THE USE OF BIOPREPARATIONS WITH BACTERIA AND FUNGI TO CONTROL DOWNY MILDEW (*Plasmopara vitícola*) AND REDUCE CHEMICAL TREATMENTS

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Abstract: This paper reviews the possible biological control of grapevine downy mildew *Plasmopara viticola*. Often, when the climatic conditions are very favorable for the emergence and manifestation of the disease, the chemical treatment has a low efficiency, this phytopathogen producing significant damage, including as a result of the formation of resistant strains. A modern method of combating it is biological control using microorganisms bacteria and fungi which can inhibit through their presence directly or indirectly the growth and multiplication of the fungus, which we present in this paper.

Key words: *Vitis vinifera*, downy milldew, biological control, *Bacillus*, *Trichoderma*, *Lysobacter*

INTRODUCTION

Downy mildew *Plasmopara viticola* (Berk. & Curt.) Berl. et De Toni is one of the main phytopathogenic diseases of grapes that cause important harvest losses. The use of large amounts of pesticides including products containing copper to control fungal diseases in agricultural crop is restricted by EU Regulation N 1473/2002. This ceiling starts with the limit of 8 kg copper per ha and, after a transitional period of four years, it will be reduced to 6 kg copper per ha. During the vegetation period, approximately 12-15 treatments with pesticides or even more are used to control this kind of pathogens in crops (Pertot et al., 2018). The International Organization of Vine and Wine found that in Europe approximately 70.000 t of fungicides are used on 3.8 million hectares of grapevine (Compant et al., 2013). An alternative treatment using Zeolite 850 WP and 800 MP was tested (Polat et al., 2018) alone or associated with *Tricoderma asperellum*. Zeolite 850 WP showed to be effective against *P. viticola*.

In general the tendency is to reduce the use of pesticides in cultures for providing healthier crops for consumers. For example older works showed that one of the methods is to induce resistance in plants against pathogens with microrganisms releasing stimulating factors (Sequiera, 1983) or to stimulate the systemic resistance in plants with PGRP bacteria and some fungi (Bent, 2006). Microbial biocontrol agents can be an real alternative to pesticide use having antagonic activity (Wilson 1989). A very good review (Lahlali et al. 2022) shows that biological control agents are more and more important in plant protection and diseases control and some strains of bacteria like *Bacillus*, *Pantothea*, *Streptomyces*, fungi as *Trichoderma* strains and some yeasts *Candida oleophila*, *Rhodotorula glutinis* strains are considered for biological control.

This paper reviews relevant literature regarding the possible biological control of grapevine downy mildew *Plasmopara viticola*.

DISCUSSIONS

A test with about 1764 microorganisms showed that only 5% of it can inhibit oospores and 0.8% sporangiophore production (Vecchione et al. 2007). In greenhouses, commercial strains such as *Bacillus subtilis* and *Trichoderma harzianum* T39 can be used (Vecchione et al, 2007). *B. subtilis* GLB191 is a strain used in biological control of *Plasmopara viticola* (Li et al., 2019) the compounds surfactin and fengycin, cyclic lipopeptides (Li et al, 2019), were isolated from culture supernatant which are acting directly on pathogen. *Clorostachis rosea* together with *T. harzianum* have been shown to be effective in combating grape downy mildew (Vecchione et al., 2007). Some bacteria (*Streptomyces viridosporum*, *S. violatus*) and fungi (*T. harzianum*, *T. viride*) have been tested for the control of *P. viticola* (El-Sharkawi et al., 2018). Considering the existence of large areas cultivated with vines, it is currently unlikely that the biological control method can be applied on large surface of culture.

Bacteria. Studies on biological control showed that 303 bacterial strains were isolated from grapevine leaves and about 12 strains showed antagonism towards phytopathogens (Zamg, 2020). One of them was the strain from the genus *Ochrobactrum* sp. showing antagonism to *P. viticola* but also to other pathogens of grapes (Zang et al., 2020).

Some bacterial strains were found to increase plant resistance to the attack of the phytopathogen e.g. plant-stimulating bacteria *Klebsiella planticola*, and some that can inhibit growth such as *Streptomyces alni* and *Pseudomonas fluorescens*. The list also contains strains of *Bacillus subtilis*, *B. circulans*, *Serratia macrescens*, *Pantotea* sp. and *Acinetobacter* which can act against other phytopathogens too (Compant et al. 2013). *Lysobacter capsici* AZ 78 is considered to be a good biological control agent because it inhibits the growth of *P. viticola* and at the same time is resistant to abiotic stress and some concentrations of copper products, having a copper oxidase that reduces the attack of *P. viticola* as well as effective as copper products (Puoppolo et al., 2014). This biological control agent is as effective as the reference fungicide Kocide© 2000 and does not affect non-target organisms and wine yeasts and could be developed as a commercial biopesticide (Markellou et al., 2022).

Fungi The endophytic fungal strain that colonizes the leaves of *Vitis vinifera*, *Acremonium byssoides* has been found in many grape varieties in Italy without obvious symptoms (Burruano et al., 2008) and other strains like *A. persicinum* and *A. sclerotigenum* with inhibition effect of sporangia germination in different degrees (Lo Piccolo et al., 2015).

Another traditional fungus used in biological control is *T. harzianum*. This fungus induces plant resistance to downy mildew *P. viticola* (Perazzolli et al., 2012). *Trichoderma* species/strains are used to control many phytopathogenic fungi (Sawant, 2014). Its mechanism of action was described (Benitez et al., 2004), indirectly, by competing for space and food, antibiosis or directly by so called mycoparasitism. In the same time, it was found that biotic stress reduces the induced resistance produced by this fungus (Roatti et al., 2013). *Trichoderma* strains appear to produce VOCs volatile organic compounds with antifungal effects against *P. viticola* (Lazarzzara et al., 2021).

A superior fungus, *Agaricus bisporus* in formulation of 10, 15 and 20% aqueous mycelial suspension inhibits and reduces sporangiospore germination by 80% (Garcia et al, 2018). *Epicoccum nigrum* species is a general saprophyte (Kortenkamp, 1997) and the author believes that this strain can be used for biological control. Another fungal strain, *Fusarium* sp. (Ghule & Sawati, 2017) highly questionable for use in biological control, would have some strains that, through their chitinase, glucanase and protease enzymes, contribute to the

distortion of the sporangiophores of *P. viticola*. The strain *Fusarium proliferatum* is also considered a good strain for biological control of the same fungus (Falk et al., 1996).

Different natural products Stilbenes are a group of organic compounds obtained from waste of grapevine cane wood and roots (Gabaston et al. 2017). There are resveratrol, ampelopsin, hopeaphenol and others, they were extracted from vegetal material and analized by UHPLC-MS and tested in different concentrations in order to identify their activity of inhibition of P. *viticola* sporulation. The extracts from plants containing stilbenes induce resistance of the plant to the fungal attack and inhibited the ability of sporangia to release zoospores (Krzyzaniak et al., 2018). Previous studies demonstrated antifungal activities of methanolic and alcoholic extract containing mainly stilbenes from cane of vine against *P viticola*, *Botritys cinerea* and *Erysiphe necator* (Schnee et al, 2013). Tests with neem azal oil, vitis cane and *Inula viscosum* extract demonstrated their direct inhibition activity against *P. viticola* (Sudiro et al, 2022). The authors also tested a new plant extract identify only as LL017 with apparent a very good results, increasing the plant tolerance comparing with plants treated with conventional pesticides. Diketopiperazines are compounds extracted in this case from the fungi *Alternaria altenata* (Musetti et al. 2007) which has an effect of inhibition of activity of *P. viticola.*

CONCLUSIONS

It has been demonstrated that there are viable alternative to chemical pesticides treatment to control of the downy mildew *Plasmopara viticola*. Unfortunately, the production of biological control products is cumbersome, it is difficult to obtain in sufficient quantities and to apply on large surfaces and quite difficult to control. We believe that they can be applied in ecological systems, and in combination with cupric fungicides in smaller quantities. It is very necessary to do investigations regarding the safety of these products for humans, as well as economic calculations.

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