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Saponaria officinalis-synthesized silver nanocrystals as effective biopesticides and oviposition inhibitors against *Tetranychus urticae* Koch

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ABSTRACT

Green-fabricated nanoparticles have been mainly tested on mosquito and tick vectors, while no information are available about their toxicity against phytophagous mites. Therefore, here it was determined whether Ag nanoparticles with acaricidal activity could be synthesized using the *Saponaria officinalis* root extract. Size, shape and crystalline structure of the nanoparticles were described. Furthermore, the toxicity of *S. officinalis* extract vs. *S. officinalis*-fabricated Ag nanoparticles was studied, comparing their activity on eggs, larvae and adults of two-spotted spider mite *Tetranychus urticae*. The impact of both treatments on *T. urticae* oviposition was investigated. Both the *S. officinalis* root extract and the nanoparticles showed a very good acaricidal efficacy. Ag nanoparticle LC_{50} was 1.2 g L^{-1} (LC_{90} = 2.8 g L^{-1}), significantly less if compared with the root extract alone ($LC_{50(90)}$ = 7.8 (11.9) g L^{-1}). Adults of *T. urticae* showed the lowest sensitivity, with LC_{50} of 6.1 and 19.9 g L^{-1} for nanoparticles and the aqueous root extract, respectively. Both treatments showed high ovicidal toxicity with LC_{50} of 3.1 and 13.8 g L^{-1} for the nanoparticles and aqueous root extract, respectively. Treatment spray residues also caused significant inhibition of oviposition in females of *T. urticae* with EC_{50} estimated as 1.4 g L^{-1}, a value significantly lower, if compared with the extract alone (EC_{50} = 6.1 g L^{-1}). No phytotoxicity of both treatments was observed in short-term tests. *S. officinalis* root aqueous extract is used by food and cosmetic industries, thus it can be considered a safe option for plant protection. In addition, the fabricated AgNP also seem highly promising as they showed high biological efficacy, and the production method is relatively simple and cheap.

1. Introduction

The frequent use of pesticides in plant protection has led to a number of major problems. Besides health-related and environmental risks associated with application of some pesticides (Karabelas et al., 2009; Fantke et al., 2012), a significant issue is represented by the development of resistant populations of harmful organisms including the two-spotted spider mite, *Tetranychus urticae* Koch (Arachnida: Acari: Tetranychidae), which has been known for its ability to rapidly develop resistance to chemical pesticides (Van Leeuwen et al., 2010). Resistance selection is accelerated by its high fecundity, inbreeding, arrhenotokous reproduction and short life cycle, resulting in many generations per year, especially in warmer conditions (Van Leeuwen et al., 2015). This species is currently considered as one of the 'most resistant' arthropods, in terms of the total number of pesticides to which populations have become resistant (Whalon et al., 2008).

Notably, *T. urticae* is a phytophagous pest that can cause significant yield losses in many agricultural crops, including vegetables, fruits, cotton and ornamentals plants (Cazaux et al., 2014; Van Leeuwen et al., 2015). To date, 1127 host species have been reported around the world in both outdoor crops and greenhouses (Migeon et al., 2011). In addition, computer modelling suggests that thanks to the intensifying global warming, the noxiousness of *T. urticae* in agriculture will markedly increase due to accelerated development at high temperatures (Van Leeuwen et al., 2010).

The above-mentioned problems are the main reasons of the current intensive efforts to seek new, suitable alternatives for plant protection with minimum negative impacts on non-target organisms and human health (Benelli and Pavela, 2016a; Benelli et al., 2016; Benelli and Mehlhorn, 2016). Promising plant protection alternatives also include the use of plant secondary metabo-

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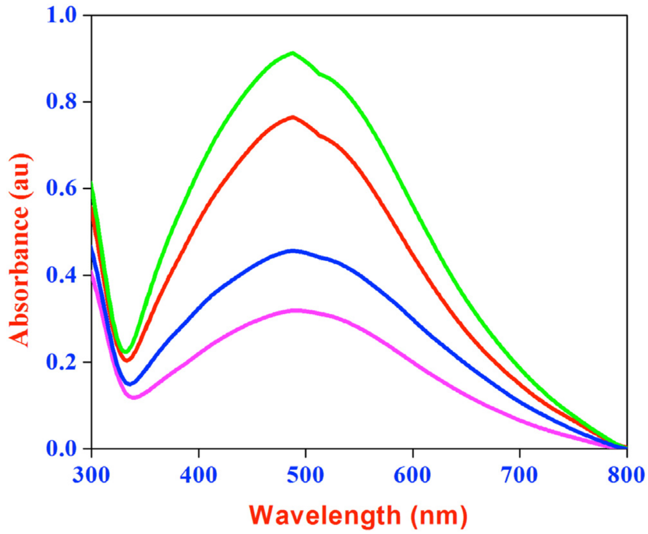

Fig. 1. *Saponaria officinalis*-synthesized Ag nanoparticles: UV–vis spectroscopy after 30, 60, 120 and 180 min from reaction (purple, blue, red and green spectrum, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lites synthesized by some plants within the framework of their natural defensive capacity against pathogens and pests (Rattan, 2010). As shown in many studies, plant secondary metabolites can exhibit significant toxic effects against arthropod pests (Isman and Grieneisen, 2014; Pavela, 2014a,b; Benelli, 2015a,b; Isman, 2015; Pavela, 2015a,b), including acaricidal effects (Attia et al., 2015; Benelli and Pavela, 2016b; Pavela, 2015c, 2016a,b). The history of using plant extracts in the protection against pests dates back to the ancient times and this tradition has been preserved until nowadays, although only to a limited extent (Pavela, 2016b). Generally, these substances are obtained from plant materials using suitable isolation methods and subsequently they are used as active ingredients in the so-called "botanical insecticides" (BIs) (Isman and Grieneisen, 2014; Pavela, 2016b; Pavela and Benelli, 2016a).

Recently, intensive research on plant extract bioactivity has resulted in the discoveries of new toic substances, which can be considered as suitable for the development of new botanical pesticides including acaricides (Bakkali et al., 2008; Isman, 2015; Pavela et al., 2016a,b). BIs are generally considered as safe for the health and the environment (Isman, 2015; Benelli and Govindarajan, 2016). In addition, given that they contain complex mixtures of active compounds often with synergistic effects (Pavela, 2014b, 2015b; Benelli et al., 2017) and different mechanisms of action (Rattan, 2010; Pavela, 2016b), it can be expected that no resistant populations of pests would develop (Pavela and Benelli, 2016a,b).

However, despite many positive properties of BIs, these products also show some negative characteristics that often prevent their prompt commercial use. Such characteristics include the need of relatively high concentrations or doses of BIs in order to achieve the required efficacy. Moreover, due to rapid biodegradation of the active compounds, most BIs show a short duration of persistence of their effect, meaning that their application must be repeated, which increases the costs for the grower (Pavela and Benelli, 2016a).

Currently, it is important to seek new active compounds with novel multiple mechanisms of action, as well as to use new technologies to increase their biological efficacy and improve their yields or extend their duration of persistence (Pavela, 2016b). For these reasons, this research is focused on the acaricidal potential of metal nanoparticles synthesized using a cheap extract obtained from the roots of soapwort, *Saponaria officinalis* L. (Caryophyllaceae). Thanks to their chemical and physical properties, soapwort extracts have been used as emulsifiers and softening agents in food industry, particularly in the production of "halva" and other sweets. Sunflower "halva" is a popular confectionery product specific to the countries of Eastern Europe (Bedigian, 2004; Korkmaz and Özçelik, 2011; Mureşan et al., 2013). In addition, extracts from *S. officinalis* have been used in traditional medicine and in the cosmetics industry as diaphoretic, antioxidant and tonic agents. They have been traditionally used for the treatment of rheumatic diseases and syphilis, and for jaundice and engorgement of the abdominal viscera (CAS No. 84775-97-3) (Kucukkurt et al., 2011).

From a pest management perspective, in a previous research good acaricidal effect of aqueous extracts obtained from the roots of *S. officinalis* was reported (Pavela, 2016a). However, as far as we know, no information has been available about lethal concentrations for individual developmental stages of *T. urticae* and no possibilities of enhancing acaricidal efficacy of the extract using the AgNP synthesis have been studied.

Here a modern and inexpensive technology was used to synthesize silver nanoparticles. The efficacy of extracts obtained using standard methods was compared with that of AgNP, specifically their biological efficacy against two-spotted spider mites, *T. urticae*. Indeed, nanotechnology has the potential to revolutionize a wide array of applications in the fields of biomedicine, pest management and parasitology (Benelli, 2016a,b). Green biosynthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, and easy to exploit for large-scale nano-synthesis (Marimuthu et al., 2011; Govindarajan et al., 2016).

To the best of our knowledge, the possibility of using AgNP plant extracts in the protection against phytophagous mites has not been studied. Until now, this technology has been studied especially in the research of larvicides with potential use in the protection against mosquito vectors or was mainly focused on ticks of veterinary importance (Marimuthu et al., 2011; Benelli, 2016b), while no information is available about the toxicity of green-fabricated nanoparticles towards phytophagous mites.

Therefore, the aim of this study is therefore to determine whether AgNP with acaricidal activity can be synthesized using *S. officinalis* extract, to describe the size, shape and crystalline structure of the biosynthesized nanoparticles, and to evaluate the efficacy of *S. officinalis* root extract vs. *S. officinalis*-fabricated Ag nanoparticles, comparing their toxic action on eggs, larvae and adults of two-spotted spider mites, *T. urticae* at the same time, their effects on *T. urticae* oviposition activity was studied.

2. Materials and methods

2.1. Preparation of S. officinalis extract and green synthesis of Ag nanoparticles

Commercially sold soapwort (*S. officinalis*) roots were obtained from Byliny Mikes (Czech Republic), a company engaged in the sale of medicinal plants. Roots were obtained from two-year old plants harvested in November 2015, which were adapted after the harvest using standard methods according to European Pharmacopoeia (Wichtl, 2004), i.e., they were dried and ground to pieces approximately 0.5 cm long.

Saponaria officinalis root aqueous extract was prepared mixing 100 g of *S. officinalis* roots with 1 L of tap water. Roots were macerated for 24 h at ambient temperature (21 ± 1 °C). Subsequently, the extract was filtered using filter paper (Whatman no. 1) and the filtrate was stored in darkness at 7 °C temperature until testing. A part of the obtained aqueous extract was used for the green synthesis of Ag nanoparticles. The extract was treated with aqueous $AgNO_3$ 1 mM (21.2 mg of $AgNO_3$ in 125 mL of Milli-Q water) in an Erlenmeyer flask and incubated at room temperature.