ANTIFUNGAL EFFECT OF SEVERAL ORGANIC ACIDS TOWARDS CONIDIOSPORES OF MONILIA FRUCTIGENA IN THE *IN VITRO* CONDITIONS

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Abstract: An *in vitro* antifungal trials with conidiospores of *Monilia fructigena* were performed for testing the ability of seven organic acids to block their germination. Tested acids were: malic acid, succinic acid, tartaric acid, citric acid, acetic acid, formic acid and lactic acid. They are with low toxicity towards humans and the environment and most of them are typical food additives for achieving the sour taste of foods. Conidiospores of *Monilia fructigena* were taken from naturally infested apple fruits and an *in vivo* phytotoxic trials with two apple cultivars ("Golden Delicious" and "Jonagold") were performed too. Conducted tests show that tested acids express different effectiveness towards *Monilia fructigena*. Some of them as acetic, formic and succinic were completely inapplicable as potential fungicides towards testes pathogen. Conducted tests prove that they can inhibit the ability of conidiospores to germinate but in very high concentrations in which they also can cause damage on treated apple plants. However other acids as tataric, lactic and citric were effective in the very low concentrations (0.1-0.3 %) which make them very appropriate as fungicides. These acids are cheap, produced in large amounts (especially citric acid), safe for the humans and the environment and soluble in water. Up to 1 % concentration no one of the tested acid cause phytotoxicity on the apple trees.
Keywords: organic acid, *Monilia fructigena*, conidiospores, antifungal, apple

1. INTRODUCTION

Monilia fructigena is a widely spread fungal plant pathogen that causes so-called brown rot on various orchard cultures as apples, cherries, sour cherries, peaches, apricot and so on. The fungus can destroy the fruits and fully compromise the yield (Liu et al., 2012). The problem with the use of typical synthetically based plant projection products is that from one side there is a very high resistance risk, and from the other: need obey to pre –harvest interval if such a product will be used (Sunding & Zivin, 2000; Hawkins et al., 2019). That's why during recent years more and more researches are conducted for founding a new, environmentally friendly and with zero resistance risk fungicides (Teodorescu et al., 2007; Elisovetskaya et al., 2015). Using the botanical extracts can be complicated due to their preparation, non persistent contend, in some cases – low water solubility, risk of phytotoxicity and sensitiveness to the hardness of the water. In the present research, several organic acids : malic acid, succinic, tartaric, citric, acetic, formic acid and lactic acid were evaluated for their possible antifungal effectiveness for inhibition of conidiospores of *Monilia fuctigena.*

Malic acid ($C_4H_6O_5$) is a dicarboxylic acid which is responsible for sour taste of fruits and its name derived because was firstly discovered in the apple juice Carl Wilhelm Scheele in 1785.This compound is used in the food industry under name E296 as food additive for providing sour taste (Kövilein et al., 2020). Malic acid together with citric acid showed an inhibitory effect on growth of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Monilia albican* to a certain extent (Wang & Zhang, 2010) and antimicrobial activity against *Pseudomonas aeruginosa* too (Borah et al., 2023).

Succinic (acid $(CH_2)_2(CO_2H)_2$ is an organic acid which pay numerous roles in the metabolism of living organisms. The compound is widely used in the chemical industry in the manufacturing of different kinds of polymers and drug production. In the food industry is used as acidity regulator and flavoring agent for sour taste (Nghiem et al., 2017). The incorporation of succinic acid (SA) in the lattice of L-Lysine monohydrochloride (LM) has opened the new avenue in the field of production and application of scintillator materials such as LED and antifungal drug. SA: LM exhibited good response towards pathogenic fungi which causes numerous types of infections and diseases in both humans and animals like *Candida albicans Candida parapsilosis and Aspergillus flaves* (Aneeba et al., 2021). Monoester of succinic acid was found to have antifungal and antibacterial activities too (Iqbal et al., 2014). Succinic acid plays a crucial role in multiple biological functions including in ATP making, and a signaling agent for cellular metabolic states and so on and shows an antibacterial activity against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus , Bacillus subtilis* and *Candida albicans* (Gizer & Sahiner, 2021).

Tartaric acid ($C_4H_6O_6$) is also a typical organic compound which can be found in may living plants especially in the grapes, citrus and bananas. Typical food additive and antioxidant under name E334 providing sour taste to the foods. The acid is also widely used in the production of pharmaceuticals, especially in the production of effervescent salts. In the chemical industry, tartaric acid is used as chelating agent (Gal, 2008). The tartaric acid together with citric acid were founded to inhibit the growth f some important pathogenic fungi in vitro conditions *as*

Trichophyton mentagrophytes var. mentagrophytes, Candida albicans, Aspergillus fumigatus, and *Malassezia furfur* (Shokri, 2011).

Citric acid ($HOC(CO_2H)(CH_2CO_2H)_2$) is very well known organic acid naturally occur in the citrus fruits. The compound has extremely wide use as acidifier, as a flavoring, and a chelating agent. In the food industry is denoted as E330 (Berovic & Legisa, 2007).

Acetic acid (CH_3COOH) is the major component of vinegar which contain at least 4 % of it. This chemical has numerous applications in the food and chemical industry in the processes of making polymers, glues, fabrics, paper manufacture. As food additive it is denoted under name E260 (Wagner & Staff, 2000). Apple cider vinegar(5%) inhibits growth of *Aspergillus niger, Aspergillus flavus, Candida albicans* and Non- Candida albicans (Abbas et al., 2011). Acetic acid (10%) showed the highest inhibition effect on *Asperguillus flavus* growth together with formic acid (Hassan et al., 2012).

Formic acid (HCOOH) s the simplest carboxylic acid and has such name because naturally occurs in the ants. Important reagent for chemical industry. In the agriculture, formic acids is used as preservative and antibacterial agent in livestock feed. Beekeepers use formic acid as a miticide against the tracheal mite (*Acarapis woodi*) and the Varroa destructor mite and *Varroa jacobsoni* mite. The acids is used in hydrogen fuel cells, in the soldering, chromatography, also in the tanning of leather and dyeing and finishing textiles. Used and as a coagulant in the production of rubber. In the medicine, formic acid is used for treatment of warts (Hietala et al., 2016).

Lactic acid ($CH_3CH(OH)COOH$) receive its name after been discovered by Swedish chemist Carl Wilhelm Scheele in 1780 from milk. The acid is produced by fermentation and pay key role for converting milk to yogurt and cheese. In the pharmacy industry, lactic acid is used to produce water-soluble lactates. Lactic acid is used in some liquid cleaners as a descaling agent for removing hard water deposits such as calcium carbonate. It is used in some antibacterial soaps and dish detergents (Axelsson, 2004). Lactic acid bacteria have a major potential for use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods (Stiles, 1996).

2. MATERIALS AND METHODS

Conidiospores of Monilia fructigena were taken from naturally infested apple fruits and were placed in the distilled water by brush for making spores suspension with density 3*104 spores per ml. A microscopic slides pattern "hanging drop" were treated by spraying with tested acid in several concentrations. The control variant was not spayed. Four slides formed one test variant, after drying the solution, the spore suspension was added (0.2 ml per slide). The microscopic slides were incubated in a thermostat (humid chamber) under 22 – 24°C. After 48 h four observations in four different directions of the slides were made with a light microscope for determination of the germination of conidia. On every observation field, the number of germinated and non-germinated conidia was counted and was determined minimal inhibitory concentration in which no germinations on conidiopores were observed. In vivo tests for phytotoxicity were performed with two apple cultivars "Golden Delicious " and "Jonagold" , BBCH = 75. The brunches of the trees were sprayed with solutions with tested acids in different concentrations prepared with distilled water and the addition of 0.1 % organosilicone surfactant. Visual observations for phytotoxic signs as necrosis, chlorosis and deformations were performed 7 days after treatments. One way ANOVA analyses were performed in order to determine the pretense or lack of statistical proven differences between tested variants by R language of statistical computing (Faraw, 2015)

3. RESULTS

In the Table 1 are presented data for pKa constant and water solubility (g/L) at 20 °C of tested organic acids:

Acid	pKa	Water Solubilty (g/L), 20 °C
Malic acid	3.4	558
Succinic acid	4.2	58
Tartaric acid	2.9	20.6
Citric acid	3.13	59.2
Acetic acid	4.75	Miscible
Formic acid	3.74	Miscible
Lactic acid	3.86	Miscible

Figure 1 presented the results from conducted antifungal trials with tested organic acids.

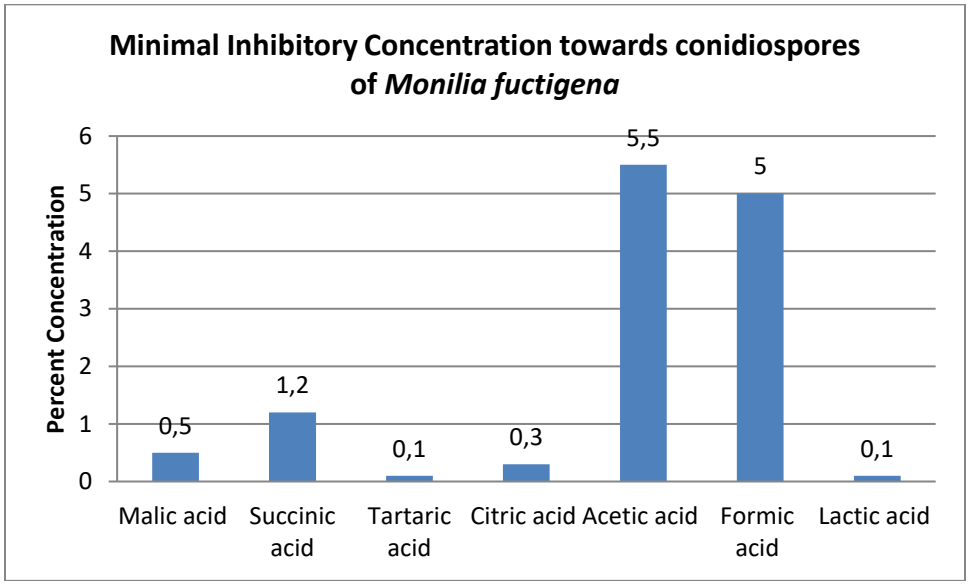

Figure.1 Antifungal action of tested ogranic acids towards conidiospores of Monilia fructigena

The tests show that all of the compounds can block the germination of conidiospores of a tested plant pathogen. However this can be achieved in quite different concentrations: while malic, tataric, lactic and citric acids fully inhibit the germination of spores in 0.1 – 0.5 % concentrations, succinic acid achives the same in 1.2 % concentration. Acetic and formic acids show antifungal effect too but in 5- 5.5 % concentrations.

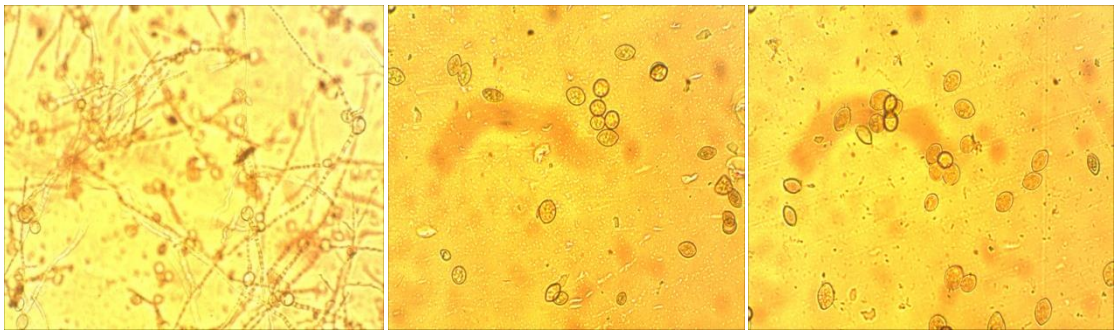

Figure.2 In the left: control variant; in the middle: tataric acid in 0.1 % concentration, in the right: lactic acid in 0.1 % concentration

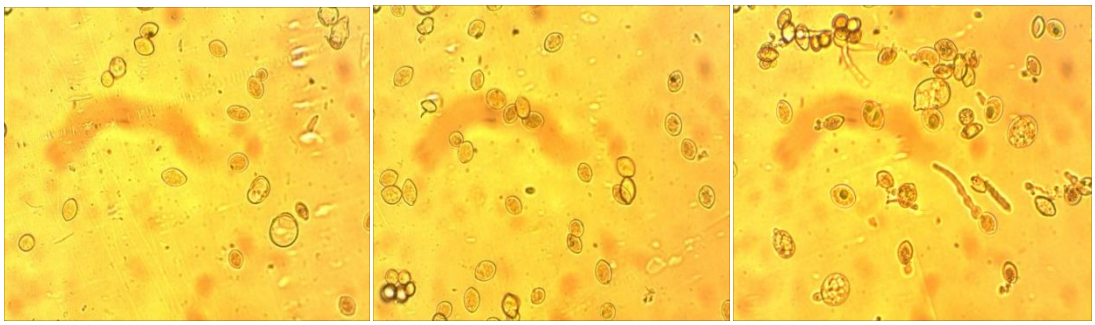

Figure.3 In the left: malic acid in 0.5 % concentration, in the middle: citric acid in 0.3 % concentration, in the right: succinic acid in 1.0 % concentration

In conducted phytotoxicity trials with apple trees variety "Golden Delicious " and "Jonagold" , BBCH = 75, all acids do not cause any damage on plants up to 1.0 % concentration. Beyond this concentration the first phytotoxic signs as necrosis in the leaves became to appear.

4. DISCUSSIONS

Tested organic acids show different effectiveness as potential fungicides. Although acetic and formic acids are proven to express antimicrobial (antifungal) activity (Adesogan & Salawu, 2004; Zinn & Bockmühl, 2020). They were able to block the germination of conidisopores of *Monilia fructigena* in 5-5.5 % concentrations although in 1 % concentration they start to express phytotoxic activity towards apple plants. On the other side, tataric, lactic and citric acids show strong antifungal effect towards tested plant pathogen.

5. CONCLUSIONS

Some of the tested acids as acetic, formic and succinic were completely inapplicable as potential fungicides towards *Monilia fructigena*. Conducted tests prove that they can inhibit the ability of conidiopsores to germinate but in very high concentrations in which they also can cause damages on treated apple plants. However other acids as tataric, lactic and citric were effective in very low concentrations (0.1-0.3 %) which makes them very appropriate as fungicides. These acids are cheap, produced in large amounts (especially citric acid), relatively safe for humans and the environment and soluble in the water.

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