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Original paper

Research on the action of fenugreek extract on the growth of the pathogen *Monilinia* spp. in vitro

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Abstract

Moniliosis caused by the species of the *Monilinia* genus attacks fruit tree orchards wherever seeds fruits tree and stone fruits tree species are cultivated, producing economically significant losses. *Moniliosis* is also present in Romanian orchards, requiring control of this disease because it can evolve both in the field and in storage conditions, too. The research aimed to test the antifungal in vitro activity of fenugreek extract against the pathogen *Monilinia* spp. *Monilinia* spp was isolated and successively replicated from fresh plant material, represented by apple fruits with specific sporodochia. The fenugreek extract (ska) was tested in concentrations of 3.3% (ska 3,3%) and 10% (ska 10%), comparing the results with the control variant. At the 10% fenugreek extract, the fungus did not grow in the first 3 days, registering a vegetative growth after 6 days of incubation. After 12 days of incubation with the 10% fenugreek extract, the diameter of the mycelium colonies was 6.3 mm compared to the control variant, where the value of the colony diameter was 56.3 mm. The effectiveness of the fenugreek extract at a concentration of 10%, as a percentage of inhibition, was 88.80%.

Keywords *Monilinia* spp., antifungal activity, efficacy

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Introduction

Moniliosis is a frequent disease in orchards, being caused by the complex of pathogens belonging to the genus *Monilinia*. The attack of *Monilinia* spp. moulds, responsible for the occurrence of grey mould and fruit rot, represent a serious economic problem, these pathogens determine important losses in fruits vegetation and storage period (OLIVEIRA & al [15]). But the losses caused during the vegetation period can also evolve in storage conditions (LEEUWEN & KESTEREN, [8]). *Monilinia fructigena* is common on apple and quince species, *Monilinia laxa* mainly attacks stone fruits tree species and *Monilinia fructicola* (G. Winter) occur frequently on plum, cherry, apricot and stone cherry (SNYDER & JONES, [5]). The symptoms of the moniliasis attack are visible in the vegetation causing the withering of the shoots and in the flowering stage causing the browning of the flowers. As for the fruits, the attack is different depending on the climatic conditions. In hot and humid weather, the attack appears in the form of brown rot, in wet and cold weather, black rot appears on the fruits and in alternating cold and warm weather, the attack appears in the form of mummified fruits. The identification of the pathogens involved in the occurrence of moniliasis in trees is carried out according to the morphological characteristics of the fungi involved (Gheorghies & Cristea, [2]) and especially through genetic methods (FULTON & BROWN, [3], FULTON & al [4], FORSTER & ADASKAVEG, [9], GELL & al, [9], ZHU & al [21]). The epidemiological studies on the spread of moniliasis have highlighted the importance of biotic and abiotic factors: vectors such as insects and birds, water droplets, fruit lesions, the infectious load on diseased fruit, hail, air currents and man through his activity (LACK, [13]; PAUVERT et al, [17], HELLMAN, [16], BYRDE and WILLETTS [20], BANNON & al [6]). Also, an important role in the transmission of diseases is played by sick fruits and the biological reserve from the orchards, the fallen and mummified fruits from the trees constitute the inoculum for the following year. Field attacks can also evolve under storage conditions (van LEEUWEN [7]). Pathogen survival, tissue colonization, *Monilinia* spore release and infection are related to environmental factors (temperature, relative air humidity, amount of precipitation) (WATSON et al, [19]; BANNON et al, [6]).

Phytosanitary intervention in the control of moniliasis takes into account a lot of prophylactic and therapeutic possibilities, which include crop hygiene, agrophytotechnical measures, genotype resistance, and chemical control (BORVE & STENSVAND, [11]). The administration of fungicides to combat brown rot is the curative measure used in the management of moniliasis, but special care must be given to the phenomenon of resistance, which can occur with the repeated application of products with systemic action. The application of integrated control schemes with the presence of chemical products has shown effectiveness in combating moniliasis in fruit trees (CHITULESCU and CRISTEA, [13]). Research on the biocontrol of *Monilinia* pathogens in the field and laboratory is an alternative to fungicide therapy (GRZEGORCZYK & al, [15]). A researched method is the use of plant extracts with antifungal activity. It was determined under laboratory conditions, the effect of steroidal glycoalkaloids extracted from *Solanum* species, as a component of a bio fungicide, on the growth of *Monilinia* spp., pathogens isolated from plum fruits (CRISTEA & al, [18]).

Materials and Methods

Our research investigated the antifungal action of fenugreek extract on the mycelial growth of the pathogen *Monilinia spp*. It was used the inclusion method in the culture medium, and the PDA (potato-dextrose-agar) culture medium (Carl Roth GmbH +Co) was prepared according to the protocol (autoclaved at 121^0C, 1.2 atm, 20 min). The micromycete was isolated directly from the diseased plant material. The affected plant material was passed twice through distilled water. The inoculum was harvested by detaching small portions (2-3mm) from the areas affected with sporodochia and then placing them on the PDA culture medium. Cultures were incubated at 22^0C. The isolates were identified based on their morphology and then replanted and kept in pure cultures at a temperature of 22^0C. The concentrations used for testing the antimicrobial activity were 3.3% and 10% of fenugreek extract in butylene glycol, these being added to the PDA medium cooled to 45^0C. After homogenization, the mixture was distributed in Petri dishes (60 mm diameter) in an amount of approximately 10 ml/dish. After solidification of the medium, the fungal rounds were placed centrally (with a diameter of 7 mm) and executed by an eyelet from the edge of the pure fungal cultures (on the 10th day of growth). The effect of the test concentrations on the mycelial growth of the pathogens was estimated in comparison with a control cultured on a PDA medium. The Petri dishes seeded were incubated at a thermostat at a temperature of 22^0C. For all variants, readings were taken 3, 6, 9 and 12 days after the start of the incubation period. The estimation was evaluated by measuring the average diameter of the mycelial growth (the average diameter of the fungal colonies). The effectiveness was calculated, by determining the average diameter (mm) of

Table 1. The influence of test concentrations on the growth of the pathogen *Monilinia* spp.

Pathogen species	**Var Ska (%)/ control**	**The incubation period and diameter were measured in mm**			
		3 days - Ø(mm)	**6 days - Ø(mm)**	**9 days - Ø(mm)**	12 days - Ø(mm)
Monilinia spp					
	ska 10%	0.00	0,20	3,30	6,30
	ska 3,3%	11,30	33,80	41,30	53,30
	Control	33,30	49,30	53,30	56,30

Fenugreek extract 10% (ska10%); fenugreek extract 3.3% (ska3.3%)

the vegetative growth around the disc of the pathogen. Efficacy (% inhibition) was calculated according to the formula:

E = [(D var mt- var test)/ D var mt]x100 (%) (Abott formulas).(1)

Results and discussion

The obtained data showed that in the control variant, the fungus grew from the first days, reaching 33.3 mm in diameter after 3 days of observation. After 6 days of incubation, the average diameter of the colonies was 49.3 mm and after 9 days its diameter was 53.3 mm. After 12 days from the start of the experiment, the average diameter of the colonies was 56.3 mm. The fungus registered a rapid growth rate in the first 6 days after incubation, and after 9 days the vegetative growth had a slower growth rate. In the case of the variant containing 3.3% fenugreek extract, the micromycete grew throughout the observation period, registering 11.3 mm after 3 days and 33.8 mm after 6 days. After 12 days of incubation, the colonies reached an average diameter of 53.3 mm.

From the data obtained, the fungus had a faster growth rate, in the monitored interval, with values close to the value of the control variant. Also, in the ska 3.3% variant, a denser growth of colonies was observed than in the control variant, with a slight effect of stimulating vegetative growth, which had a more compact and richer appearance, with growth especially vertically, in culture vessels. The density of the vegetative mass recorded in the case of this extract variant tested (ska 3.3%) led to the conclusion of a possible stimulation of vegetative growth at this concentration. According to the results obtained regarding the variant in which the concentration of 10% fenugreek extract (ska 10%) was tested, the fungus did not develop until after 3 days when slight traces of mycelium were re-

Figure 1. Monitoring the vegetative growth of the micromycete *Monilinia* spp. at 3, 6, 9 and 12 days

Table 2. The effectiveness of the test concentrations on the growth of pathogens

Pathogen species	Var Ska	(%)/ control	3 days / Ø (mm)	Efficacy (%)	6 days/ Ø (mm)	Efficacy (%)	9 days/ Ø (mm)	Efficacy (%)	12 days/ Ø (mm)	Efficacy (%)
Monilinia spp	ska 10%	0	100	0,20	99,59	3,3	93,80	6,3	88,80	
	ska									
3,3%	11,3	66,06	33,8	31,44	41,3	22,51	53,3	5,32		
	Control	33,3	-	49,3	-	53,3	-	56,3	-	

Fenugreek extract 10% (ska10%); fenugreek extract 3.3% (ska3.3%)

corded, so we can say that the micromycete was inhibited in the first 3 days and recorded a significant increase after 6 days of incubation (Table 1, Figure 1). CRISTEA et al., 2017 [18] showed that plant extracts had an inhibitory effect on the mycelial growth of the pathogen *Monilinia* spp. PERISOARA et al [1] demonstrated the antifungal activity of the hydroalcoholic extract obtained from *Tagetes erecta* on the pathogenic species of *Molinilia laxa* and *Fusarium graminearum*, where in the case of the species *F. graminareum*, the highest percentage of inhibition (54.17 ± 5.89%, compared to the solvent, $p < 0.05$) was obtained at the highest tested extract concentration (5%), while in the case of *Monilinia laxa*, the inhibition of the colony diameter was observed (52.29 ± 2.60%, compared with the solvent, $p < 0.05$%) at the lowest tested extract concentration (0.5%).

The efficacity of the tested concentrations on the growth of micromycete colonies (% inhibition) was also calculated and it was found that compared to the control, the efficacy of the ska 10% variant was 88.8 %, which we consider a high percentage of inhibition of the growth of the fungus colonies. For the variant containing ska 3.3%, a very low efficacy was calculated, confirming that at this concentration the fungus was not significantly inhibited, the abundant mycelial growths ensuring a stimulation of the growth of the vegetative mass, the source of subsequent infection (Table 2).

Conclusions

The research on the influence of fenugreek extract on the mycelium growth of *Monilinia spp* showed an inhibition of the vegetative mass of the micromycete at a concentration of 10%. The efficacy of the concentration was 88.80% compared to the control. At the concentration of 3.3%, the percentage of inhibition was insignificant, the vegetative growths of the micromycete being abundant.

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