In vitro fungicidal activity of plant oil based soaps towards apple scab
(\textit{Venturia inaequalis})

Donyo H. Gacnhev, Agricultural University - Plovdiv, Bulgaria, donyo@abv.bg

Abstract - The plant oils and soaps are known from many years as promising eco friendly insecticides used for control of the various soft-body insects. In present study \textit{in vitro} trials with three soap formulations on the base of sunflower oil were conducted in order to be evaluated their potential to be developed as naturally friendly fungicides for controlling of apple scab. This formulations, in the future can be viable alternative of present days commercial fungicides used for the management of apple scab as cheap, low toxic and easy to be manufacture plant protection products. All formulations were tested at 1.0 \% (v/v) concentrations by the method of leaf disks and germ tube inhibition method. The results show that tested soap on the base of sunflower oil amended with potassium chloride was able to achieve 100 \% inhibition of the mycelium and germ tube germination of the apple scab pathogen (\textit{Venturia inaequalis}) at 1.0 \% (v/v) concentration. Other soap formulations do not show any effectiveness to the tested phytopathogen. No phytotoxic manifestations onto treated apple leaves were observed during the leaf disks test. The conducted trials reveal that although the created soap formulation are very similar (all are on the base of sunflower soap neutralized with boric acid) the addition (mixing) of different substances like potassium chloride can completely change their action and effectiveness. This make soaps promising and good alternative to the expensive fungicides especially in the area of organic agriculture.

I. INTRODUCTION

Soap is one of the most important accent discovery which is the key milestone in the human civilization. In the region of Babylon, the first recipe for making soap date back since 2800 B.C. [1]. During medieval ages one of the most famous and important center for soap making was the town of Aleppo (present day Syria). The soap was made by mixing and after that – heating the olive oil with wood ash. The key point was the addition of the essential oil from Laurel (\textit{Laurus Nobilis L.}) at the end of the possess. Even during that time, the soap was used as for personal hygiene goals as for treatment of various dermatic disorders as acne, psoriasis, insects bites and others [2]. One of the oldest plant protection product on the base of soap was the soap prepared from whale oil successfully used against mites, aphids and other pests [3].

However, even today there is several plant protection products on the base of soaps used as insecticides and miticides [4], there is only a few which can be used as fungicides primary against plant pathogens with ectotrophy mycelium [5].

Apple scab (\textit{Venturia inaequalis}) is the most economic important disease for the culture especially in the temperate climate areas [6]. The management of disease include using of the some fungicides as dithianon, difenoconazole, captane, tebuconazole, metiram, dodine, and others which from one hand are
too expensive (especially for the farmers from developing countries like Bulgaria) and from other cab
cause resistance and / or to be dangerous for the humans and the environment [7].

The soaps as plant protection products have several advantages:

- They are biodegradable [8], [9]
- After drying the soap solution onto treated plants, the soaps are practically harmless for organisms
- They are harmless for the bees [10]
- They have low or no toxicity for the humans, birds, mammals [11]
- They can be used without or with minimal post-harvest intervals
- They have multi-cite mode of action – there is zero risk for developing resistance to them and such kind plant protection products can be used without rotation with other pesticides with different mode of action. In this aspect, they are especially suitable for use in the integrated pest management and integrated resistance management schemes [12], [13], [14]
- They can be easily manufacture and are low-cost pesticides with make them especially suitable for developing countries
- They can be used as ready-to-be-used solutions which make them very suitable for amateur farms or so called urban farming
- They can have multicide action – can act as insecticides, fungicides, herbicides, miticides
- They can be combined with other pesticide substances, especially with plant extracts [15], [16], [17]

The soaps are recognized as viable fungicides against apple scab (Venturia inaequalis) [18], [19] as cheap, low toxic and easy to be manufacture plant protection products. The similar activity have saponins from various plants like Saponaria officinalis, Sapindus mukorossi [20] and Yucca schidigera [21]. The purpose of the present study was to be evaluated the eventual fungicidal action of three soap formulations on the base of sunflower oil created in the Laboratory of Pesticide Science and Ecotoxicology - Agricultural University, town of Plovdiv, Bulgari, in the in vitro conditions against apple scab (Venturia inaequalis) and toe reveal their future potential to serve as plant protection products.
II. Materials and Methods

Three different formulations of soaps on the base of sunflower oil mixed with potassium hydroxide were created by so called "cold process" [22]. The soaps were liquidized with distilled water and neutralized with boric acid up to pH=7 and by this way first formulation with working name SLBOR was created. In liquidized sunflower soap potassium chloride was added and then neutralization with boric acid was performed - SLBORKCL formulation was created, in the third formulation, in the liquidized sunflower soap calcium carbonate was added and then neutralization with boric acid was performed - SLCAO formulation was created.

All formulations were tested in the 1.0 % (v/v) concentrations in in vitro conditions against *Venturia inaequalis* by the method of leaf disks - the method was adapted from those of Wong and Wilcox [23]

Soap solutions with 1.0% (v/v) concentration were prepared with distilled water with added standard organosilicone surfactant Silwet®L-77 manufactured by General Electric Company at concentration 0.015 % (v/v) for improvement of wetting ability [24]. The control variant was treated with solution of surfactant in distilled water, the using standard was Dithane M-45 ® on base of mancozeb as active substance [25].

Fresh leaves from 5 year old apple trees variety “Golden Delicious ” growth stage 72 [26] naturally infected with the tested phytopathogen were used for the purposes of test.

Leaf disks (parts) with visual apple scab symptoms with 3 cm diameter were soaked in the fungicide solutions for 5 seconds and after that placed in wetted with distilled water filter papers. Each test variant was set in five replicates. The effectiveness was measured on 24 and 48 hours after treatment by lack or presence of visual symptoms of phytopathogen (presence of mycelium) on leaves by digital microscope at 10x magnification.

Germ tube inhibition tests were conducted in order to be determined a possible protective activity. Fresh infected with inspected pathogen plant parts were collected and were incubated in a humid chamber for the purpose of stimulation the conidial sporulation of the phytopathogens. Conidial suspensions were prepared with the density 3*104 spores/ml. Microscopic slides kind
“handing drop” were sprayed with tested soaps solutions at 1.0 % (v/v) concentration and after drying, 20 µl of conidial suspension was applied. The slides were incubated in a humid chamber, in thermostat and after 24-48 h. The number of germinated conidia were counted with a light microscope. The effectiveness was determined with formula of Abbott [27].

III. RESULT AND DISCUSSIONS

In the conducted *in vitro* leaf disks and germ tube inhibition tests, soap on the base of sunflower oil amended with potassium chloride was able to achieve 100 % inhibition of the mycelium and germ tube germination of the apple scab pathogen (*Venturia inaequalis*) at 1.0 (v/v) concentration. The other soap formulations - SLBOR and SLCAO do not show any effectiveness according to the tested phytopathogen. The used standard Dithane - M 45 on the base of mancozeb as active substance, also was able to inhibit the tested plant pathogen at 0.3 % (m/v) concentration - Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig.5

Figure 1. Apple scab leaf disk test - control variant
Figure 2. Apple scab leaf disk test - Dithane M-45 - 0.3 % (m/v)

Figure 3. Apple scab leaf disk test - SLBORKCL - 1.0 % (v/v)

Figure 4. Germ tube inhibition tests with conidiospores of *Venturia inaequalis* - Control

Variant
On Fig. 5 can be seen the vacuolization of the conidiospores, explicit sign for the toxic action of tested fungicide.

No phytotoxic manifestations onto treated apple leaves were observed during the leaf disks test. The additional trials with SLBORKCL with same pathogen, but in the lower concentrations reveal that in this case (concentrations below 1.0% (v/v)), the soap have no sufficient effectiveness according to the apple scab.

III. Concussion

The conducted trials reveal that although the created soap formulation are very similar (all are on the base of sunflower soap neutralized with boric acid) the addition (mixing) of different substances like potassium chloride can completely change their action and effectiveness.

The cheap and simple process of the manufacture of soaps and respectively their low prices combined with their low toxic action and danger for the environment, plus their non-specific action according to the phytopathogens, which guarantee the lack of development of resistance to them, make soaps promising and good alternative to the expensive fungicides especially in the area of organic agriculture.
REFERENCES

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