DOI: 10.22620/agrisci.2022.35.006

IN VITRO ANTIFUNGAL EVALUATION OF L-ASCORBIC ACID (VITAMIN C) TOWARDS MONILIA FRUCIGENA, ALTERNARIA SOLANI AND VENTURIA INAEQUALIS

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Abstract

In vitro antifungal tests with one of the most popular medical substance in the world – L – ascorbic acid, widely known as vitamin C, were conducted with three of the most spread plant pathogens in Bulgaria: *Monilia frucigena, Alternaria solani* and *Venturia inaequalis* in order to evaluate the direct antifungal action of the acid used in agriculture as ISR promoter (phytovaccine). The expression of pesticide action of ISR promoters is not unusual unlike the vaccines for the animals and humans. Conducted tests shows the strong antifungal activity of L – ascorbic acid towards mycelium and conidiospores of *Alternaria solani* and *Venturia inaequalis* and a very weak antifungal action towards *Monilia frucigena* which means that vitamin C can be used as a direct fungicide agent and phytovaccine in the pest management simultaneously. The substance has excellent water solubility which make is very appropriate for formulation as a plant protection product.

Keywords: L-ascorbic acid, antifungal, ISR promoter, *Monilia frucigena, Alternaria solani, Venturia inaequalis*

INTRODUCTION

L – ascorbic acid or vitamin C are water soluble white crystals with no smell, with chemical formula $C_6H_8O_6$ and a molecular mass 176,13. The density of the substance is 1.65 g/ml and octanol – the water coefficient (log P) = 1.85 (Shamim & Khoo, 1979). The acid is extremely popular in medicine as a remedy for healing cold and influenza (Pauling, 1971; Karlowski et al., 1975; Zarubaev et al., 2017). It was discovered in 1912 by Albert Szent-Györgyi and Walter Norman Haworth who were awarded with Nobel Prizes in 1937 for this discovery (Zetterström, 2009). In 1945, Linus Pauling established the influence of vitamin C on the treatment of cold and influenza (Pauling, 1976). The chemical is cheap, soluble in water, produced in large quantities all over the world.

Vitamin C is *de facto* a substance nontoxic to humans with LD50 = 11900 ppm. However, large doses of the ascorbic acid can cause some health effects such as stomach pain and headache. As for the effects on the environment, the substance is also nontoxic and in the soil it can be disintegrated to 97% for approximately 5 days (Olson & Hodges, 1987; Levine et al., 1995; Naidu, 2003).

Although the L-ascorbic asid is exremelly popular and famous as a remedy for cold and influenza, it's role for the prevention and treatment of COVID - 19 is disputable (Abobaker et al., 2020; Simonson, 2020; Milani et al., 2021).

However, the acid can play important role for the treatment of other diseases on humans like cancer, infarct, insult and Alzheimer (Bendich et al., 1986; Packer, 1997; Boothby & Doering, 2005). Vitamin C is also a common food additive under the name "E300" (Fredriksen et al., 2009).

During recent years there is an increased interest towards the l-ascorbic acid in the area of pest management. It was established that the vitamin C together with the salicylic acid is a key component in the so called "oxygen burst" which is an essential part of the System Activated Resistance (SAR) and the Induced System Resistance (ISR) in plants (Davey et al., 2000; Khan et al., 2011; Boubakri, 2017).

Other researches show the strong stimulation effect of the substance on the growth and development of plants and their resistance to adverse abiotic conditions like drought and especially – cold (Guo et al., 2005; Gallie, 2013; Kaur & Nayyar, 2014). There are also an increasing number of agrochemical products which contain vitamin C as an active substance like:

- SNS-DC^{tm} fungicide – contain active substances: L-ascorbic acid, silicon dioxide, potassium sorbate and soap bark
- Sunonic® broad spectrum fungicide and bactericide - contain active substances: L-ascorbic acid, Ethyl lactate, glycerin, sodium chloride
- Biolife fungicide, bactericide and viricide - contain active substances: L-ascorbic acid, citric acid, lactic acid

In 2013, the European commission approved vitamin C as an active substance for the treatments of potatoes and tomatoes against plant pathogens as: *Phytophthora infestans, Botrytis sp.* and *Fusarium sp.*

The treatments of foliar diseases on vegetables with ascorbic acid were able to significantly reduce the infestations from powdery mildews and downy mildews (Abdel-Kader et al., 2012; Abdel-Kader & El-Mougy, 2014).

Butylated hydroxy toluene, tannic acid, ascorbic acid and dimethyl sulfoxide (DMSO) at a concentration of 1.0 mm were found to successfully able to control the grey mould of tomato fruits (Elad, 1992).

Other studies reveal that vitamin C can express significant fungicidal activity against *Fusarium graminearum, Alternaria alternata* and *Pyrenophora tritici-repentis* in *in vitro* conditions (Shomeet et al., 2018) and also against *Magnaporthiopsis maydis*, causing maize late wilt disease (Abdel-Kader et al., 2022).

Other research also confirms that ascorbic acid can reduced the linear growth of plant pathogens such as *Fusarium oxysporum, F. solani*, and *Macrophomina phaseolina* which infest Roselle (*Hibiscus sabdariffa L.)* (Hassan et al., 2014).

In his research, Christian Chervin confirms that several small molecules of natural origin like acetaldehyde, acetic acid, ascorbic acid, ethanol, ethylene, jasmonic acid and methyl jasmonate, salicylic acid and methyl salicylate, and est., have been shown to act as alternatives to synthetic fungicides for biological control of fungal diseases of grapes and other crops (Chervin, 2012).

In the conducted study it was established that the treated sugar beet plants with 50.0 ml K_2HPO_4, 3 g/l ascorbic acid, 10 g/l $KHCO_3$ and Fungicides (Ridomil-Plus at 2 g/l) resulted in reducing *Cercospora* leaf spot severity more than 50.0%. All treatments also significantly increased the sugar beet yield (Abd-El-Kareem et al., 2010).

The purpose of the current research is to evaluate the possible direct antifungal action of the l-ascorbic acid (vitamin C) towards three of the most spread plant pathogens in Bulgaria: *Monilia frucigena*, *Alternaria solani* and *Venturia inaequalis*. The expression of the pesticide action of ISR promoters as vitamin C is not unusual unlike the vaccines for the animals and humans.

MATERIALS AND METHODS

Radial growth assays *in vitro* trials were conducted according to the methods of Thornberry (Thornberry, 1950). The tested plant pathogens were isolated from the naturally infected plants (quince tree (*Cydonia vulgaris*) – for *Monilia fructigena*, and tomato plants – for

Alternaria solani). In sterile Petri dishes 1 ml of the tested distilled water solution of L-ascorbic acid were added preliminarily sterilized (1 ml sterile distilled water for the control variant); followed by an addition of 9ml PDA (potato dextrose agar). After vigorously shaking for good mixing of the solution with PDA, inoculation with 10 mm PDA disks with developed mycelium of tested pathogen was conducted (one disk per Petri). Each test variant consisted of 5 repetitions. The L-ascorbic acid was tested in different concentrations in order to determine at which one there will be a full inhibition of mycelium of the tested pathogens. The inoculated Petri dished were incubated in thermostat under 22-25°C. The observations were conducted on 3, 7, 10, and 14 days after the inoculation with a ruler, measuring the mycelium zone around the inoculated disks. Effectiveness was calculated by the formula of Abbot (Abbot 1925).

For the conidiospores germination inhibition tests, a microscopic slides pattern “hanging drop” was used (four slides formed one variant). The slides were preliminary treated by spraying with a distilled water solution of L-ascorbic acid in the tested concentrations. After drying the solution, a conidial suspension made from relevant plant pathogen (conidiospores from naturally infected plants of apples, tomatoes and quince tree for *Venturia inaequalis*, *Alternaria solani* and *Monilia frucigena*) and ($3*10^4$ spores/ml – 20 μl) was added. The microscopic slides were incubated in the thermostat (humid chamber) under 22 – 24°C.

After 48h four observations in four different directions of the slides were done with a light microscope for determining the germination of conidia. On every observation field, the number of germinated and non-germinated conidia was counted.

The percent of germination in each observation field was calculated with the formula:

Percent germinated conidia=Number of germinated *100 / (Number of germinated + Number of non – germinated).

The mean value percent of the germinated conidia for each variant was calculated. According to these values (of tested variants and controls) the effectiveness of the formulations was calculated with the formula of Abbot:

Effectiveness = ((Percent of germination of the control – Percent of germination of the variant) / Percent of germination of the control)*100.

The major toxicological indexes were calculated as follows:

- NOAEL (No Observed Adverse Level) = LD 05
- LOAEL (Lowest Observed Adverse Level) = LD 25
- LD 50 – Dose at which 50% the effectiveness (antifungal action) towards the testes plant pathogens was observed
- LD90 - Dose at which 90% the effectiveness (antifungal action) towards the testes plant pathogens was observed

For the phytotoxicity trials, plant parts (leaves and branches) from different plant cultures (tomatoes, potatoes, cucumbers, pepper plants, onions, common pumpkin, wheat, apples, quince tree, cherry, sour cherry, peaches, grapes, oil – yielding rose) were treated with distilled water solutions of vitamin C in different concentration (from 0.01% to 5% m/v). 14 days after treatments, visual observations were performed for the detection of deleterious manifestations of ascorbic acid on plants like: chlorose, necrosis, whitening's and deformations.

RESULTS

The received results from the conducted *in vitro* tests with the mycelium of *Monilia fructigena* and *Alternaria solani* are presented on the figure below:

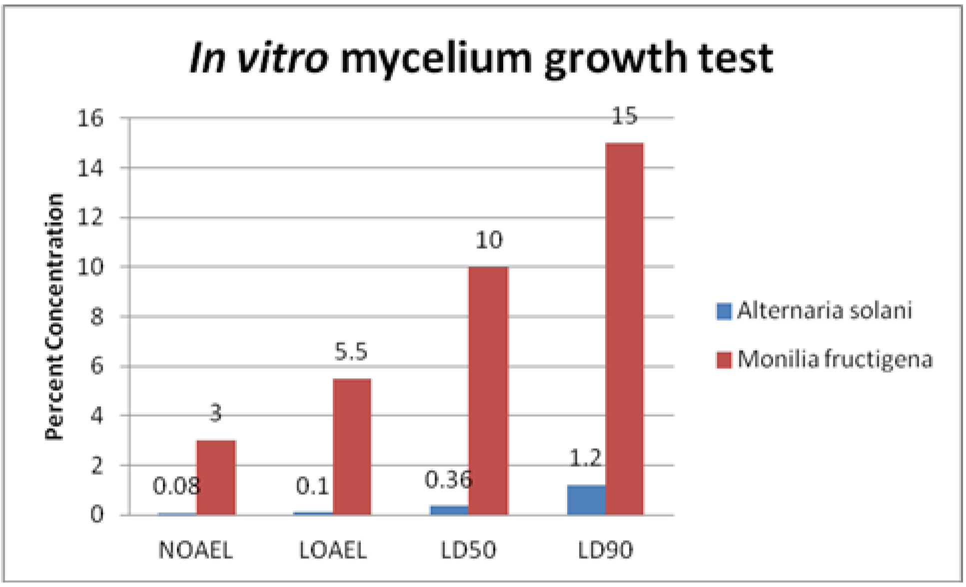

Figure 1. Antifungal action of water solutions of the L-ascorbic acids towards *Monilia fructigena and Alternaria solani*

The results from Figure.1 show that the L-ascorbic acid except ISR action on plants can express significant antifungal effectiveness against *Alternaria solani*. However, the tests reveal that the action towards mycelium of *Monilia fructigena* was extremely weak – LD90; it was 15% (m/v), the full inhibition of mycelium was achieved at 20% (m/v) concentration of vitamin C in the water solutions. In comparison – towards *Alternaria solani*, this concentration was 1.5% (m/v) – Figure.2

In the Figure. 3 there are results from the conducted *in vitro* tests for the inhibition of conidiospores germination of the tested plant pathogens.

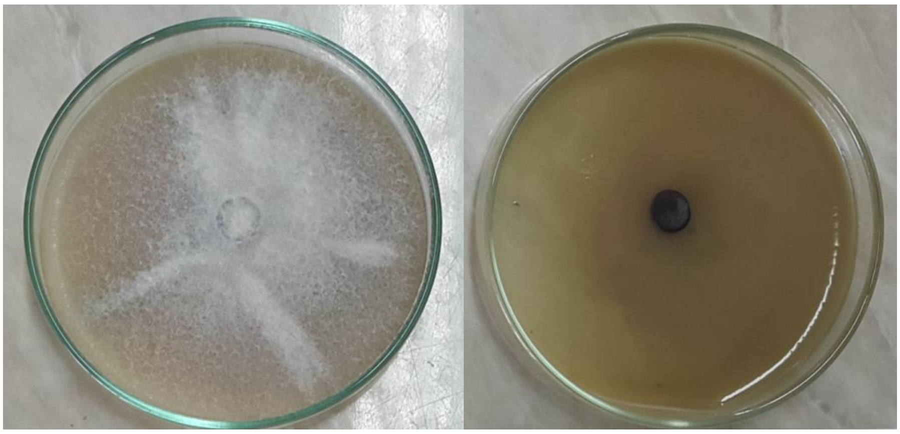

Figure. 2 Antifungal action of the L-ascorbic acid towards *Alternaria solani* on the left – control variant, on the right – 1.5% (m/v) water solution of L-ascorbic acid

The received results confirm the established fact that vitamin C has a very weak activity against *Monilia fructigena* – the concentration at which 100% inhibition of conidiophores was achieved was 15% (m/v). For *Alternaria solani*, the tested substance was able to fully inhibit the spores germination at 0.08% (m/v) – a concentration which has a very strong antifungal effect.

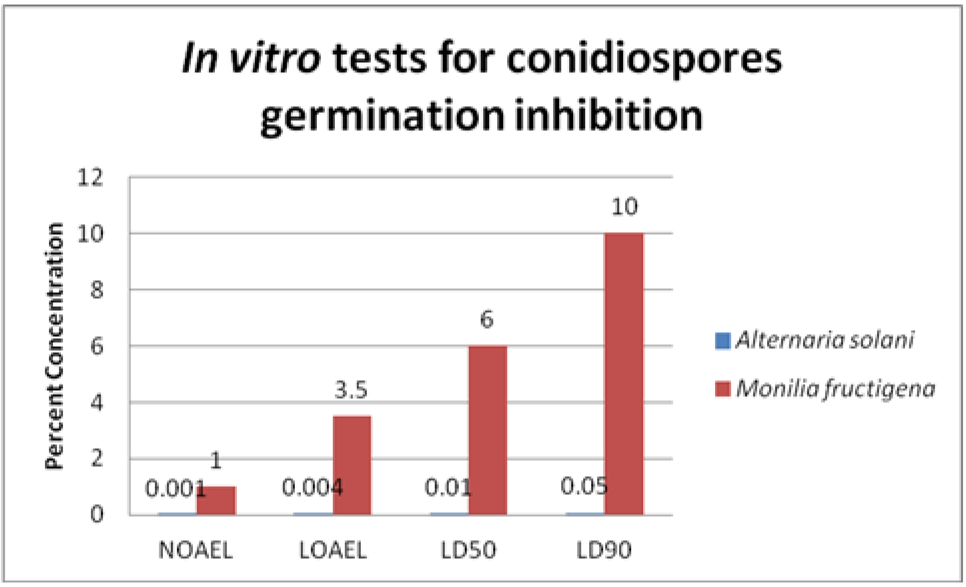

Figure 3. Inhibition of conidiospores germination action of water solutions of L-ascorbic acid towards *Monilia fructigena* and *Alternaria solani*

According to the conidiospores of *Venturia inaequalis*, the received results from the conducted trials are presented below:

- NOAEL = 0,045%
- LOAEL = 0,22%
- LD50 = 0,75%
- LD90 = 1,2%

Vitamin C has a weaker antifungal effect in comparison with *Alternaria solani,* but still strong – the full inhibition spores germination was achieved at 1.5% (m/v) concentration – Figure. 4.

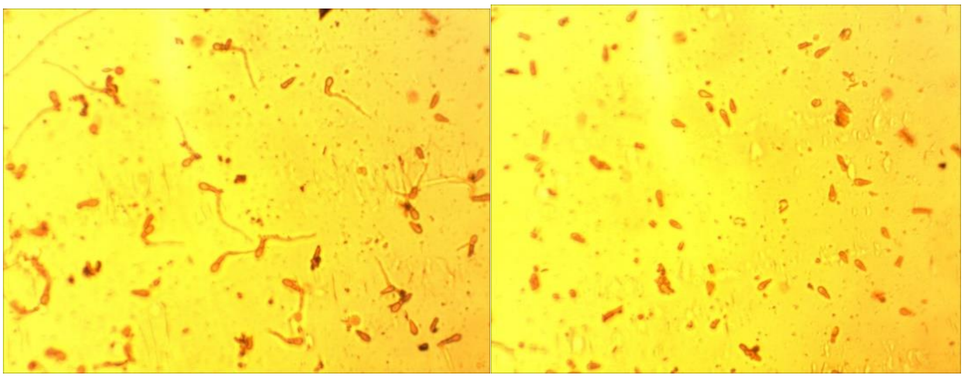

Figure. 4. Antifungal action L-ascorbic acid towards conidiospores of apple scab (*Venturia inaequalis*). On the left – control variant, on the right – 1.5% (m/v) water solution of L-ascorbic acid

The conducted *in vivo* test for phytotoxicity reveal that even 5% water solution of vitamin C does not cause any deleterious effect on treated tomato plants, cucumbers, potatoes, pepper plants, onions, wheat, apples and peaches.

However, on the cherry and sour cherry plants for the same concentration there were observed minimal (0.5 – 1%) phytotoxicity manifestations like necrosis on the treated leaves.

The conducted tests also show that the common pumpkin (*Cucurbita maxima*) is very sensitive to vitamin C treatments. Only 1% water solution do not cause phytotoxicity. Above this concentration, necrosis and deformations on leaves were observed. The same was established for oil-yielding rose.

However, the most sensitive to the L-ascorbic acid was grape – every concentration above 0.5% (m/v) achieves deleterious effects on treated plants. However even at 5% (m/v) concentration, the phytotoxic manifestations were minimal – Figures 5 and 6.

Figure.5 – Phytotoxic action of 3% (m/v) water solution of L – ascorbic action towards oil – yielding rose

Figure.6 – Phytotoxic action of 5% (m/v) water solution of L – ascorbic action towards grape leaves

DISCUSSION

The conducted *in vitro* trials reveal the strong antifungal action of the L-ascorbic acid towards *Alternaria solani* and *Ventiria inaequalis* and extremely weak activity towards *Monilia frucigena*. Simultaneously, vitamin C was established to be relatively nontoxic for the treated plants. However, some of the tested cultures like grapes, oil-yielding rose and common pumpkin were very sensitive to the substance.

CONCLUSION

The current study shows that the substance can express a direct antifungal action towards some plant pathogens. There is however a big difference of this antifungal action with respect to different pathogens. The acid expressed a very weak antifungal activity against *Monilia fructigena*, even more – produce phytotoxicity on the cherry and sour cherry plants which are very commonly affected by *Monilia fructigena*. According to *Alternaria solani* and *Venturia inaequlis*, vitamin C can be used as a safe, cheap and natural alternative to the commercial fungicides. The expression of a pesticide action of ISR promoters as vitamin C is not unusual unlike the vaccines for the animals and humans. The solubility of water makes it very appropriate for formulation as a plant protection product due to the ability of the formation of true solutions without sedimentation or separation.

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