Research & Reviews: Journal of Botanical Sciences
e-ISSN:2320-0189
p-ISSN:2347-2308

# Botanical Compounds to Combat Vineyards Mealybugs: An Ideal Alternative for Organic Vitiviniculture

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## Review Article

**Received:** 21/08/2018

**Accepted:** 14/09/2018

**Published:** 18/09/2018

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**Keywords:** Organic pest control, Essential oil, Botanical extract, Mealybug

**ABSTRACT**

The vine mealybug (*Planococcus ficus*) is a major pest in vineyards and is widespread in most grape-growing areas, however, to date; few organic products have been tested to combat them. Mealybugs have an important waxy cover, which protects them from most synthetic insecticides. This class of insecticides could cause damage to the ecosystem and human health, and therefore the current need for viticulture is centered in searching novel formulations based on botanical products to fight pests in a healthier way and maintaining the sustainability of agricultural systems. In this review we show that, despite the negative impact of mealybugs against economically important crops, such as vineyards, there are few studies related to organic control using natural compounds (botanical extracts and/or essential oils). However, many of these studies are incomplete because they did not include field application or phytotoxicity tests in crops. We discuss the extent of knowledge of botanical compounds for mealybug control and provide main research lines for the development of environmentally friendly pest control products.

## INTRODUCTION

The vine (*Vitis vinifera* L.) is the world's most economically important fruit culture [1]. Viticulture is one of the most significant activities worldwide in terms of, not only the area with vineyards, but also the amount of money involved in this market in wine-producing countries [2]. However, this huge production presents pest problems. Around 150 arthropod species worldwide have been considered as vineyard pests, being phytophagous mites, phylloxera, leafhoppers, grape moths and mealybugs among the main pests that attack vineyards. Mealybugs (*Hemiptera: Pseudococcidae*) are phytophagous insects that constitute a family with about 2,000 species, many of which are major pests of other agricultural plants [3-7]. Particularly, the species *Planococcus ficus* Signoret, causes losses of major economic importance in different wine-growing zones around the world: Mediterranean regions of Europe, North and South Africa, East California, Mexico and Argentina [8,9]. Vine mealybugs could cause direct damage through sap suction and injection of phytotoxic saliva, affecting the normal growth and development of the plants [10]. In addition, this insect could cause indirect damages due to the transfection of virus between plants, which produces a decreased sugar content and diminished pigmentation, reducing the productivity and altering the quality of the grape [11,12]. Hence, wines made with high percentages of infested clusters possess undesirable organoleptic characteristics [13-15]. *Planococcus ficus* reproduces at a higher rate than other mealybug species, allowing a small number of insects to reach harmful levels in one growing season. Also, these insects are well protected from high summer temperatures, natural enemies and from most foliar insecticides because they hide under bark or in the roots of the plants [16]. Given that traditional *P. ficus* control uses synthetic insecticides that have undesirable effects on environmental and human health, it is necessary to develop environmentally friendly and pesticides from natural products. However, to our knowledge, the state of the art and the prospect of the development of natural products for

*P. ficus* control have not yet been reviewed. Consequently, the aim of this work was to revise the current knowledge about the organic products used to protect vineyards, in order to identify the main limitations in mealybug management, and to propose new strategies to control this pest **(Figure 1)**.

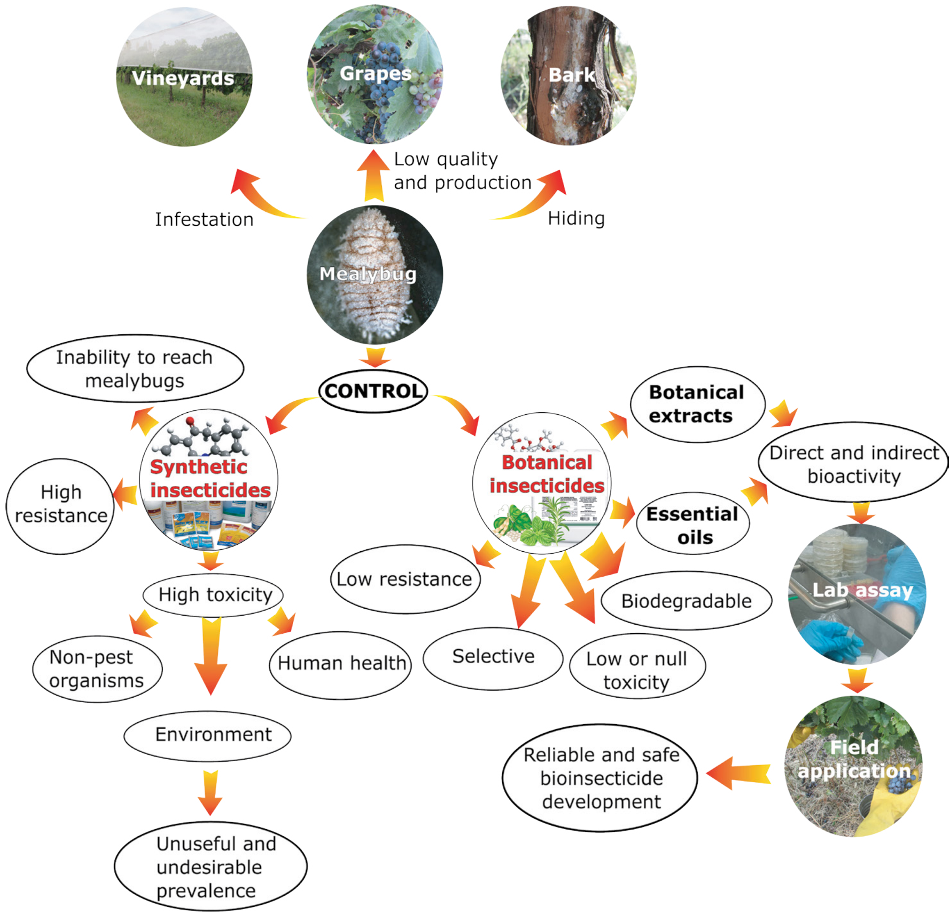


**Figure 1**. Visual summary of the Review content. Some mealybugs, such as *Planococcus ficus*, are important pests of vineyards. These insects decrease the quality and production of the grapes. Currently, synthetic insecticides are used for mealybug control; however, they have many disadvantages such as inability to penetrate either the waxy coating of mealybugs or certain parts of the plant, such as the vine bark, under which mealybugs are typically found. These compounds have high toxicity to non-pest living organisms and to the environment, they can generate pest resistance with its subsequent use, and they represent a potential risk to human health. Thus, it is necessary to develop environmentally friendly and effective botanic insecticides. Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. These compounds have lower resistance and are more selective towards pest target. Few studies have focused on the effect of organic compounds on the development of life cycle, mortality and repellence of mealybugs. Most of these studies are laboratory tests and very few included field trials and phytotoxicity tests, which are indispensable for reliable and safe bioinsecticide development.

## LITERATURE REVIEW

### Synthetic *Versus* Botanical Insecticides for Mealybug Management

Chemical control is the most common strategy used by wine producers to reduce the mealybug population in their crops, particularly, systemic insecticides, such as the group of neonicotinoids (Imidacloprid, Clothianidin and Dinotefuran) [17-19]. Nevertheless, the effectiveness of conventional insecticides is often limited due to its inability to penetrate either the waxy coating of mealybugs or certain parts of the plant, such as the vine bark, under which mealybugs are typically found [20-22]. On the other hand, pest control with synthetic insecticides is dangerous due to their high toxicity to non-pest living organisms and to the environment, and because they can generate pest resistance with its subsequent use [23-25]. Nevertheless, the main problem of using synthetic pesticides is its potential risk to human health. The entire population is completely unprotected against exposure to pesticides and their effects [26]. In fact, recent studies have proved a relation between the use of synthetic pesticides with an increasing incidence of Amyotrophic lateral sclerosis and attention deficit- hyperactivity disorder cases [27]. Global use of pesticides increased dramatically between 1960 and 1990, and more slowly thereafter, although large increases still occur in many developing countries. Therefore, deaths worldwide and the incidence of chronic diseases due to pesticide poisoning are also increasing, supporting the urgent need for safer alternatives to agrochemicals. In this perspective, sustainable and insecticide-free control strategies have been tested against the vine mealybug [28-32].

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals [33]. These new compounds must be more selective towards pest target, thus avoiding toxicity to non-target organisms. Also, to overcome pest resistance, it is necessary to come up with new biochemical modes of action. Finally, for being biodegradable, they must have less impact on ecosystems. In this way, universities, research institutes and agrochemical companies are attaching special importance to the study of natural products for the development of sustainable agriculture [34].

**State-of-Art of Botanical Insecticides Knowledge**

It is well-known that aromatic plants have important pharmacological and insecticidal actions [35,36]. However, few works have assessed its insecticidal and repellent activity on mealybugs **(Figure 2)**. Among more studied species, *Pseudococcus jackbeardsleyi* was tested in 21% of the cases, *Planococcus citri* in 19%, whereas *P. ficus* (the main vineyards pest) was only tested in 16% of cases **(Figure 2)**. The most recent studies of nymph and adult mortality of *P. ficus* show that this species could be controlled with some natural products such as citrus essential oil with limonene as the main component [29,32] and other volatile compounds such as cinnamaldehyde [37].

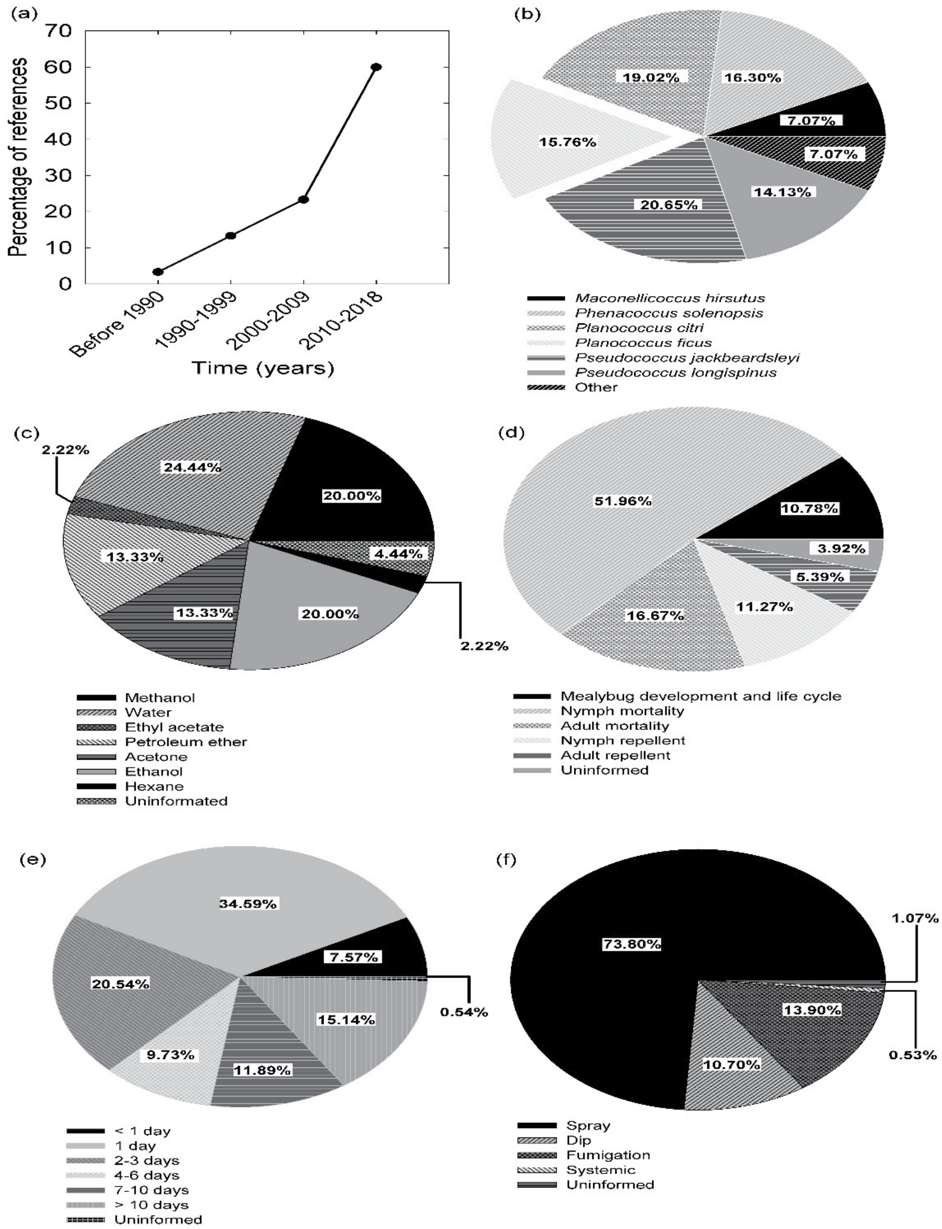


**Figure 2.** (**a-f**) Indicates percentages of total number of experiments performed to test the effectiveness of organic compounds in mealybugs **(a)** Publications referring to the study of organic compounds as insect repellents and/or insecticides as a function of time. **(b)** Mealybug species used in the different studies with organic products. **(c)** Solvents used for the preparation of the botanical extracts. **(d)** Effect of the organic product on the mealybugs. **(e)** Number of days in which the effect of the organic product was measured. **(f)** Application method of the organic product. Figure performed is based on information obtained from study various journal articles and official reports. We build the figure using search engines (academic google and scopus) with the following keywords: "*Planococcus ficus*", "*Planococcus*", "Mealybug", "Essential oil", "Botanical extract", "Insecticide", "Organic agriculture", "Mealybug control", "Mealybug mortality", "Mealybug repellency".

On the other hand, *Azadirachta indica* was the most used plant species in the different studies on mealybugs, followed by *Eucalyptus globulus*. Also, we highlight the use of four *Citrus* species and two *Ocimun* species. Azadirachtin is the most important of the neem limonoids (*A. indica*), which has a repellent and insecticidal action against many insect species [38,39]. Several neem products have toxic effects on pests, affecting behaviour, physiology and development stages of insects, by their influence on the hormonal system, especially in ecdysteroids, leading to growth inhibition, malformations and insect mortality [39].

**Insecticides of Botanical Origin: Plant Extracts Vs. Essential Oils**

Botanical extracts can be prepared by maceration or percolation of fresh green plants or dried powdered plant material (leaves, stems, flowers), using water and/or organic solvents [40]. Different solvents are available to extract the bioactive compounds from natural products, and the selection of a given solvent largely depends on the specific nature of the compound of interest. According to this, the extraction of hydrophilic compounds requires polar solvents such as methanol, ethanol or ethyl-acetate, while the extraction of lipophilic compounds, requires dichloromethane or a mixture of dichloromethane/methanol in ratio of 1: 1 are preferred.

On the other hand, special care must be taken when making interpretations about the bioactivity of botanical extracts. Even with extracts obtained from the same plant, they may not have the same bioactivities if they are prepared from different organs, because they may present different (type or quantity) compounds. For example, *Balanites aegyptiaca* root extracts are more active against *Maconellicoccus hirsutus* than extracts obtained from stems, fruits, flowers and leaves [41]. Also, using different extraction solvents may cause variation in bioactivities because each solvent may extract selectively the different chemical compounds of the plant. Botanical extracts were mostly prepared using water, methanol and ethanol as solvents **(Figure 2)**. Particularly, methanol was highly used to prepare plant extracts against mealybugs, because its effect on mealybugs was higher with this solvent compared to others. Despite water is the universal solvent, resins do not dissolve in water. This may be the reason why Badshah et al. found that *Phenacoccus solenopsis* mortality increased significantly when acetone and n-hexane were used for the preparation of neem seed extracts instead of water. The exact composition of a particular plant extract and its bioactivity is dependent on the plant species, the plant organ and the solvent used for the extraction, as explained before.

Unlike botanical extracts, the essential oils (EOs) are extracted from plants using conventional methods such as Distillation methods such as Steam-Distillation, Hydro-Distillation and Water–Steam-Distillation, and solvent extraction methods [42-44]. Besides, the separation and analysis of these volatile compounds can be performed by gas chromatography–mass spectroscopy, a fast efficient and relatively simple technique is largely used to perform analyses of a mixture of volatile compounds [45,46]. The practicality of EOs extraction and identification methods make EOs easier to be standardized and industrially produced. Nevertheless, even those EOs containing the same active compounds, have considerable variation in mealybug mortality. For example, mealybug mortality values using two products of canola oil Garden Safe Houseplant and Garden Insect Spray and Pyola were 74% and 50%, respectively [47]. The major component of a particular EO does not increase the mortality effectiveness itself, but the result of the synergistic effect of all compounds present in the oil [47,48]. For example, clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum bejolghota*) and lemon grass (*Cymbopogon citratus*) EOs were highly toxic against *P. Jackbeardsleyi* nymphs, presenting mortality values greater than 80% at 3 μL/L of air. Clove EO had the highest fumigant toxicity, followed by cinnamon EO and lemon grass. The major component of clove and cinnamon EOs is eugenol (97% and 82%); while the major component of lemon grass is trans-citral (38%) and cis-citral (32%). Eugenol and citral presented less fumigant toxicity against insects than EO containing eugenol or citral as a main component (such as clove and lemon grass). In other words, eugenol and citral do not increase the mortality effectiveness themselves, but efficacy in mortality occurs as a result of the combination of different chemicals in EO [48].

**Natural Products as Insecticides and Repellents**

Few studies have focused on the effect of organic compounds on the development of mealybug life cycle **(Figure 2)**. Determination of 52% of nymph mortality is the most performed study, possibly because it is the most susceptible stage to the insecticides. The covering of wax that adult females produce makes them difficult to combat because it forms a physical barrier preventing chemical penetration [49]. Studies carried out using nymphal instars could be the most appropriate because they would ensure the mortality of the majority of insect pest population. Wax content can be strongly reduced after treatment with extracts of *B. aegyptiaca* and saponins, which cause an alteration in lipid synthesis. The effectiveness of a product can be increased by adding a low concentration soap solution to the botanical product. Soap facilitates the solubility of the active ingredient, acting as an adherent agent which breaks the protective wax coating, while acting as a surfactant [50-53]. Mealybugs become more sensitive to pesticides and chemical action when their covering lipid content is reduced. The reduction of mealybug lipid/wax content through plant metabolite applications may be one of the potential tools for pest control, something that is difficult to achieve with some synthetic insecticides. Hollingsworth and Tacoli et al. demonstrated that limonene, a major component of *Citrus* EO, is an effective natural alternative to mineral oils that can be used to moisten and kill insects with a waxy coating such as scale insects, mealybugs and white flies. Limonene is often incorporated as an ingredient in cleaning solutions, particularly those that are designed to cut grease or remove wax or oil [54,55]. Due to this excellent property, mealybug control was very effective (close to 100% of mortality). However, limonene was not effective in adult females of *P. ficus* in fumigant test at 600 μL $L^{-1}$ [56].

Hollingsworth found that the addition of Silwet L-77 to the treatment solutions with APSA-80 (emulsifier/surfactant) improved the efficiency against mealybugs without producing phytotoxicity. The addition of this silicone-based nonionic surfactant reduced

e-ISSN:2320-0189
p-ISSN:2347-2308

the size of the air bubbles in the vicinity of individuals that were immersed in the treatment solutions, apparently due to a reduction of the surface tension. Another example of a more effective and between removal of mealybug wax is the addition of sodium lauryl sulfate (surfactant) and anhydrous citric acid (insect dehydrator) to organic products. Both products have the ability to interact synergistically to remove the wax, by means of a mechanism that is not yet understood but could be related to the ionic between of the two compounds [49]. The current challenge is finding organic products with insecticidal action and the property to dissolve the mealybug waxes to improve their effectiveness.

Repellency studies are less abundant, in relation to the studies of insect mortality **(Figure 2)**. Extracts of *A. indica*, *E. globulus*, *Prunus persica* and *Polyalthia longifolia* showed repellence against mealybugs. *Azadirachta indica* extract is globally accepted as a good bioinsecticide containing bioactive alkaloids, azadirachtin and other tetranortriterpenoid compounds responsible for repellency [57-61]. *Eucalyptus globulus* is a good insect repellent, because it contains as main components 1, 8-cineol, α-pinene and p-cymene [62]. Time of exposure to the botanical compounds, is also another factor that influences on their effectiveness. Most of the experiments, evaluated compound activity after 24 hours of application, while very few assessed the activity after more than 30 days of application **(Figure 2)**.

Certain EOs and/or their constituents have a broad spectrum of activity against different pests, such as insects, mites, viruses and plant patogenic fungi and nematodes, however in many cases selectivity is not well documented [63,64]. Despite current information indicates that they are safe for the user and the environment, EOs that are most active against pests are often the most phytotoxic [65]. This fact requires serious attention when formulating products for agricultural and landscape use. In very few cases, the compound phytotoxicity was analysed, considering the importance of innocuous pesticides for crops. Moreover, studies that evaluated the biopesticides phytotoxicity were only qualitative, based on observations of the external symptoms, such as visual effect in leaves. However, in most cases, these organic compounds do not cause visible injury in terms of chlorosis, necrosis, and tissue damage until after several days of treatment [66]. Organic insecticides can have an immediate effect on plant physiology, reducing chlorophyll and water content, decreasing cellular respiration and causing oxidative damage, structural and functional damages to cellular membrane lipids, proteins, enzymes and DNA [67,68]. The mechanism of action of most organic compounds in insects is not well understood. This is one of the reasons why it is very difficult to predict what will happen when testing natural compounds in pests. Given this situation, the studies that are carried out are usually a traditional trial-error procedure. It is therefore necessary to broaden the knowledge about the mechanisms underlying the effectiveness of each compound before performing laboratory tests.

Given that assays are carried out mostly in laboratory conditions and very few are replicated in greenhouse or in the field, generally these studies show little representation of field situations. Mortality and repellency field studies using by EOs and botanical extracts were less abundant because of the additional difficulty of climatic and biotic variables; factors that can be easily controlled in a laboratory. One of the few field experiments was carried out by Prishanthini, Vinobaba, applying a 0.6% solution of *Ocimum sanctum* on plants infested with *P. solenopsis*. Mealybug mortality under field conditions was lower than mortality values under laboratory conditions [32,53]. "probably" because the contact of the insects with the extract was lower. Additional protection of insects is provided by mealybugs habits of hiding and its waxy layer. Many of the foliar applications in general do not establish direct contact with the insect when they are in protected places, lacking effectiveness. In addition, repeated applications of the product are required under field conditions, because organic compounds such as neem show limited persistence in the environment. Residual effects of neem-based products usually last from 5 to 7 days [69,70]. Some of the new compounds currently used in mealybug control have systemic properties, if they are applied through an irrigation system or as a leaf spray [71]. In sustainable crop programs, products derived from neem, light mineral oils, cal-sulfur, citrus products and fatty acid soaps are being used. However, according to bibliographic references, works performed with these compounds have provided ambiguous results. Contact insecticides have little effectiveness for mealybug control due to insect ability to hide in protected locations of plants. Despite this, most experiences that evaluated the effectiveness of organic compounds were carried out by spraying methods and immersion methods, with very few studies assessing other modes of action different to contact, such as systemic and fumigation **(Figure 2)**. It is therefore necessary to carry out further studies about the efficacy of natural products against pests such as mealybugs, including field experiments. Also advances in the screening of high-performance crystallization proteins would allow X-ray crystallography to provide information on the action modes of organic compounds [72].

#### Towards a More Sustainable Grapevine Crop

An urgent strategic approach is needed to reduce the number of synthetic products used, and to achieve the implementation of sustainable practices. Furthermore, current agriculture has to implement environmentally friendly practices, with fewer public health risks. Conventionally produced grapes are usually sold to the local market while organic produced grapes are more easily sold in the international market, as certified organic grapes. This suggests the existence of an interaction between the commercial system and novel pest management strategies. A lot of problems associated with *P. ficus* conventional control methods have highlighted the need of alternative, economical, and reliable biological methods of control.

## DISCUSSION AND CONCLUSION

In this context, the highly bioactive organic compounds derived from plants offer an opportunity for the development of a

e-ISSN:2320-0189
p-ISSN:2347-2308

useful and sustainable strategy to protect vineyards from mealybugs, contributing to the management of organically certified plantations. However, many obstacles still need to be overcome, particularly those related to the mode of action of organic compounds, the methods of application, the cost and effectiveness of field applications, the potential toxicity to non-target organisms, crop phytotoxicity and environmental sanitation, in order to develop a reliable, effective and safe bioinsecticide for extensive application in vineyards [73].

## ACKNOWLEDGEMENTS

This work complies with Argentinean laws. The authors also declare that there is no conflict of interest. Financial support for this work came from the following sources: FONCyT (pict 2012-2146), CONICET (PIP 11220120100661CO) and Universidad Nacional de Córdoba (SECyT).

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