ANTIFUNGAL EFFECT OF SOME STEROIDAL GLYCOALKALOIDS ON *MONILINIA FRUCTIGENA* (ADERH. & RUHL.) HONEY FUNGUS

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Abstract

Monilinia fructigena is the pathogen responsible for the appearance of brown rot and mummification of apple fruit present anywhere this species is grown. In Romania, the fungus attack is frequently in the apple orchards every year, depending on the environmental conditions, variety resistance, pathogen control management. The antifungal activity aainst this pathogen of steroidal glycoalcaloids extracted from *Solanum* species was tested in vitro. The steroidal glycoalkaloids considered were: solanine, solanidine, tomatine, solamargine, chaconine. Of these, a structural group, encoded GLY, was selected as one of the active components of a patented biofungicide. It was tested in concentrations of 0.1%, 0.5% and 1% and compared with the control. The biological material was represented by the isolated *Monilinia fructigena* (Mf 7), collected on apple fruit, the Idared variety, originated from RIFG Maracineni. The fungus did not develop in the first 3 days of incubation in any of the experimental variants. After 6 days of observation, the fungus developed at variants of GLY 0.1%, GLY 0.5% and control. The maximum mycelial diameter was determined in the control variant with 73.3 mm after 12 days of observation. In the GLY0.1% variant the diameter of the colony was 50 mm and in the GLY 0.5% variant the diameter of the fungus colony reached 8.2 mm, after 12 days of observation. In GLY 1% variant fungus did not develop throughout the observation period. Efficacy was maximal (E = 100%) in the GLY1% variant, followed by the GLY0.5% variant, with the efficacy of 88.8%. The EC 50 and EC 90 values were 0. 21% and 0.75% for the data obtained after 12 days of observation.

Key words: fungus, efficacy, steroidal glycoalkaloids

Monilinia fructigena (Aderhold & Ruhland) Honey ex Whetzel is commonly found on apple, peanut, quince. The attack of *Monilinia* species is dangerous in blooming, on fruit in vegetation and in fruit storage conditions (van Leeuwen G.C.M., van Kesteren H.A., 1998, van Leeuwen G.C.M., 2000).

The distinction of the pathogens involved in these symptoms is achieved by genetic methods (Fulton C.E., Brown A.E., 1997) and morphological identification (Gheorghies C., Cristea S., 2001). The brown rot caused by the fungus *Monilinia fructigena* produces serious damage and has been dominant throughout the apple vegetation period (Ivić D. *et al*, 2012). Studies on fungal epidemiology in the genus *Monilinia* have shown that an important role in spreading lies with vectors (Lack J.K., 1989), and environmental factors favor the spread of conidia (Bannon F. *et al*, 2009). Control of pathogens of the genus *Monilinia* is based on multiple interventions, including the use of fungicides. Plant extracts are active against pathogens and can be sources of products or preparations used for plant protection (Cristea S., 2004, Pârvu M., Pârvu A., 2011, Ichim E. *et al*, 2017). Steroidal glycoalkaloids, extracted from species of the genus *Solanum* have antimicrobial properties (Iijima Y. *et al*, 2013).

The objective of our research was to test under laboratory conditions the effect of steroidal glycoalcaloids extracted from *Solanum* species, as a component of a biofungicide, on the growth of the *Monilinia fructigena* pathogen.

MATERIAL AND METHOD

The research has been aimed at testing the in vitro effect of steroidal glycolcloids extracted from *Solanum* species on the growth of the

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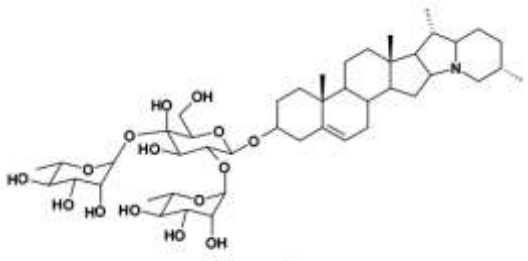
Monilinia fructigena fungus. The steroidal glycoalcaloids that we considered were: solanine, chaconine, solanidine, tomatine, solamargine. Of these, a certain structural group, encoded GLY, was selected as one of the active components of a patented biofungicide. The biological material was isolated from *Monilinia fructigena* (Mf 7), collected on apple fruits, Idared variety, originated from RIFG Maracineni, Romania. The morphological characteristics of colonies and asexual fructifications of the pathogen led to the identification of the fungus *Monilinia fructigena* (Gheorghies C., Cristea S., 2001). From the mature cultures of *Monilia fructigena*, the 0.5 mm diameter rounds were harvested which were centrally located in the culture medium. The Petri dishes of 90 mm and potato-dextrose-agar culture medium were used. The concentrations of 0.1%, 0.5% and 1% were tested, each variant being placed in three repetitions.

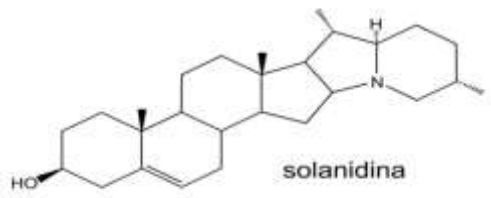
Incubation was done at 22°C. The food poison technique was used (Schmitz H., 1930). Measurements of myelin growth were performed at 3, 6, 9, 12 days. The efficacy of the product was determined after 12 days of observation as the rate of inhibition of mycelial growth in the treated variants compared to the control variant (Pandey, D.K. *et al*, 1982). For the results obtained at 12 days of observation, the effective concentration EC 50 and EC 90 was calculated (after determining the regression curve).

REZULTS AND DISCUSSIONS

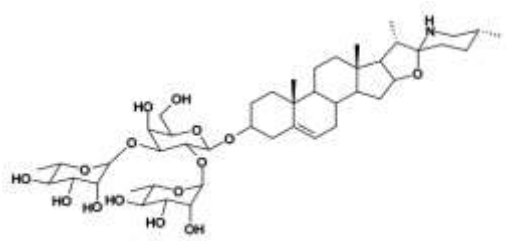
Our research followed the influence of the GLY-coded structural group in which the steroidal glycoalkaloids extracted from *Solanum* species are added to the development of the *Monilinia fructigena* fungus under laboratory conditions (in vitro). The glycoalcaloses considered were:



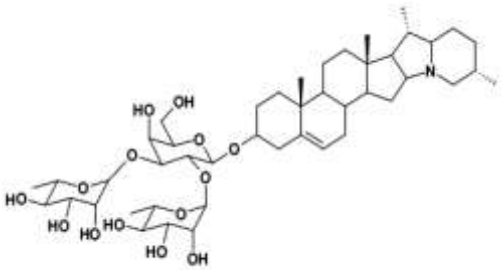
Chaconine



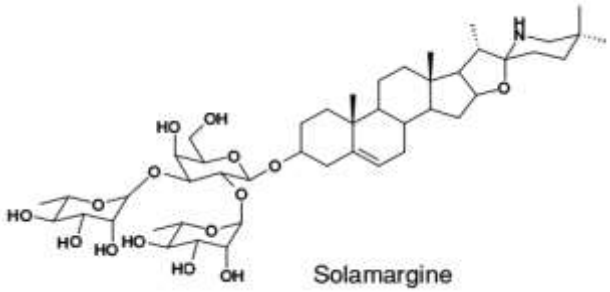
solanidina



Tomatine



Solanine



Solamargine

Data obtained after 3 days inoculation show that the fungus did not increase in any of the variants.

After 6 days of observation, the fungus developed at variants of GLY 0.1% and GLY 0.5% at an average diameter of 17.2 mm and 4.3 mm.

The untreated fungus developed the most, reaching 20.7 mm. After 9 days of observation, the fungus mycelium developed colonies that reached a diameter of 43.2 mm in the control without the tested product included in the medium, and also developed a larger colony at the GLY 0.1% variant (*figure 1*).

A less relevant increase was noted for the GLY 0.5% variant. In GLY 0.5%, the growth rate of the fungus was reduced, with a value of 4.3 mm in diameterafetr 6 days of observation and 6.2 mm in diameter after 9 days of observation. After 12 days of observation in this variant the diameter of the fungus was 8.2mm.

In the control variant the fungus occuped the culture vessel recording 73.3 mm diameter at the end of the observation period. Also, the fastest rhythm of growth of the fungus was observed in the control variant.

The GLY 1% variant at which the pathogen did not develop over the observation period was noted. *(table 1).*

Table 1

Antifungal activity of GLY on mycelial growth of *Monilinia fructigena* (in vitro)

Variant / concentration (%)	Diameter colony (mm) / 3 days	Diameter colony (mm) / 6 days	Diameter colony (mm) / 9 days	Diameter colony (mm) / 12 days
GLY/0.1	0	17.2	33	50
GLY/0.5	0	4.3	6.2	8.2
GLY/1	0	0	0	0
Control	0	20.7	43.8	73.3

Efficacy was 31.8% and 88.8% at the GLY 0.15 and GLY 0.5% after 12 days of incubation. A slower growth rate of the fungus was noted in the range of 9-12 days at a concentration of 0.5%. Efficacy was maximal, 100% at the GLY 1% variant (*table 2*).

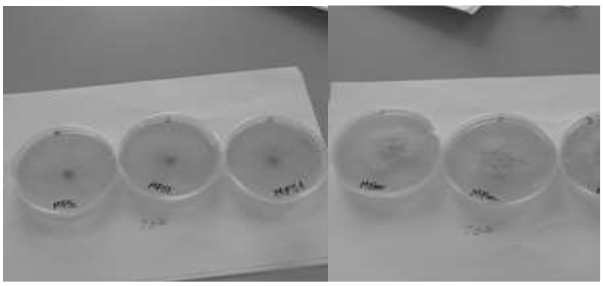


Figure 1 **In vitro grouth micelial of *Monilinia fructigena* at GLY 1% and control variants (after 9 days)**

Table 2

GLY eficacy on mycelial growth of *Monilinia fructigena* (in vitro)

Variant/ concentration (%)	Diameter colony (mm)/ 12 days	Efficacy (%)	EC 50 Values for mycelial growth (%)	EC 90 Values for mycelial growth (%)
GLY /0.1	50	31.8	0.21	0.75
GLY /0.5	8.2	88.8		
GLY/1	0	100		
Control	73.3	-	-	-

Calculation of the concentration inhibiting 50% and 90% mycelial growth determined EC 50 = 0.21% and EC 90 = 0.75% for data obtained after 12 days of observation (*table 2*).

Studies of the losses caused by *Monilinia fructigena* in organic apple orchards show that losses can reach 41.6% by fruit harvest in 2002 in Hungary. Holb's surveys for 2011-2012 show that 70-80% of infected fruits were damaged by codling moth in organic apple production (Holb I.J., 2004).

Vegetal extracts are an alternative to pathogen control (Pârvu M., Pârvu A., 2011, Cristea S., 2004) and the specialty literature has revealed the steroidal glycoalcaloids extracted from *Solanum* species have antimicrobial properties (Iijima Y. *et al*, 2013, Itkim M. *et al*, 2011).

Plants of the genus *Solanum* have been used in experiments on the virulence of pathogens (Ichim E. *et al*, 2016). Our research can lead to extensive biocontrol possibilities of the *Monilinia fructigena* fungus.

CONCLUSSIONS

Our research led to the following conclusions:

The research presented showed that the GLY structural group had an effect on the growth of the fungus *Monilinia fructigena*.

GLY 1% had maximum efficacy against the growth of the *Monilinia fructigena* fungus.

Research will continue with testing the final product under laboratory conditions and testing it under field conditions.

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REFERENCES

Bannon F., Gort G.,. van Leeuwen G.C.M, Holb I.J., and Jeger., 2009. *Diurnal patters in dispersal of Monilinia fructigena conidia in apple orchand in relation to weather factors*. Agricultural and Forest Meteorology 148:8.

Cristea S., 2004. *Testing „in vitro" of some phyto-extracts with antifungal action*. Lucrări ştiintifice, USAMVB. Seria A, vol XLVII, 288-290.

Fulton C.E., Brown A.E.,1997. *Use of Ssu rDNA group-I intron to distinguish Monilinia fructicola from M.laxa and M.fructigena*. FEMS Micriobiology Letters 157:307-312.

Gheorghies C., Cristea S., 2001. *Fitopatologie* vol 1, Ed Ceres, Bucuresti.

Holb I.J. , 2004. *Yield loss and disease development of Monilinia fructigena (Aderh.& Ruhl.) Honey in organic apple orchard*. Journal of Agriculture Sciences 15:2,6-8.

Ichim E., Maruntescu L., F. Manole, Cristea S., 2016. *Correlation of different Cms strains virulence with the ability to induce a non–host hypersensitive response and in vitro cellulolytic activity.* European Biotechnology Conference Latvia, May 05-07, 2016. Journal of Biotechnology Volume 231 Suplement: S Pages: S 83-S 83.

Ichim E., Maruntescu L., Popa M., Cristea S., 2017. *Antimicrobial efficacy of some plant extracts on bacterial ring rot pathogen, clavibacter michiganense ssp. sepedonicus.* The Eurobiotech Journal, vol 1 Issue 1, 93-96.

Iijima Y, Watanabe B, Sasaki R., Takenaka M., Ono H., Sakurai N., Umemoto N., Suzuki H., Shibata D., Aoki K.,2013. *Steroidal glycoalkaloid profiling and structures of glycoalkaloids in wild*

tomato fruit. Phytochemistry, Volume 95, 145-157.

Itkim M., Rogachev I., Alkan N., Rosenberg T., Malitsky S., Masini L., Meir S., Iijima Y., Aoki K., deVos R., Prusky D., Burdman S., Beekwider J., Aharoni A., 2011. *GLYCOOLKALOID METABOLISM1 is required for steroidal alkaloid glycosylation and prevention of phytotoxicity in tomato*. Plant Cell, 23, pp. 4507-4525.

Ivić D., Sever Z., Milicevic T., 2012. *Estimation of economic loss due to postharvest diseases of apple (cv.Idared) during four seasons*. Pomologia croatica, vol. 18.

Lack J.K.,1989.*The spread of apple brown rot (Monilinia fructigena) by insects*. Annals of Applied Biology 115 (20): 221-227.

Pandey D.K., Tripathi N.N., Tripathi R.D., Dixit S.N.,1982. *Fungitoxic and pfytotoxic of essential oil of Hyptis suaveolens*. Z. Planzenk, vol. 89, 344-349.

Pârvu M., Pârvu A., 2011. *Antifungal plant extract. In : Science against microbial pathogens: communicating current* research and technological advances A. Méndez-Vilas (Ed.) Formatex, Microbiology Book Series Number 3: 1055-1062.

Schmitz H., 1930. *Poisoned food technique*. Industrial and Engineering Chemistry Analyst. Ed. 2: 361 .

van Leeuwen G.C.M., van Kesteren H. A.,1998. *Delineation of the three brown rot fungi of fruit crops (Monilinia spp) on basis of quantitatif characteristics*. Canadian Journal of Plant Botany 76:2042-2050.

van Leeuwen G.C.M.,Stein A., Holb I., Jeger M. J., 2000. *Yield loss in apple caused by Monilinia fructigena (Aderh.& Ruhl.) Honey, and spatio-temporal dynamics of disease development*. European Journal of Plant Pathology 106: 519-528.