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Bioactivity of a matrine-based biopesticide against four pest species of agricultural importance

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ABSTRACT

The bioactivity of a matrine-based biopesticide was assessed against four pest species (*Diaphorina citri*, *Panonychus citri*, *Sitophilus zeamais* and *Spodoptera frugiperda*) of agricultural importance in different systems of production. For this purpose, we conducted bioassays under laboratory and field (commercial farming) conditions. In our laboratory bioassay, the matrine-based biopesticide caused lethal and sublethal effects on all the studied pest species. However, its acaricidal activity against *P. citri* was between ~10 and 100 times greater than its insecticidal activity (on *D. citri*, *S. zeamais* and *S. frugiperda*). In the tests with seedlings cultivated in vases, the acaricidal activity of this formulation was similar to that of spirodiclofen (the positive control). Despite its high efficacy, the biopesticide showed a short residual effect (~1 day). In a commercial citrus farm in Brazil (with natural infestation of *P. citri* and *Eutetranychus banksi*), this biopesticide formulation (at 150 ppm) showed high efficacy (~90%) in reduces mite populations, with acaricidal activity similar to that of spirodiclofen. This matrine-based biopesticide is a useful component for crop protection where the studied pest species occur, mainly in ecological systems of food production, which are in need of efficient tools for pest management.

1. Introduction

The demand for environmentally suitable products for use in integrated pest management (IPM) programs has grown considerably in recent years. Such demand results from the expansion of ecologically based food production systems (including organic production), which occurred due to the growing consumer awareness and pressure for safer and healthier products (Isman, 2006; Moreno et al., 2012). However, the control of pest arthropods is still one of the main challenges for the feasibility and consolidation of high productivities in these systems (Ribeiro et al., 2012), considering the limitations of both the knowledge of the complex interactions that occur in diversified systems and of efficient management tools in consonance with the precepts and standards of certifying organizations.

In addition to biological control, botanical pesticides may perform an important role in the regulation of the main pest species of different production systems, especially in those where the use of synthetic compounds is not allowed (Zehnder et al., 2007; Bernardi et al., 2013). Considering such potential, several studies have been performed to explore the enormous richness of the tropical and subtropical flora with its complex diversity of secondary metabolites (Stefanazzi et al., 2011; Moreno et al., 2012; Fernandes et al., 2013; Ribeiro et al., 2013). Despite the growing number of studies and patents in this area, a very limited number of biopesticides has been formulated, tested in semi-field and field conditions and released in the market (Isman and Grieneisen, 2014). In Brazil, the small share in the agricultural inputs market (possibly <0.5% of the pesticide market) is attributed to the lack of standardization, the lack of quality control of formulations and, mainly, the difficulty in registering these phytochemicals (Corrêa and Salgado, 2011). The recent promulgation and implementation of a specific legislation for the registration of products for use in organic agriculture (Brasil, 2009) is enabling the registration of new

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biopesticides based on the Brazilian flora as well as the introduction of formulations already commercially available in countries with a longer tradition in this area (India and China). However, before any recommendation, it is necessary to thoroughly evaluate these products on the target species under different conditions (laboratory, semi-field and field) and their interactions in the different trophic levels of the distinct agroecosystems, guaranteeing the sustainability and credibility of the technology.

Recently, a pre-commercial formulation based on extracts from dry roots of *Sophora flavescens* Ait. (Fabaceae), leaves of *Pterocarya stenoptera* C. DC. (Juglandaceae), and essential oils from leaves of *Platycladus orientalis* (L.) Franco (Cupressaceae) was developed in the United States for use in control of pest arthropods. The major bioactive component of this formulation is the alkaloid matrine, one of the main heterocyclic compounds ($C_{15}H_{24}N_2O$) derived from quinolizidine, which is abundantly found in roots of some species from the genus *Sophora* (Fabaceae) (Mao and Henderson, 2007), one of the most used derivatives in traditional Chinese medicine (Zhang et al., 2012). This alkaloid possesses reported activity on ecto- and endoparasites of animals (Wang et al., 2001). More recently, matrine has been used in isolation or in mixtures with other botanical extracts and synthetic pesticides for the control of termites, aphids, leafhoppers, caterpillars and mites, fungal and bacterial diseases and nematodes in areas of production of vegetables, fruits, flowers and teas in China (Yang and Zhao, 2006; Mao and Henderson, 2007; Wang et al., 2007; Marčić et al., 2012) as well as in the management of pests of stored grains (Liu et al., 2007). In addition to the acute toxicity (upon residual contact) on different pest species of agricultural importance for the cultivation systems of eastern countries, Mao and Henderson (2007) and Bakr et al. (2012) demonstrated that the alkaloid matrine promotes an antifeedant effect against the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) and the twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). In another study, Hwang et al. (2009) demonstrated the efficacy of a formulation (KNI3126) composed of a mixture of matrine and neem oil on *Nilaparvata lugens* Stal (Hemiptera: Delphacidae), *Aphis gossypii* Glover (Hemiptera: Aphididae), *Thrips palmi* Karny (Thysanoptera: Thripidae) and *T. urticae*, corroborating the insecticidal and acaricidal potential of this natural compound and its spectrum of biological action on arthropods with different feeding habits.

The aim of this study was to evaluate, under laboratory conditions, the lethal and sublethal effects of a matrine-based formulation on four species of pest arthropods with distinct feeding habits and of importance in different agricultural systems: Asian citrus psyllid [*Diaphorina citri* Kuwayama (Hemiptera: Liviidae)], citrus red mite [*Panonychus citri* (McGregor) (Acari: Tetranychidae)], maize weevil [*Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)] and fall armyworm [*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)]. Considering the results obtained in the laboratory, the efficacy of the product was also evaluated in field (commercial farming) conditions.

2. Materials and methods

2.1. Insects and mites

Insects and mites originating from populations maintained under controlled laboratory conditions [25 ± 2 °C, relative humidity (RH) 60 ± 10% and photoperiod of 14 L: 10 D h] were used in the bioassays. Seedlings of orange jasmine [*Murraya paniculata* (L.) Jack (Rutaceae)] cultivated in pots (2 L) were initially pruned to a height of 25 cm and after producing sprouts (2–3 cm in length), they were placed in rearing cages (60 × 60 × 50 cm length, width, and height, respectively) and used as substrate for *D. citri* feeding and oviposition, according to the method proposed by Nava et al. (2007). The population of *P. citri* was maintained on leaves of sweet orange [*Citrus sinensis* (L.) Osbeck (Rutaceae)] cv. Valencia placed on a foam layer moistened with distilled water. The leaves were surrounded with moistened strips of absorbent cotton to prevent the escape of mites and to maintain leaf turgor as described by Ribeiro et al. (2014). The rearing of *S. zeamais* was performed in glass jars (5 L) containing wheat grain [*Triticum aestivum* L. (Poaceae)] according to the procedure described by Ribeiro (2010). Additionally, *S. frugiperda* was kept as the artificial diet proposed by Kasten-Junior et al. (1978) during its larval phase, whereas a solution of honey 10% was used to feed the adults.

2.2. Biopesticide characterization

The biopesticide is available in an emulsifiable concentrate (EC) formulation at the total concentration of 3% (w/v), including 0.36% (w/v) of matrine (main bioactive component obtained of dry roots of *S. flavescens*), 0.02% (w/v) of juglone (5-hydroxy-1,4-naphthoquinone) (main component obtained of leaves of *P. stenoptera*) and 0.20% (v/v) of α-pinene (main component of essential oils of the leaves of *P. orientalis*).

2.3. Bioassays

2.3.1. Laboratory tests

For each of the target species, the biopesticide was tested in different concentrations (6 or 7) established according to preliminary tests and following the procedure described by Finney (1971). The bioassays were performed in a climatized room at a temperature of 25 ± 2 °C, 60 ± 10% RH and photoperiod of 14 L: 10 D h.

2.3.1.1. Diaphorina citri. The insecticidal and antifeedant effects of the matrine-based biopesticide were evaluated on adults of *D. citri* using different bioassays:

2.3.1.1.1. Evaluation of the lethal effect. Seedlings of Rangpur lime [hybrid of *Citrus reticulata* Blanco × *C. sinensis* (Rutaceae)], cultivated in tubes, were previously pruned and used in the bioassay as experimental units. After buds had formed (2–3 cm in length), the seedlings were sprayed with 2 mL of solution, concentrated at different treatment levels, with the aid of a microatomizer coupled to a pneumatic pump, adjusted to provide a pressure of 0.7 kg cm^{-2}. The product concentrations tested were 625, 1250, 2500, 5000, 7079 and 10000 ppm (μL of formulation per L of solution), defined according to previous tests. Deionized water, the solvent used to solubilize the product, was used as a control.

After the application of treatments, the seedlings were maintained in a climatized room (25 ± 2 °C and 60 ± 10% RH) for 2 h to allow the residues to dry. Later, the seedlings were placed in cages (experimental units) prepared from transparent "PET" (polyethylene terephthalate) bottles (2 L) with a frontal opening of approximately 100 cm^2, covered with voile-type fabric to allow gas exchange and avoid excess humidity. These cages were attached over plastic jar with a Styrofoam disk with a central orifice of 3 cm diameter in their upper rim. These jars contained water (200 mL) to maintain the turgor of the plants. In each cage, 10 *D. citri* adults of ages varying from five to eight days were released, with five replicates per treatment level ($n = 50$).

Insect mortality was evaluated every 24 h for a period of 5 days. The insects that did not react to the touch of a fine brush were considered dead. Considering the data obtained the concentrations necessary to kill 50 and 90% of the population, the LC_{50} and LC_{90}, respectively, were estimated in the different exposure times.