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In vitro antifungal activity of some steroidal glycoalkaloids on *Monilinia* spp.

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Abstract

Monilinia spp is the plant pathogen responsible for the occurrence of grey mold and fruit rot in stone fruits species, and it is present in all cultivated areas. In Romania, it occurs each year in plum orchards, depending on the environmental conditions, varieties' behavior and control treatment management. It was tested in vitro the antifungal activity of some glycoalkaloides steroids extracted from species belonging to Solanum genre towards Monilinia spp. isolated from plum fruits. Of these, a structural group encoded as GLY was selected and tested in 0.1%, 0.5% and 1% concentration compared to control variant. After 12 days from treatment, the colonies diameter was 47 mm in control plate and 34.4 mm in 0.1% GLY plate. In variants with 1% GLY and 0.5% GLY the mold growth was weak, with 2.8 mm and 5 mm diameter after 12 days. The efficacy was 94% in the 1% GLY variant, followed by 0.5% GLY variant where the efficacy was 89%. The EC 50 and EC 90 values were 0.25% and 0.82%.

Keywords: antifungal activity, efficacy, micelial growth

1. Introduction

Monilinia spp. is a complex of plant pathogens which causes significant losses to pome fruits orchards. *Monilinia laxa* (Aderhold & Ruhland) Honey and *Monilinia fructicola* (G. Winter) Honey occur frequently on plum, cherry, apricot and stone cherry (SNYDER & JONES) [1]. The attack of these species is dangerous on flowering stage, on vegetation fruits and in fruits storage (LEEUWEN & KESTEREN [2], VALIUSKAITE & al. [3]). An accurate determination of the pathogens inducing these symptoms is established through genetic methods (FULTON & BROWN [4], FULTON & al. [5], FORSTER & ADASKAVEG [6], GELL & al. [7], ZHU & al. [14] and morphological identification (GHEORGHIES & CRISTEA [28]). The attack of *Monilinia* spp. molds, responsible for the occurrence of grey mold and fruit rot, represents a serious economic problem, these pathogens determining important losses in fruits vegetation and storage period (OLIVEIRA & al. [8]). The host plants range includes apple, pear, cherry, apricot, plum and nectarines (BYRDE & WILLETTS [9]). *Monilinia laxa* is the most dangerous pathogen inducing blossom wilt and twig blight in stone fruits, causing significant losses in all fruits producing regions (BALAŽ

[11]). Studies on fungal epidemiology of the genus *Monilinia* have shown that vectors, such as insects, birds (LACK) [12] and environmental factors favor the spread of conidia (BANNON & al. [13]).

The control of pathogens of the *Monilinia* genre is based on multiple interventions, cultural hygiene measures, cultural practices, breeding of resistant varieties, use of fungicides (BORVE & STENSVAND [15]). Chemical control is still effective, but can lead to the appearance of pathogenic resistance, phytotoxicity, toxicity to other organisms. Research in the field is increasingly focused on the use of biocontrol (GRZEGORCZYK & al. [16]). An alternative for the control of *Monilinia* spp. fungi can be the use of plant extracts with antifungal activity. Plant extracts are active against pathogens and may be sources of plant protection bioproducts or formulations (PARVU & al. [17], CRISTEA [18], CALVO & al. [22], ICHIM & al. [27]). Plants of the genus *Solanum* have been used to study the pathogenicity and virulence of some pathogens (ICHIM & al. [29]). Steroidal glycoalkaloids, secondary metabolites extracted from *Solanum* species have antimicrobial properties (IIJIMA & al [19], ITKIN & al. [20], MILNER & al [21]). The objective of our research was to assess under laboratory conditions the effect of steroidal glycoalkaloids extracted from *Solanum* species, as a component of a biofungicide, on the growth of *Monilinia* spp. pathogens isolated from plum fruits.

2. Materials and Methods

The research aimed the *in vitro* assessment of the activity on the growth of *Monilinia* spp. of some steroidal glycoalkaloids extracted from *Solanum* species. The steroidal glycoalkaloids taken into account were:

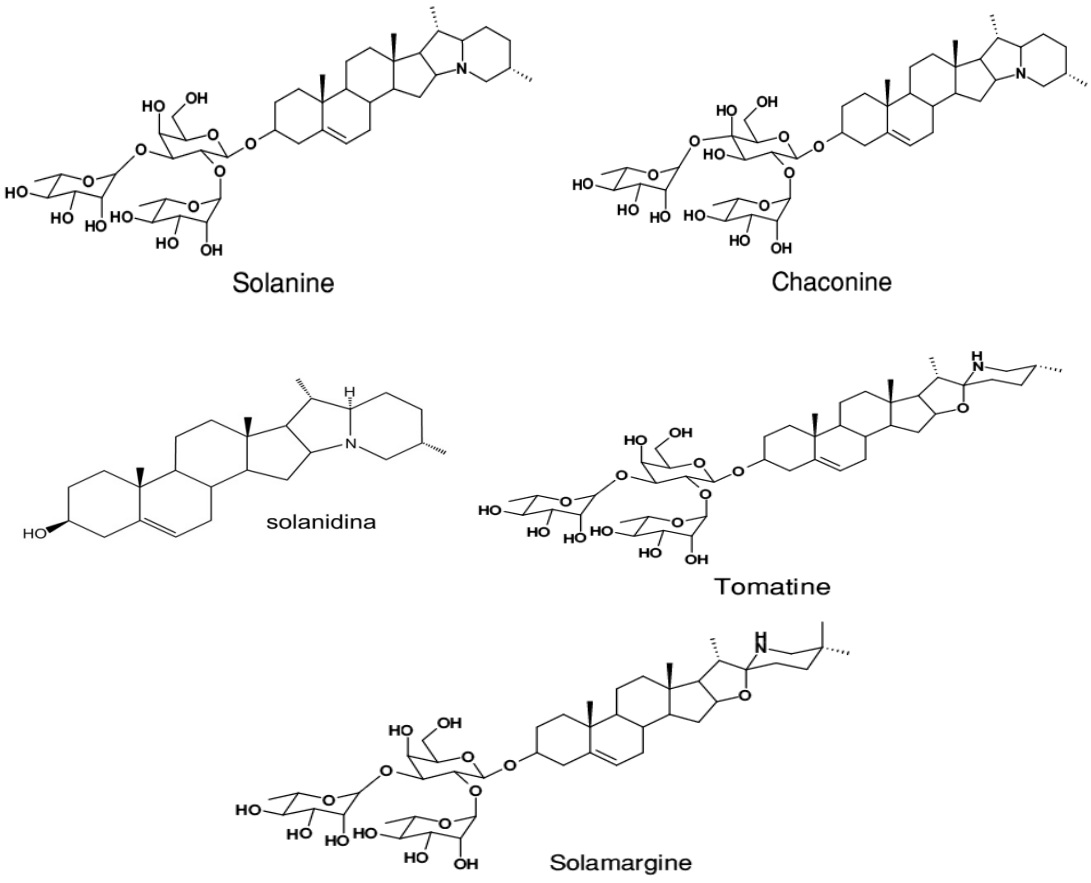

Figure 1: Steroidal glycoalkaloids from *Solanum* species

In order to prove the antifungal activity, we selected a standardized structural group, encoded as GLY, obtained from specific technological processes of extraction and purification from different sources of vegetal raw materials. The biological material consisted in isolates of *Monilinia* genus (Ml 13) from collected samples of plum fruits, Stanley variety, originated from RIFG Mărăcineni, Romania. The method food poison technique was used (SCHMITZ)[24]. From *Monilinia* spp. fresh fungal cultures, 0.5 mm diameter plots were harvested and placed centrally in 90 mm Petri dishes. Potato-dextrose-agar culture medium was used and the test product was included in concentrations of 0.1%, 0.5% and 1%; each variant was placed in three repetitions. Incubation was done at 22°C. Measurements of mycelial growth were performed at 3, 6, 9, 12 days. The efficacy of the product was determined after 12 days of observation, as the rate of inhibition of mycelial growth from the treated variants compared to the control variant, without the product included in the culture medium, according to the formula: I%=[(Dc-Dt)/Dc]x100, where, I % is micelian growth inhibition, Dc is average micelian growth diameters of fungus colony in control, Dt is average micelian growth diameters of fungus colony in tratament (PANDEY & al.[23]. For the results obtained after 12 days form treatment, the effective concentration of CE 50 and CE 90 was calculated (the concentration that reduced the mycelial growth by 50% and 90% determined by regressing the inhibition of radial growth values).

3. Results and discussion

Among the presented steroidal glycoalkaloids a GLY-coding group was selected for *in vitro* testing of the effect on *Monilinia* spp. fungi, isolated from Stanley variety plums. The data in Table 1 shows that at 3 days after replication the fungus had no growth in any of the variants. After 6 days of observation, the fungus developed in all variants reaching 11.6 mm diameter in the 0.1% GLY concentration and 18 mm in the control variant (figure 2). After 9 days of observation, fungal mycelium developed colonies that reached a 31.6 mm diameter in the control variant colony, with an average diameter of 27.6 mm in the 0.1% GLY concentration variant, 3.9 mm diameter in the 0.5% GLY concentration variant and 1.4 mm diameter in the 1% GLY concentration variant (figure 3). After 12 days of observation in the control variant, the *Monilinia* spp. fungal growth was 47 mm diameter. It was observed that in 1% GLY and 0.5% GLY variants the analyzed fungi developed very slowly during the observation period, reaching 2.8 mm and 5 mm diameter respectively, after 12 days of observation (Table 1).

Table 1. Antifungal activity of GLY on mycelial growth of *Monilinia* spp. (*in vitro*)

Variant / concentration (%)	Average colony diameter (mm) / 3 days	Average colony diameter (mm) / 6 days	Average colony diameter (mm) / 9 days	Average colony diameter (mm) / 12 days
GLY/0.1	0	11.6	27.6	34.4
GLY/0.5	0	1.5	3.9	5.0
GLY/1	0	0.8	1.4	2.8
Control	0	18.0	31.6	47.0

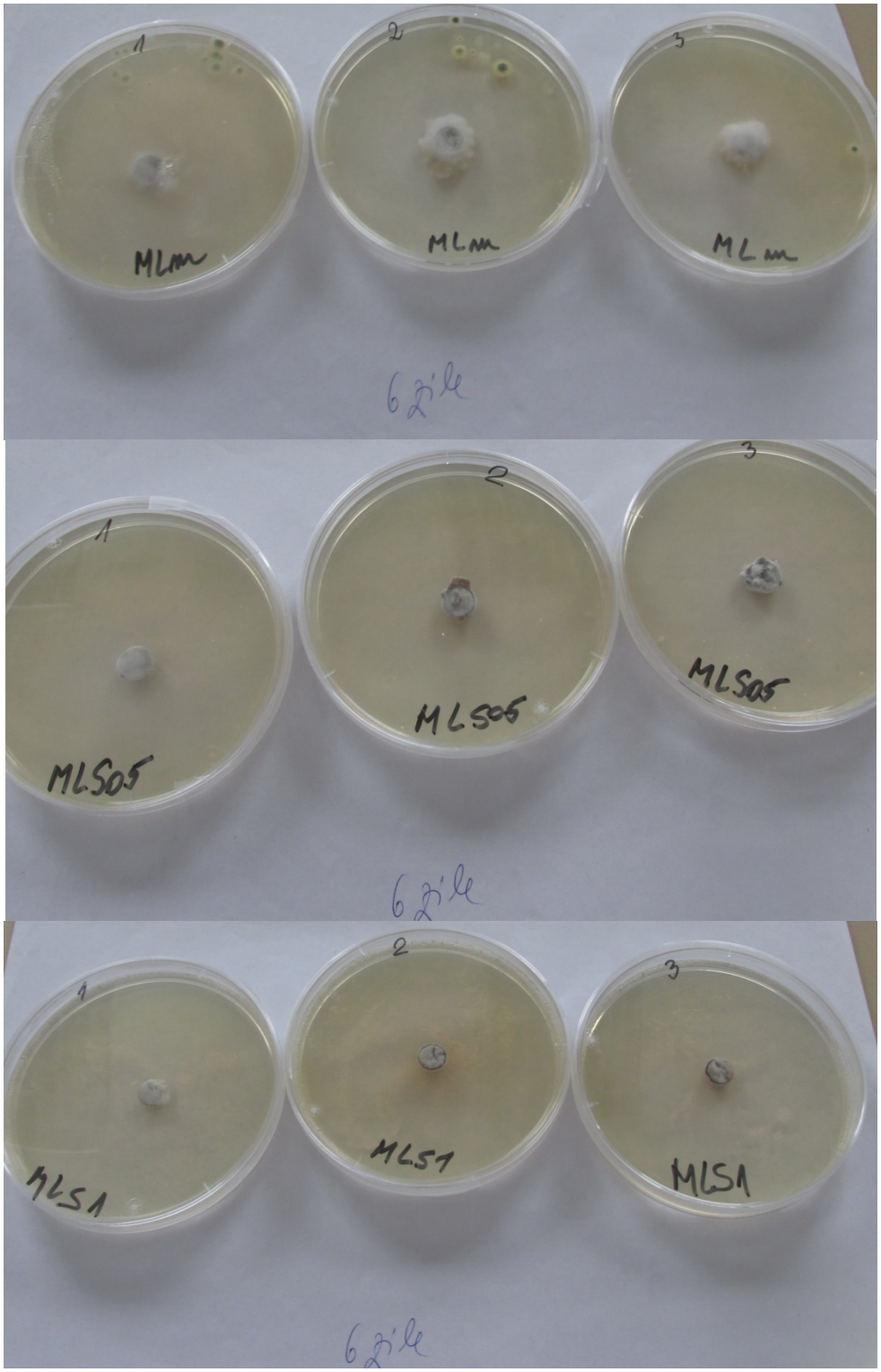

Figure 2. Growth mycelial of *Monilinia* spp. at GLY 1% , GLY 0.5% and GLY control variants (after 6 days)(in vitro)

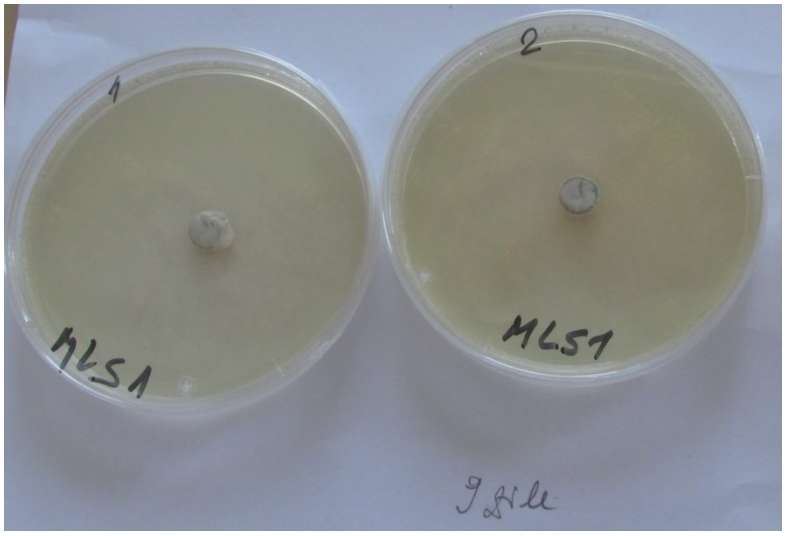

Figure 3. Mycelial growth of *Monilinia* spp. at 1% GLY and control variants (after 9 days) (*in vitro*)

The efficacy of the GLY complex on the *Monilinia* spp. mycelial growth after 12 incubation days was 89% for 0.5% GLY variant and 94% for 1% GLY variant (Table 2). The concentrations values which inhibit 50% and 90% of the mycelial growth were EC 50 =0.25% and EC 90 = 0.82%.

Table 2. GLY efficacy on mycelial growth of *Monilinia* spp. (*in vitro*)

Variant/concentration (%)	Average colony diameter (mm) / 12 days	Efficacy (%)	EC 50 Values for mycelial growth (%)	EC 90 Values for mycelial growth (%)
GLY /0.1	34.4	26.8	0.25	0.82
GLY /0.5	5.0	89.0		
GLY /1	2.8	94.0		
Control	47.0	-	-	-

Monilinia spp. causes important economic losses in orchards. Moniliosis affects primarily fruits producing brown mold, black mold and fruit rot. The rotted fruits that remain in trees until spring are the source of inoculum (BYRDE & WILLETTS [9]). Environmental conditions as well as microbial load are important in disease extension (XU & ROBINSON [25]). The identification of the *Monilinia* species is based on the morphological and biological characteristics of the pathogens (GHEORGHIES & al. [28]), but an accurate identification is performed by genetic methods (COTÉ & al.[26]). Chemical control is still effective but can lead to the appearance of pathogenic resistance, phytotoxicity, toxicity to other organisms. Researches are increasingly focusing on the use of biological control (HOLB [10]).Vegetal extracts are a promising alternative for pathogens control (PARVU & al. [17], CRISTEA [18], ICHIM et al. [27]), and steroidal glycoalkaloids extracted from *Solanum* species have antimicrobial properties (IIJIMA & al. [19], ITKIN & al. [20], MILNER & al. [21]). Our research has shown that these glycolalkaloids have efficacy on the attack of *Monilinia* spp. pathogens.

4. Conclusions

The research presents the inhibition of Monilinia spp. growth isolated from plum fruits, in 0.5% GLY and 1% GLY variants with 89% and 94% efficacy, which recommends the complex for use in biotechnology applications for the innovation of biofungicides.

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