_9%; A4 = leaf grapevine Fz_A_15%; B1= grapevine canes of Fetească neagră 6 Șt. Fn_A_0,5%; B2 = grapevine canes of Fetească neagră 6 Șt., Fn_A_2%; B3 = grapevine canes of Fetească neagră 6 Șt., Fn_A_9%; B4= grapevine canes of Fetească neagră 6 Șt., Fn_A_15%; C1 = grapevine canes of Fetească regală 6 Șt., Fr_A_0,5%; C2 = grapevine canes of Fetească regală 6 Șt., Fr_A_2%; C3 = grapevine canes of Fetească regală 6 Șt., Fr_A_9%; C4 = grapevine canes of Fetească regală 6 Șt., Fr_A_15%).

Statistical analysis of the obtained data, demonstrated that the hydroalcoholic extract obtained from tested leaf waste at the maximum concentration (F_A_15%) exhibited a 100% inhibition against the pathogenic agent *A. solani* compared to the control. Hydroalcoholic extracts obtained from canes waste of Fetească regală 72 Șt. (Fr_A_15%) and Fetească neagră 6 Șt. (FN_A_15%) tested at the concentration of 15% recorded inhibition percentages ranging between 63.33% and 77.08%. Additionally, it was observed that the hydroalcoholic extract from leaves at the concentration of 9% showed an inhibition of over 80% compared to the control. These results indicate that the leaf waste extract has significantly stronger antifungal activity compared to the other tested extracts (Fig. 3).

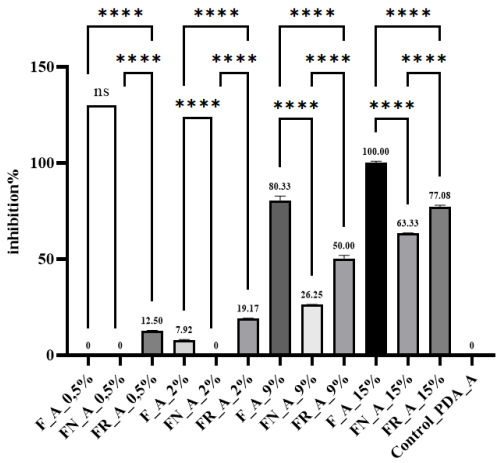

Fig. 3. The effect of hydroalcoholic extracts at different concentrations on the pathogenic fungus *A. solani*. The data are expressed as the mean ± SD, and the p-values were calculated using the one-way ANOVA method followed by Šídák's multiple comparison test. $^{****}p < 0.0001$, $^{ns}p > 0.9999$ (F - grapevine leaf, FN - grapevine canes of Fetească neagră 6 Șt., FR - grapevine canes of Fetească regală 72 Șt., PDA - potato-dextrose agar, *A* - *A. solani*)

For a comprehensive study and to determine the antifungal effectiveness of silver, hydroalcoholic extracts were enhanced with silver nanoparticles (NPAg) and also tested *in vitro* at the four concentrations (0.5%, 2%, 9%, and 15%). Fig. 4 illustrates the impact of the three hydroalcoholic extracts + NPAg on the mycelial development of the *A. solani* pathogenic agent.

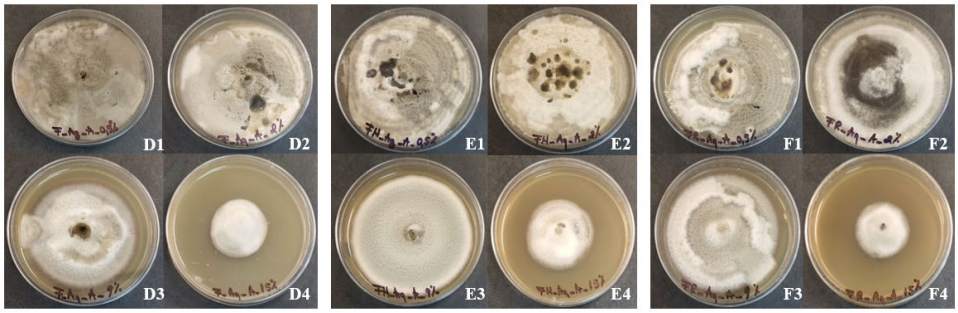

Fig. 4. Growth of the *A. solani* pathogenic fungus on PDA medium amended with hydroalcoholic extracts enhanced with silver nanoparticles. (D1= grapevine leaf Fz_Ag_A_0,5%; D2 = grapevine leaf Fz_Ag_A_2%; D3 = grapevine leaf Fz_Ag_A_9%; D4 = grapevine leaf Fz_Ag_A_15%; E1= grapevine canes of Fetească neagră 6 Șt. Fn_Ag_A_0,5%; E2 = grapevine canes of Fetească neagră 6 Șt., Fn_Ag_A_2%; E3 = grapevine canes of Fetească neagră 6 Șt., Fn_Ag_A_9%; E4= grapevine canes of Fetească neagră 6 Șt., Fn_Ag_A_15%; F1 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_A_0,5%; F2 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_A_2%; F3 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_A_9%; F4 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_A_15%).

According to studies from the related literature, silver nanoparticles have demonstrated their antibacterial effectiveness, and less antifungal. These have been previously recognized for their remarkable antimicrobial activity against a diverse range of pathogenic bacteria, including *Staphylococcus aureus* (Gram-positive), *Bacillus cereus*, *Escherichia coli* (Gram-negative), *Pseudomonas aeruginosa*, and *Salmonella choleraesuis*, as well as against yeasts of the *Candida* genus and various fungi, including *Aspergillus*, *Fusarium*, and *Pythium* [26].

Following the statistical analysis of the data, it was revealed that the three hydroalcoholic extracts enhanced with silver nanoparticles, at the maximum concentration used, had similar effects against the pathogen *A. solani*. Thus, for extracts obtained from the Fetească regală 72 Șt. and Fetească neagră 6 Șt. canes waste, an inhibition of 50% was recorded for the hydroalcoholic extract enhanced with silver nanoparticles (FR_NPAg_A_15%), followed by the hydroalcoholic extract enhanced with silver nanoparticles from Fetească neagră 6 Șt. canes (FN_NPAg_A_15%) with an inhibition of 49.17%. The inhibition percentage for the hydroalcoholic extract enhanced with silver nanoparticles obtained from leaves (F_NPAg_A_15%) was 44.58%.

The same situation was reported at the concentration of 9%, where the hydroalcoholic extract from Fetească neagră 6 Șt. canes (FN_NPAg_A_9%) recorded the highest inhibition percentage of 11.25%, followed by the F_NPAg_A_9% extract with a percent of 9,58%, and the FR_NPAg_A_9% extract with a percent of 7,08%. At concentrations of 0.5% and 2%, no significant differences were observed in controlling or combating the *A. solani* pathogenic agent with the help of the three extracts. These findings show that hydroalcoholic

extracts obtained from waste, both from grapevines canes and leaves, enhanced with silver nanoparticles, tested at concentrations of 9% and 15%, present significant differences in the antifungal activity against the *A. solani* pathogenic agent, as shown in Fig. 5.

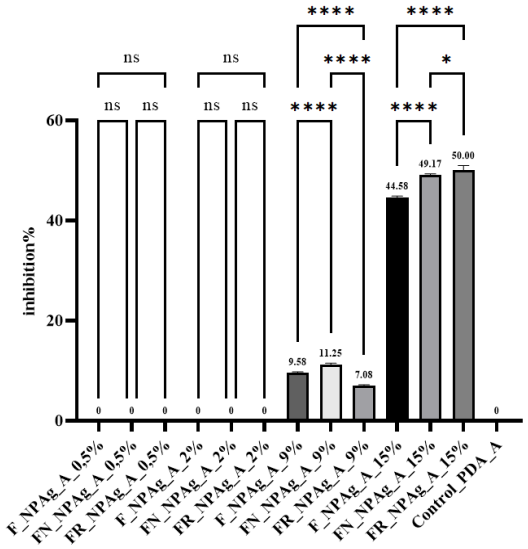

Fig. 5. The effect of hydroalcoholic extracts enhanced with silver nanoparticles (at different concentrations) on the pathogenic fungus *A. solani*. The data are expressed as the mean ± SD, and the p-values were calculated using the one-way ANOVA method followed by Šídák's multiple comparison test. $^{****}p < 0.0001$, $^{*}p = 0.0334$, $^{ns}p > 0.9999$ (F- grapevine leaf, FN - grapevine canes of Fetească neagră 6 Șt., FR - grapevine canes of Fetească regală 72 Șt., PDA- potato-dextrose agar, NPAg-silver nanoparticles, *A -A. solani*)

The effect of the three hydroalcoholic extracts obtained from viticultural waste through the conventional extraction method was also tested on the mycelial growth of the pathogenic agent *F. oxysporum* (Fig. 6).

The statistical analysis of the obtained data, indicated that from the four concentrations of hydroalcoholic extracts obtained from viticultural waste, only concentrations 2%, 9%, and 15% showed significant differences compared to the control variants. At the 0.5% concentration, no significant inhibition of mycelial growth of the *F. oxysporum* pathogenic agent was observed. In this context, at a concentration of 15%, the hydroalcoholic extract from leaves (F_F_15%) completely inhibits the mycelial growth of the *F. oxysporum* pathogenic agent, suggesting its antifungal effect with an effectiveness of 100%. Inhibition percentages of 77.50% were recorded for the FR_F_15% extract and 75.42% for the FN_F_15% extract at the same concentration.

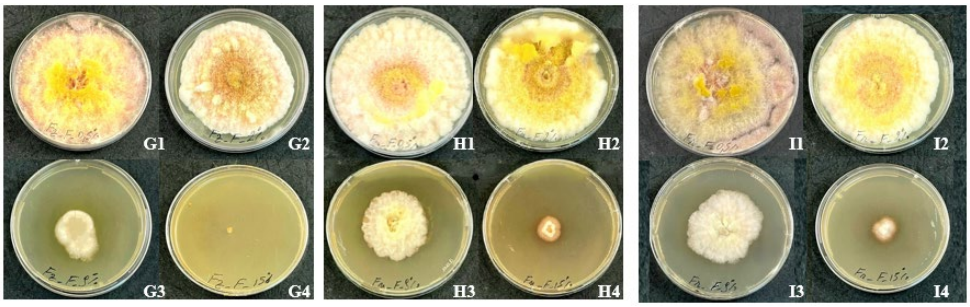

Fig. 6. Growth of the *F.oxysporum* pathogenic fungus on PDA medium amended with hydroalcoholic extracts. (G1= grapevine leaf Fz_F_0,5%; G2 = grapevine leaf Fz_F_2%; G3 = grapevine leaf Fz_F_9%; G4 = grapevine leaf Fz_F_15%; H1= grapevine canes of Fetească neagră 6 Șt. Fn_F_0,5%; H2 = grapevine canes of Fetească neagră 6 Șt., Fn_F_2%; H3 = grapevine canes of Fetească neagră 6 Șt., Fn_F_9%; H4= grapevine canes of Fetească neagră 6 Șt., Fn_F_15%; I1 = grapevine canes of Fetească regală 6 Șt., Fr_F_0,5%; I2 = grapevine canes of Fetească regală 6 Șt., Fr_F_2%; I3 = grapevine canes of Fetească regală 6 Șt., Fr_F_9%; I4 = grapevine canes of Fetească regală 6 Șt., Fr_F_15%).

At a concentration of 9%, inhibition up to 65% was observed for the F_F_9% extract, followed by percentages of 45.83% for the FN_F_9% extract and 37.50% for the FR_F_9% extract. At the 2% concentration, the inhibition percentage was 4.17% for the F_F_2% extract, followed by 2.92% for the FR_F_2% hydroalcoholic extract, while the FN_F_2% extract did not inhibit the growth of the pathogenic agent (Fig. 7).

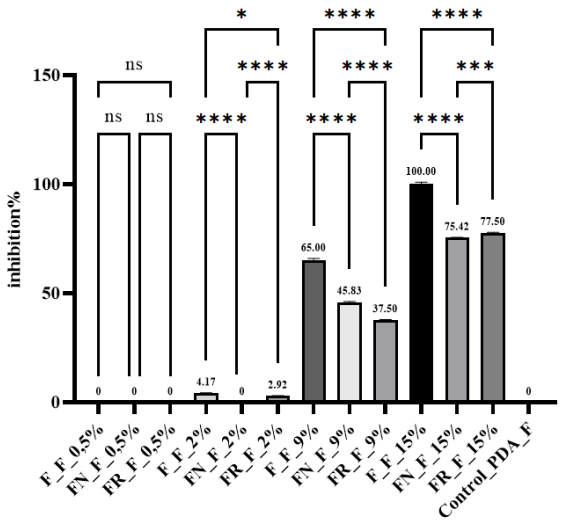

Fig. 7. Hydroalcoholic extracts effect (at different concentrations) on the *F. oxysporum* pathogenic fungus. The data are presented as the mean ± SD, and the p-values were calculated using the one-way ANOVA method followed by Šídák's multiple comparison test. Significance levels are indicated by asterisks: $^{****}p < 0.0001$, $^{***}p = 0.0001$, $^{*}p = 0.0318$, $^{ns}p > 0.9999$ (F - grapevine leaf, FN - grapevine canes of Fetească neagră 6 Șt., FR - grapevine canes of Fetească regală 72 Șt., PDA - potato-dextrose agar, *F - F. oxysporum*).

The results of this study are in correlation with those obtained by Din et al. (2022), where it was observed that the extract from Cabernet Sauvignon pomace obtained during the winemaking process had a high content of polyphenols. Regarding the impact on phytopathogenic fungi, *in vitro* test data revealed a strong inhibitory effect on the mycelial growth rate and a significant reduction in spore germination capacity in *Fusarium* sp., up to 91.56% [27].

In order to emphasize a significant inhibition of pathogen development, four different concentrations were investigated for each type of hydroalcoholic extract improved with silver nanoparticles (NPAg) obtained from grapevine waste (canes and leaves). These extracts include hydroalcoholic extract from leaves + NPAg, hydroalcoholic extract from canes of Fetească neagră 6 Șt. + NPAg, and hydroalcoholic extract from canes of Fetească regală 72 Șt. + NPAg. Fig. 8 illustrates the impact of the three hydroalcoholic extracts + NPAg on the mycelial development of the *F. oxysporum* pathogen.

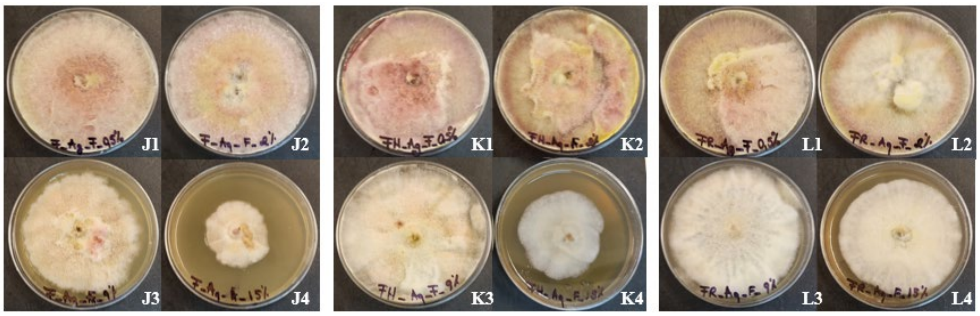

Fig. 8. *F. oxysporum* pathogenic fungus growth on PDA medium amended with hydroalcoholic extracts enhanced with silver nanoparticles. (J1= grapevine leaf Fz_Ag_F_0,5%; J2 = leaft grapevine Fz_Ag_F_2%; J3 = leaft grapevine Fz_Ag_F_9%; J4 = grapevine leaf Fz_Ag_F_15%; K1= grapevine canes of Fetească neagră 6 Șt. Fn_Ag_F_0,5%; K2 = grapevine canes of Fetească neagră 6 Șt., Fn_Ag _F_2%; K3 = grapevine canes of Fetească neagră 6 Șt., Fn_Ag_F_9%; K4= grapevine canes of Fetească neagră 6 Șt., Fn_Ag_F_15%; L1 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_F_0,5%; L2 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_F_2%; L3 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_F_9%; L4 = grapevine canes of Fetească regală 6 Șt., Fr_Ag_F_15%).

In Fig. 9 it can be seen that hydroalcoholic extracts from canes of Fetească neagră 6 Șt. + NPAg and Fetească regală 72 Șt. + NPAg, at the concentration of 9%, did not significantly inhibit the development of the pathogenic fungus. However, at a higher concentration, namely 15%, the extract FN_NPAg_F_15% inhibited the pathogen's development by 40%, followed by the extract FR_NPAg_F_15%, which showed an inhibition of 8.75%. Hydroalcoholic extract from leaves + NPAg inhibited the development of the *F. oxysporum* pathogenic fungus by 54.58% at a concentration of 15% and up to 11.25% at a concentration of 9%.

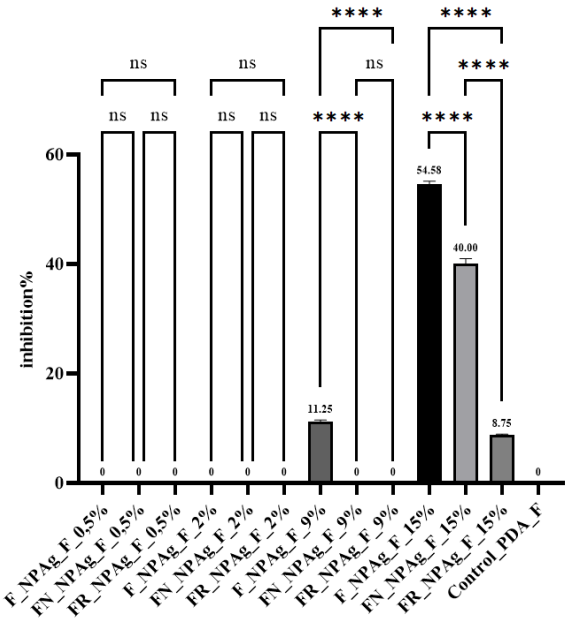

Fig. 9. Effect of silver nanoparticle-enhanced hydroalcoholic extracts at different concentrations on the *F. oxysporum* pathogenic fungus. The data are expressed as the mean ± SD, and the values of p were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test. $^{****}p < 0.0001$, $^{ns}p > 0.9999$ (F - grapevine leaf, FN - grapevine canes of Fetească neagră 6 Șt., FR - grapevine canes of Fetească regală 72 Șt., PDA-potato-dextrose agar, NPAg-silver nanoparticles, *F* - *F. oxysporum*).

According to the results obtained in the *in vitro* tests, the extracts derived from grapevine waste (Fetească regală 72 Șt. and Fetească neagră 6 Șt. canes, leaves) showed significant effects in inhibiting the development of the two studied pathogens. However, the most remarkable results were observed with hydroalcoholic extracts obtained from leaves, which exhibited significant efficacy against the studied phytopathogenic fungi.

4. Conclusions

Grapevine plantation waste is an important source of bioactive compounds with significant antifungal properties.

The hydroalcoholic extracts obtained from cane waste have a significantly higher polyphenol content compared to the extracts obtained from grapevine leaves. The hydroalcoholic extract obtained from the leaves had a lower content of polyphenols, with 48.23%, respectively 45.27% lower than the one obtained from the canes.

The research results certified that natural extracts obtained from grapevine waste represent a promising direction for the efficient control and combat of

pathogens that cause significant damage to tomato crops. This finding opens promising perspectives for the future of agriculture and the development of measures for the protection of horticultural plants.

Thus, hydroalcoholic extracts obtained from Fetească regală 72 Șt. and Fetească neagră 6 Șt. grapevine canes and those from grapevine leaves had a better effect against the *Alternaria solani* pathogen than against the *Fusarium oxysporum* pathogen.

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