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BIOFORMULĂRI DE PRODUSE FITOSANITARE ÎMPOTRIVA FITOPATOGENILOR *PODOSPHAERA LEUCOTRICHA* ȘI *VENTURIA INAEQUALIS*

BIOFORMULATIONS OF PLANT PROTECTION PRODUCTS TO CONTROL *PODOSPHAERA LEUCOTRICHA* AND *VENTURIA INAEQUALIS* PHYTOPATHOGENS

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Abstract

Currently, the legislation in the field of organic farming on combating pests in apple orchards requires the use of products of vegetal origin, with low impact on the environment and human health, on a larger scale. In this study was investigated the phytosanitary potential of some emulsions based on vegetal oils and extracts (cinnamon oil, thyme oil, neem oil, clove oil, and ivy vegetable extract) against apple scab and apple powdery mildew at *Malus domestica* Bork. The products intended for foliar application were obtained as heterogeneous dispersed systems, mechanically emulsified by homogenization at high energies. A mixture of lecithin and Tween 80 was used as surfactant, which was found to have the closest hydrophilic-lipophilic balance **to that of the oils used. The products thus obtained were tested *in vitro*. The formula with the highest efficiency in combating apple scab (*Venturia inaequalis* Cooke) was ivy vegetable extract,** inhibition zone (**IZ) = 35 ± 0.52 mm, and for apple powdery mildew (*Podosphaera leucotricha*) was mixed emulsion, IZ = 40 ± 0.64 mm.**

Cuvinte cheie: agenți fitopatogeni, bioformulări, făinarea mărului, rapănul mărului.
Key words: phytopathogen agents, bioformulations, apple powdery mildew, apple scab.

1. Introduction

Synthetic pesticides are often misused, leading to adverse effects on ecosystems and human health, especially in developing countries. The use of biocontrol options, such as plant extracts, has long been argued to be more sustainable and more appropriate for small farmers in developing countries and data from Tembo *et al*., 2018 support this and shows that the use of biopesticides based on vegetal extracts, can effectively control pests and can be integrated into sustainable agricultural practices. They also showed that bioformulations from plant extracts used in vegetable crops could sustain yields similar to synthetic pesticides. Bosshard, 1992, tested the effect of aqueous ivy extracts and reported that a 1% ivy leaf extract diluted with water at 1:8 and even lower 1:16, completely inhibited the development of the *Venturia inaequalis* Cooke phytopathogen. Northover and Schneider, 1993, tested several vegetable oils against *V. inaequalis* and reported that soy or canola oil, emulsified with Agral 90, applied every 7-10 days at a rate of 1%, reduced the severity of apple scab between 66 and 81%. Kumar and Chandel, 2018, tested the efficacy of 8 extracts from different plant species, against *Podosphaera pannosa* (rose powdery) under *in vitro* conditions. The most effective treatment was *Allium sativa* L. extract, with a conidial germination inhibition area of 66.65%, followed by *Sapindus mukorossi* L. extract. *Populus* species extract 1% gave similar efficacy to synthetic fungicides (Balint *et al*., 2014; Thiesz *et al*., 2007). Pfeiffer, 2002, investigated several plant extracts against apple scab under greenhouse conditions. The extract of *Saponaria officinalis* L. showed a high inhibition of conidia germination.

Thus, the goal of this study, was to test certain bioformulations, based on extracts and vegetal oils, against 2 phytopathogens, apple scab and powdery mildew, which cause severe economic loss in apple orchards in order to control apple scab in organic fruit growing, copper or sulphur products are widely used (Jamar et al., 2010; Mitre et al., 2009, 2010). These have several disadvantages, such as copper accumulation in soil, reduced efficiency at low temperatures, low fruit quality in terms of commercially important appearance etc. Therefore, a replacement for copper and sulphur products must be considered more environmentally friendly and economical in apple production (Kelderer et al., 2008).

2. Material and methods

To obtain the biofungicides were used cinnamon oil, thyme oil, neem oil, clove oil, ivy extract, distilled water, lecithin and surfactant Tween 80, under the following formulations:

V1. Thyme oil (1.5 ml) + H_2O (498 ml) + Tween 80 (0.5 ml) + lecithin (0.5 ml)

V2. Clove oil (1.5 ml) + H_2O (497.5 ml) +Tween 80 (0.5 ml) + lecithin (0.5 ml)

V3. Cinnamon oil (1.6 ml) + H_2O (498 ml) +Tween 80 (0.5 ml) + lecithin (0.5 ml)

V4. Ivy extract 10% (5 ml) + H_2O (545 ml)

V5. Neem oil (25 ml) + H_2O (450 ml) + Tween 80 (12.5 ml) + lecithin (12.5 ml)

V6. Mixt emulsion = clove oil (1 ml) + thyme oil (1 ml) + cinnamon oil (1 ml) + ivy extract (10 ml) + H_2O (974 ml) + Tween 80 (6.5 ml) + lecithin (6.5 ml)

The internal phase (oil), surfactant Tween 80 (emulsifier) and lecithin were added to the external phase, represented by distilled water. As a device, for the preparation of the emulsions, a high energy Silverson type homogenizer was used, for 5-7 minutes at 20,000 rpm. We used the mixture of lecithin and Tween 80, as it was found to have the closest HLB (15) (Hydrophilic-lipophilic balance) index to that of the oils used, thus ensuring good emulsion stability.

In vitro testing of bioformulations - Zone of Inhibition Test. In order to test the efficacy of the biofungicides, strains of two types of pathogenic microorganisms *Podosphaera leucotricha* and *Venturia inaequalis,* were isolated from leaves and fruits of `Idared` apple cultivar. Then, these isolates were cultivated on the culture medium Potato-Dextrose Agar (PDA) from Sigma-Aldrich, having the following composition: agar, 15 g l^{-1}, dextrose, 20 g l^{-1} and potato extract, 4 g l^{-1}. The standard medium was supplemented with 0.1 g l^{-1} chloramphenicol, a broad-spectrum antibiotic for bacteria, to prevent infection with microorganisms other than those to be investigated.

In a test tube with the microorganisms to be tested, 10 ml of sterile water was added and after scraping, 1 ml of suspension was taken and inoculated on the entire surface of the Petri plate with a sterile Drigalski spatula. The plates were then incubated at 37°C. In the center of the culture medium, three orifices with a diameter of 6 mm were made, in which 50 µl of sample was pipetted. After 72-92 hours, was measured the diameter of the inhibition zone around the orifices where the sample was placed and it was compared with the untreated control plate. Antimicrobial activity was determinated by measuring the size of inhibition zone on agar petri dish and was compared with the control plate (Fig. 1-2).

Statistical analysis. All experiments were carried out in triplicate, and the data derived was analyzed for statistical significance using analysis of variance (one-way ANOVA) and Tukey test to determine significant differences among means. Significant differences were set at $P \leq 0.05$. The results presented represent the Mean ± standard error of mean (SEM) of independent replicates.

3. Results and discussions

Assessment of the Table 1, reveal that, the most effective tested variant against apple scab - *Venturia inaequalis*, was ivy extract (V4), with an inhibition zone of 35 ± 0.52 mm followed by neem oil (V5) where inhibition zone was 20 ± 0.22 mm. Against powdery mildew - *Podosphaera leucotricha,* the best results were obtained with mixt emulsion (V6), with an inhibition zone of 40 ± 0.64 mm, followed by thyme oil (V1) where the inhibition zone was 24 ± 0.35 mm.

Following the experiments carried out, the formula with the highest efficiency in the control of apple scab (*Venturia inaequalis* Cooke) was **V4** [10% ivy extract (5 ml) + H_2O (545 ml)] with an **inhibition zone (IZ) = 35 ± 0.52 mm** (Fig. 1)

For control of powdery mildew (*Podosphaera leucotricha* Ellis & Everhart. Salmon), the best biofungicide was **V6** [mixt emulsion = clove oil (1 ml) + thyme oil (1 ml) + cinnamon oil (1 ml) + ivy extract (10 ml) + H_2O (974 ml) + Tween 80 (6.5 ml) + lecithin (6.5 ml)], with an **inhibition zone (IZ) = 40 ± 0.64 mm** (Fig. 2).

4. Conclusions

Despite the effective allocation of synthetic pesticides, their continued, excessive and inappropriate use, can lead to harmful effects on humans and the environment, having a negative impact on biodiversity.

Taking these aspects into account, we can say that the plant protection products obtained, based on vegetable oils and extracts, can be an important basis for the development of biofungicides against apple scab and apple powdery mildew.

The application of such products can reduce or even eliminate synthetic fungicides, resulting in an efficient growth of organic apple trees.

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References:

1. Bálint J., Nagy S, Thiesz R., Nyárádi I.I., Balog A., 2014. Biocontrol strategy to reduce asexual reproduction of apple scab (*Venturia inaequalis*) by using plant extracts. Turk. J. Agric. For. 38(1), 91–98.
2. Bosshard E., 1992. Effect of ivy (*Hedera helix*) leaf extract against apple scab and powdery mildew. *ActaPhytopatholEntomol* Hung.27: 135-140.
3. Jamar L., Lefrancq B., Lateur M., 2007. Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment. J. Plant Dis. Prot. 114, 221–227.
4. Kelderer M., Casera C., Pedri U., 2008. Potassium bicarbonate: a new fungicide for organic agriculture? Notiziario ERSA 20, 50–52.
5. Mitre I., Mitre V., Ardelean M., Sestras R., Sestras A., 2009. Evaluation of old apple cultivars grown in central Transylvania, Romania. Not. Bot. Horti Agrobot. Cluj-Napoca 37, 235–237.
6. Northover J., Schneider K.E., 1993. Activity of plant oils on diseases caused by *Podosphaera leucotricha*, *Venturia inaequalis*, and *Albugoo ccidentalis*. *Plant Dis* 77: 152-157.
7. Pfeiffer B., 2002. Greenhouse experiments on control of *Venturia inaequalis* – first results. In: X. International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing and Viticulture. 4–7 February 2002. Weinsberg, Germany, 81–85.
8. Tembo Y., Mkindi A.G., Mkenda P.A., 2018. Pesticidal Plant Extracts Improve Yield and Reduce Insect Pests on Legume Crops Without Harming Beneficial Arthropods. *Front Plant Sci*. 9:1425.
9. Thiesz R., Balog A., Ferencz L., Albert J., 2007. The effects of plant extracts on apple scab (*Venturia inaequalis* Cooke) under laboratory conditions. Rom. Biotech. Lett. 12(4), 3295–3302.

Tables and figures

Table 1. Inhibition zone values for tested vegetal oils and extracts

Variant (vegetal oils/extracts)	Inhibition zone (IZ) for apple scab - *Venturia inaequalis* (mm)	Inhibition zone (IZ) for powdery mildew - *Podosphaera leucotricha* (mm)
V1 - Thyme oil	-	24±0.35
V2 - Clove oil	-	10±0.62
V3 - Cinnamon oil	-	12±0.10
V4 - Ivy extract	35 ± 0.52	18±0.31
V5 - Neem oil	20± 0.22	22±0.52
V6 - Mixt emulsion	-	40 ± 0.64

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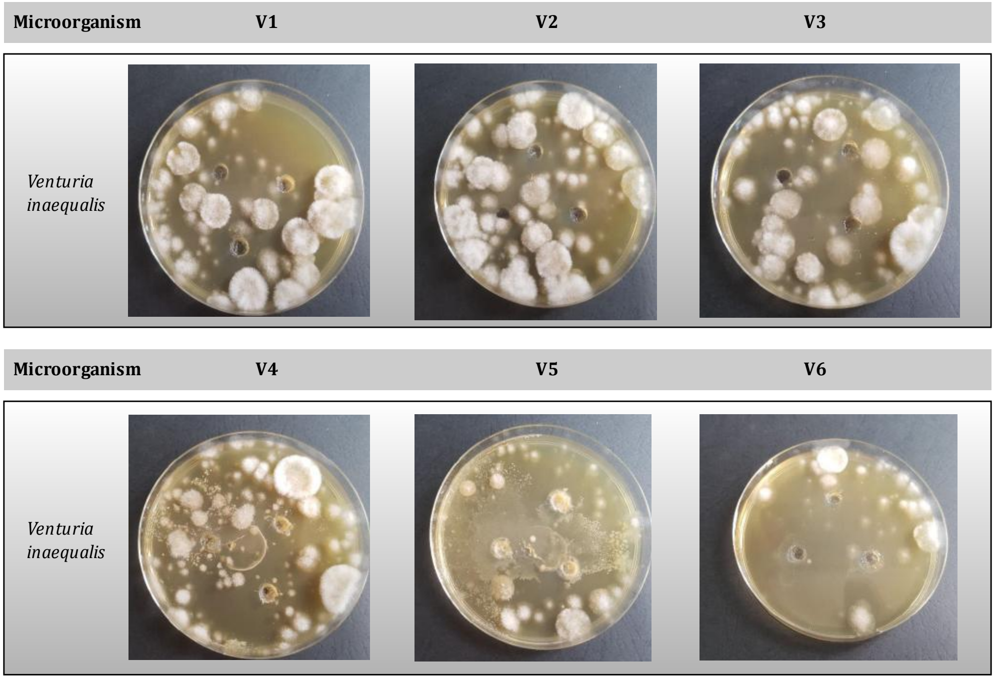

Fig. 2. Influence of bioformulations on apple scab - *Venturia inaequalis* Cooke

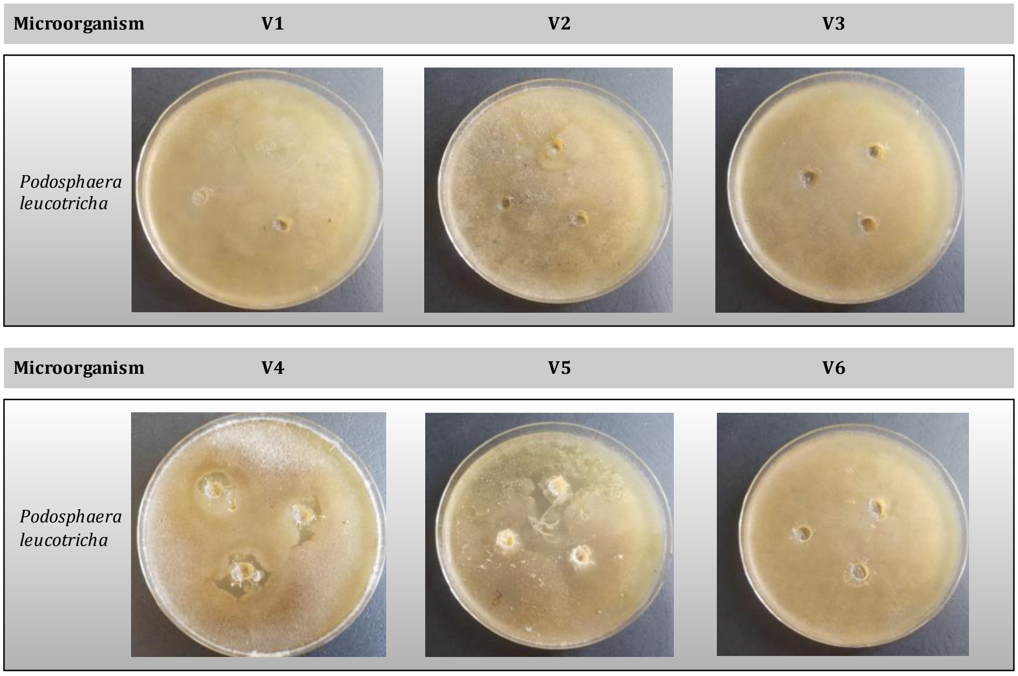

Fig. 3. Influence of bioformulations on apple powdery mildew - *Podosphaera leucotricha* Ellis & Everhart. Salmon