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RESEARCH ON LIMITING THE MANIFESTATION OF FRUIT STORAGE DISEASES THROUGH THE USE OF TREATMENTS WITH BIOLOGICAL PRODUCTS

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Abstract

In the context of organic farming, the control of plant diseases is done by applying certain methods that, depending on the time and method of application, can be preventive and curative. Deposits are usually additional sources of infestation with diseases that begin in the orchard. This paper proposes laboratory cultures of pathogens such as Venturia inaequalis, Monilinia fructigena, Gloeosporium fructigenum, Botrytis cinerea, Penicilium expansum and Pezicula alba, in an attempt to limit the evolution of the diseases produced until the total cessation of evolution. After cultivation on MS culture medium enriched with vitamins and sucrose in a sterile manner, the pathogenic material is allowed to develop. After 6 days, treatments were made with colloidal silver and with a biopesticide solution based on essential oils made according to our own recipe. In both cases, the area of infection was considerably reduced, the best results being obtained, however, after the treatment with the LIVAL 1 Biopesticide.

Key words: *organic farming, deposit diseases, biopesticide.*

INTRODUCTION

In the chemical composition of some plants from spontaneous or cultivated flora there are some biologically active substances with antimicrobial action (Ardelean, 2000; Docea, 2008). They act either directly on pathogens, inhibiting their development (fungistatic) or killing them (fungicide), or directly on the attacked plant, which stimulates them, morphologically or functionally, an adequate system of defence (strengthening its immune system), for example, soy lecithin prevents the attack of cucumber powder by spraying the leaves of the plants weekly with a solution in a concentration of 0.15% (Ion et al., 2013).

Some studies have also shown that some species of *Streptomyces* sp. are characterized by the ability to produce substances with antibiotic action (streptomycin) and control many pathogenic fungi of the root system (*Pyrenochaeta lycopersici*, *Fusarium oxysporum, Pythium debaryanum*, *Verticillium dahliae*, *Phytophthora capsici*, etc.).

These methods are particularly important in plant protection because they capitalize on the natural (genetic) properties of plants, have no impact on the environment and are relatively inexpensive. Although the name is common to all categories of pathogens, genetic methods are very different, consistent with the characteristics of each category and species of pathogens and cultivated plants (Beers et al., 1993). Plant improvement results in new varieties with superior qualities, including increased resistance to pathogens (Ion, 2007). Resistance is not only mechanical, but also chemical, under the action of a pathogen giving rise to various substances with a defensive role. These mechanisms are genetically determined and can be manipulated by various specific techniques (Hatman, 1989; Bruma, 2004).

Chemical control, which can be carried out using:

- sodium silicate, which is used to control the species *Botrytis, Sclerotinia, Pythium*;
- compost extract, which can be used to control powdery mildew;
- potassium permanganate, which is used only for fruit trees and vines to control powdery mildew;
- products based on copper, in the form of copper hydroxide, copper oxychloride, copper (tribasic) sulphate, copper oxide, copper octanoate, which are used, for example, to control downy mildew in grape

vines or scab to pomaceous before flowering. The total dose of copper must not exceed 6 kg/ha/year;
- sulfur-based products, which are used to control powdery mildew on vines and fruit trees;
- calcium hydroxide, which is used only in fruit trees, including nurseries, to control branch ulceration (Ion, 2017).

Various products have been used in the past: Trichodermin with spores of the fungus *Trichoderma lignorum*, to control the fungi *Fusarium* sp., *Rhizoctonia solani*, *Pythium debaryanum*; Sandstone-extract from the capitulas of the plant *Helichrysum arenarium*, with stimulating effect for culture plants and pathogen against fungi *Corynebacterium michiganense* and *C. insidiosum* (Hatman, 1989; Gareth, 2005; Amzar, 2008). Phytobacteromycin extracted from *Actinomyces lavandulge*, with complex effect on bean diseases; Unanin obtained from St. John's wort, very effective against mosaic, stolbur and brown spot of tomatoes (Munteanu, 2008; Stoleru, 2008). *Clonostachys* is a fungus that acts in the soil as a saprophyte, and is characterized by a specific spiral growth. Due to the production of toxic metabolites, this fungus has a good antagonistic activity against several pathogens in the soil. It can also invade and destroy resistant forms of pathogens, such as sclerotia (Stoleru, 2008; Hatman, 1989).

Potassium permanganate. Action: disinfectant, inhibits the growth of fungi and bacteria. Use: it is used in a concentration of 0.01-0.03% (1-3 g per 10 l of water) for the treatment of seedling roots (Bălan, 2003; Munteanu, 2000).

According to Calin (1989), Duza (2008) and Toncea (2002), over 1450 species of plants with insecticidal effect grow worldwide, of which only about 50 are useful. As far as our country is concerned, too few of the 200 species credited with this action have been or are actually used for this purpose and still less studied from this point of view (Davidescu, 1994).

One way to control plant diseases is to induce and increase their own protection mechanisms (phytoalexins and elicitors), which would avoid the use of toxic components for plants (Pop, 1975).

One of the objectives of the research of new compounds with phytosanitary uses is to look for new structures from natural plant sources. Many researchers, especially from countries with increased biodiversity, have contributed to the detection of new compounds derived from medicinal plants for phytosanitary uses (Rosca et al., 2008).

MATERIALS AND METHODS

The biological material used refers to 5 pathogens that can cause storage diseases, thus causing major damage to many fruits during their storage period.

Figure 1 and Figure 2. Samples of pathogenic material taken for the experiment (*Venturia inaequalis* and *Monilinia fructigena*)

The experiment was performed in the virology laboratory of the HORTINVEST Research Center. Samples of pathogenic material were taken from pathogenic microorganisms, namely:
- *Venturia (Endosigme) inaequalis*, which produces brown or rotting spots and is manifested on the fruit by more or less circular spots, from a few millimeters to a centimeter in diameter, gray, covered by a velvety layer consisting of conidiophores and conidia mushrooms.
- Brown rot or *Moniliosis* comes in three forms: brown rot, black rot, and heart rot.
- Bitter rot, caused by the fungus *Gloeosporium fructigenum*, which infects fruits in full maturity. In the deposit, it is manifested by the appearance of large, brown spots, developed on any part of the fruit, around the lentils, but especially in the peduncular area.
- *Botrytis cinerea* manifests itself on the fruit during the growing season and continues to grow during the storage period

- *Pezicula alba* with the conidial shape *Gloeosporium album* is very common in some apple stores. The infection occurs in the orchard but continues during storage, the fungus penetrating the fruit, especially through the lentils, but also through unhealed wounds.


Figure 3 and Figure 4. Samples of pathogenic material taken for the experiment (*Gloeosporium fructigenum* and *Botrytis cinerea*)

- The blue rot, produced by the fungus *Penicilium expansum* is manifested in conditions of high humidity and temperature and lack of aeration by the appearance on the surface of the fruit of circular, brown spots, which spread rapidly and on which develop piles of conidiophores and conidia, arranged irregular, at first white, then bluish green. The pulp of infected apples and pears rots.


Figure 5 and Figure 6. Samples of pathogenic material taken for the experiment (*Penicilium expansum* and *Pezicula alba*)

The culture medium represents the physical and chemical support necessary for the growth and development of any living organism, including pathogenic ones.
The culture medium must respond through its composition, nutritional and hormonal requirements of the body.
Following our own research, the composition of the culture medium that ensures an optimal germination of pathogenic spores is presented in Table 1.

Table 1. Establishing the recipe for the optimal culture medium for the growth of pathogenic microorganisms

No.	Substance	Concentration	Quantity
1	Macro MS	10 x	100 mL
2	Micro MS	1000 x	1 mL
3	Iron chelate	200 x	5 mL
4	Vitamins MS	100 x	10 mL
5	Inositol	100 x	10 mL
6	BAP	-	2 mg/L
7	GA	-	1 mg/L
8	IBA	-	0.1 mg/L
9	Sucrose	-	30 g/L
10	Agar	-	6 g/L
Culture medium pH = 5.8 (corrected with 1 N NaOH)			

Inoculation was performed under aseptic conditions, with sterile instruments under a laminar flow hood. In each petri dish with culture medium, an essay with pathogenic biological material from the 6 pathogenic species was inoculated on the same day.


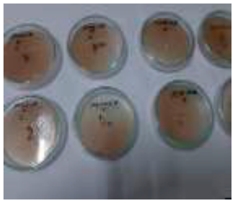

Figure 7 and Figure 8. Inoculation of the pathogenic material on culture medium

RESULTS AND DISCUSSIONS

On March 3, 2020, the inoculations were made with the culture medium established in its own recipe that supports the growth and development of any living organism. For 5 days the samples were left to incubate, then the infected areas were measured at intervals of 4 days for each pathogen, resulting in a percentage of the area of infection that was reduced after treatment.
Table 2 shows the results regarding the limitation of the infection surface following the colloidal silver treatment. Thus, following the incubation of pathogens on March 5, 2020, the initial area of infection was measured. For *Pezicula alba*, with the conidial form *Gloeosporium album*, the initial infection surface was 12 mm^2, which after the treatments with colloidal silver was reduced to 4 mm^2, respectively 2 mm^2, with a reduction of the

infection surface by 66.67% and 83.33%, at intervals of 4 days. For *Monilinia fructigena*, the initial infection area was 6 mm^2 which after the colloidal silver treatments was reduced to 4 mm^2 and 2 mm^2, respectively, with a reduction of the infection area by 33.33% and 66.67%, respectively, at intervals of 4 days. For *Venturia inaequalis*, the initial area of infection was 4 mm^2 which after the treatments with the initial colloidal silver was not reduced remaining 4 mm^2, then was reduced to 2 mm^2, with a reduction of the infection area by 0.0% respectively 50.00%, at intervals of 4 days. For *Penicilium expansum*, the initial infection area was 12 mm^2 which after the colloidal silver treatments was reduced to 2 mm^2 and 1 mm^2, respectively, with a reduction of the infection area by 83.33% and 91.67%, respectively, at intervals of 4 days.

Table 2. Results regarding the limitation of the infection surface following the treatment with colloidal silver

Infection area (mm^2) before and after treatment with colloidal silver					
Storage disease	Initial	After treatment with colloidal silver mm2)	After treatment with colloidal silver mm2)	After treatment with colloidal silver (%)	After treatment with colloidal silver (%)
	March 5	March 9	March 13	March 9	March 13
Pezicula 1	12	4	2	66.67	83.33
Monilinia 1	6	4	2	33.33	66.67
Venturia 1	4	4	2	0	50
Penicilium 1	12	2	1	83.33	91.67
Gloeosporium fructigenum	4	1	0.25	75	93.75
Botrytis 1	9	1	0.25	88.89	97.22
Average	47	16	7.5	57.87	80.44

For *Gloeosporium fructigenum*, the initial area of infection was 4 mm^2 which after treatments with the initial colloidal silver was reduced to 1 mm^2, then was reduced to 0.25 mm^2, with a reduction in the area of infection by 75.0%, respectively 93.75%, at intervals of 4 days.

For *Botrytis cinerea*, the initial area of infection was 9 mm^2 which after treatments with the initial colloidal silver was reduced to 1 mm^2, then was reduced to 0.25 mm^2, with a reduction in the area of infection by 88.89%, respectively 97.22%, at an interval of 4 days. At an average infection area of 47 mm^2, in the end this area was reduced by 80.44% in two applications every 4 days. The results are also observed spatially in Figure 9.

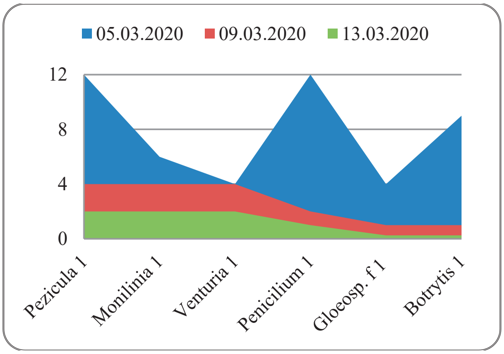

Figure 9. Results regarding the evolution of the infection surface after the colloidal silver treatment

Regarding the results obtained from the treatments with essential oils, the determinations were made after the incubation period on March 5, 2020 and are presented spatially in Figure 10.

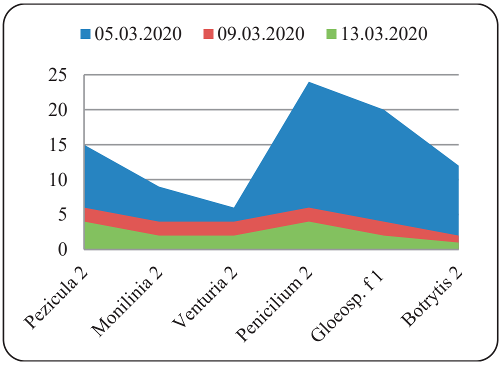

Figure 10. Graph regarding the evolution of the infection surface following the treatments with essential oils

Table 3 presents results regarding the evolution of the infection surface following the treatments with essential oils, measured at a distance of 4 days. For *Pezicula alba*, with the conidial form *Gloeosporium album*, the initial infection area was 15 mm^2, which after the treatments with essential oils was reduced to 6 mm^2 and 4 mm^2, respectively, with a reduction of the infection area by 60.00% and 73.33%, at intervals of 4 days. For *Monilinia fructigena*, the initial area of infection was 9 mm^2 which after the treatments with essential oils was reduced to 4 mm^2 and 2 mm^2, respectively, with a reduction of the infection area by 55.56% and 77.78%, respectively, at intervals of 4 days. For *Venturia inaequalis*, the initial area of infection was 6 mm^2 which, after treatment with essential oils, was reduced to 4 mm^2, then reduced to 2 mm^2, with a reduction in the area of infection by 98.0% and 66. 67%, respectively, at intervals of 4 days.

Table 3. Results regarding the evolution of the infection surface following the treatments with essential oils

Infection area (mm^2) before and after treatment with essential oils					
Storage disease	Initial	After treatment with essential oils (mm^2)	After treatment with essential oils (mm^2)	After treatment with essential oils (%)	After treatment with essential oils (%)
	March 5	March 9	March 13	March 9	March 13
Pezicula 2	15	6	4	60	73.33
Monilinia 2	9	4	2	55.56	77.78
Venturia 2	6	4	2	98	66.67
Penicilium 2	24	6	4	75	83.33
Gloeosporium fructigenum	20	4	2	80	90
Botrytis 2	12	2	1	83.33	91.67
Average	86	26	15	75.31	80.44

For *Penicilium expansum*, the initial area of infection was 24 mm^2 which after the treatments with essential oils was reduced to 6 mm^2 and 4 mm^2, respectively, with a reduction of the infection area by 75.00% and 83.33%, respectively, at intervals of 4 days.
For *Gloeosporium fructigenum*, the initial area of infection was 20 mm^2 which after the treatments with essential oils was reduced remaining 4 mm^2, then was reduced to 2 mm^2, with a reduction of the infection area by 80.0%, respectively 90.00%, at intervals of 4 days.
For *Botrytis cinerea*, the initial area of infection was 12 mm^2 which after the treatments with essential oils was initially reduced remaining 2 mm^2, then was reduced to 1 mm^2, with a reduction of the infection area by 83.33% respectively 91.67%, at intervals of 4 days.
Regarding the treatments with essential oils at an average infection area of 86 mm^2, there is a reduction of the infected area in proportion of 80.46% applied in 2 treatments at intervals of 4 days, which demonstrates a good effectiveness.

CONCLUSIONS

Medicinal plants and essential oils represent the oldest category of therapeutic remedies that has accompanied mankind throughout its historical evolution, now used in organic farming. More recently, colloidal silver is also used in integrated control and organic farming. It is already known worldwide that silver nanoparticles (AgNPs) are the most widely used and widespread nanotechnological material. Colloidal silver solutions can contain several types of material, as far as silver is concerned, because the notion of "colloidal" only defines the type of solution (a colloid) and not its content. In the case of colloidal silver treatments, at an average infection area of 47 mm^2, in the end this area was reduced by 80.44% in two applications every 4 days. Regarding the treatments with essential oils at an average infection area of 86 mm^2, there is a reduction of the infected area in proportion of 80.46% applied in 2 treatments at intervals of 4 days, which demonstrates a good effectiveness. The differences between the 2 products is not very large which concludes that both forms are equally effective.

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