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 1.2g.L^{-1} (LC_{90}=2.8g.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 19.9g.L^{-1} for nanoparticles and the aqueous root extract, respectively. Both treatments showed high ovicidal toxicity with LC_{50} of 3.1 and 13.8g.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 1.4g.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.

Keywords: soapwort; acaricidal activity; botanical insecticides; plant extracts; nanotechnology

47 **1. Introduction**

48

49 The frequent use of pesticides in plant protection has led to a number of major
50 problems. Besides health-related and environmental risks associated with application of
51 some pesticides (Karabelas et al., 2009; Fantke et al., 2012), a significant issue is
52 represented by the development of resistant populations of harmful organisms including
53 the two-spotted spider mite, *Tetranychus urticae* Koch (Arachnida: Acari:
54 Tetranychidae), which has been known for its ability to rapidly develop resistance to
55 chemical pesticides (Van Leeuwen et al., 2010). Resistance selection is accelerated by
56 its high fecundity, inbreeding, arrhenotokous reproduction and short life cycle, resulting
57 in many generations per year, especially in warmer conditions (Van Leeuwen et al.,
58 2015). This species is currently considered as one of the 'most resistant' arthropods, in
59 terms of the total number of pesticides to which populations have become resistant.
60 Notably, *T. urticae* is a phytophagous pest that can cause significant yield losses
61 in many agricultural crops, including vegetables, fruits, cotton and ornamentals plants
62 (Cazaux et al., 2014; Van Leeuwen et al., 2015). To date, 1,127 host species have been
63 reported around the world in both outdoor crops and greenhouses (Migeon et al., 2011).
64 In addition, computer modelling suggests that thanks to the intensifying global
65 warming, the noxiousness of *T. urticae* in agriculture will markedly increase due to
66 accelerated development at high temperatures (Van Leeuwen et al., 2010).
67 The above-mentioned problems are the main reasons of the current intensive
68 efforts to seek new, suitable alternatives for plant protection with minimum negative
69 impacts on non-target organisms and human health (Benelli and Pavela, 2016a; Benelli
70 et al. 2016). Promising plant protection alternatives also include the use of plant

71 secondary metabolites synthesized by some plants within the framework of their natural
72 defensive capacity against pathogens and pests (Rattan, 2010). As shown in many
73 studies, plant secondary metabolites can exhibit significant toxic effects against
74 arthropod pests (Isman and Grieneisen, 2014; Pavela, 2014a,b; Benelli, 2015a,b; Isman,
75 2015; Pavela, 2015a,b), including acaricidal effects (Attia et al., 2015; Benelli and
76 Pavela, 2016b; Pavela, 2015c, 2016 a,b). The history of using plant extracts in the
77 protection against pests dates back to the ancient times and this tradition has been
78 preserved until nowadays, although only to a limited extent (Pavela, 2016b). Generally,
79 these substances are obtained from plant materials using suitable isolation methods and
80 subsequently they are used as active ingredients in the so-called "botanical insecticides"
81 (BIs) (Isman and Grieneisen, 2014; Pavela, 2016b; Pavela and Benelli, 2016a).
82 Recently, intensive research on plant extract bioactivity has resulted in the
83 discoveries of new toic substances, which can be considered as suitable for the
84 development of new botanical pesticides including acaricides (Bakkali et al., 2008;
85 Isman, 2015). BIs are generally considered as safe for the health and the environment
86 (Isman, 2015; Benelli and Govindarajan, 2016). In addition, given that they contain
87 complex mixtures of active compounds often with synergistic effects (Pavela, 2014b,
88 2015b; Benelli et al., 2017) and different mechanisms of action (Rattan, 2010; Pavela,
89 2016b), it can be expected that no resistant populations of pests would develop (Pavela
90 and Benelli, 2016a,b).
91 However, despite many positive properties of BIs, these products also show
92 some negative characteristics that often prevent their prompt commercial use. Such
93 characteristics include the need of relatively high concentrations or doses of BIs in order
94 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

142

143 Commercially sold soapwort (*S. officinalis*) roots were obtained from Byliny
144 Mikes (Czech Republic), a company engaged in the sale of medicinal plants. Roots
145 were obtained from two-year old plants harvested in November 2015, which were
146 adapted after the harvest using standard methods according to European Pharmacopoeia
147 (Wichtl, 2004), i.e., they were dried and ground to pieces approximately 0.5cm long.
148 *Saponaria officinalis* root aqueous extract was prepared mixing 100 g of *S.*
149 *officinalis* roots with 1 L of tap water. Roots were macerated for 24h at ambient
150 temperature (21±1°C). Subsequently, the extract was filtered using filter paper
151 (Whatman no. 1) and the filtrate was stored in darkness at 7°C temperature until testing.
152 A part of the obtained aqueous extract was used for the green synthesis of Ag
153 nanoparticles. The extract was treated with aqueous $AgNO_3$ 1mM (21.2mg of $AgNO_3$ in
154 125mL of Milli-Q water) in an Erlenmeyer flask and incubated at room temperature.
155 Eighty-eight millilitres of an aqueous solution of $AgNO_3$ 1mM was reduced using 12
156 mL of *S. officinalis* root extract at room temperature for 10 min, resulting in a brown-
157 yellow solution indicating the formation of Ag nanoparticles. The Ag nanoparticle
158 suspension was stored in darkness at 7°C temperature until the experiments.
159 Furthermore, the *S. officinalis*-fabricated Ag nanoparticles were characterized following
160 the method recently described by Rajaganesh et al., (2016), including UV-vis
161 spectroscopy (UV-160v, Shimadzu, Japan), FTIR spectroscopy (Perkin-Elmer Spectrum
162 2000 FTIR spectrophotometer.) ESEM (Environmental Electron Scanning Microscope
163 FEI Quanta 200), EDAX, and XRD analyses (Murugan et al., 2016a,b).
164

165 2.2. Mite rearing

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167 *Tetranychus urticae* mites were obtained from the cultures maintained at the
168 Crop Research Institute (Czech Republic). They were reared on bean plants (*Phaseolus*
169 *vulgaris* L.) in a growth chamber (22-25°C; 80%R.H., 16:8h L:D).

170

171 2.3. Bioassays

172

173 Before establishing the assays, the contents of substances dissolved in water
174 were determined. One mL of the extract was removed using a pipette; this amount was
175 subsequently dried for 12h at 80±1°C. The residue was weighed, and the mass was used
176 to calculate the percentage of substances dissolved in the extract, expressed in the text
177 as % of the extract dry mass weight in the volume of water ($g.L^{1}$). The entire
178 experiment was repeated 5 times.

179 The obtained aqueous extract was found to contain, on average, 61.7$g.L^{-1}$ and
180 the Ag nanoparticle suspension, on average, 7.7$g.L^{-1}$ of dissolved substances. Based on
181 the determined contents of the dissolved substances, the extracts were diluted with
182 water in a way to obtain the following concentration series: for the root aqueous extract
183 30.0, 20.0; 15.0, 10.0; 5.0, 2.0, 1.0 and 0.5$g.L^{-1}$ and for the Ag nanoparticles 7.7, 5.0,
184 2.0, 1.0 and 0.5$g.L^{-1}$ of dissolved substances. Then, prepared extracts were immediately
185 used in bioassays.

186 In order to determine acute toxicity, individual concentrations of products were
187 applied to bean plants (*P. vulgaris*) with a defined number of adults, nymphs or eggs.
188 The bean plants were adapted in such a way that they had only one fully developed leaf.
189 20 adults (age: 3-7 days) were introduced onto every leaf 12h before application using a

fine brush, and the number of living adults was ascertained again immediately before application. Eggs or nymphs were prepared as follows: ten females were allowed to lay eggs on every bean leaf for 12h. Subsequently, the females were removed, and the eggs were left for 3 days at 21±2°C. Application then followed. Alternatively, the eggs were left to develop naturally until the birth of the nymphs. The nymphs were left on the plants to develop for an additional five days, and then the plants with a defined nymph count were treated using the prepared extracts.

Treatments were applied to the plants using a manual electronic atomizer in a dose approximately equivalent to the application of 600L of water per hectare. Control plants were treated using only water. The experiment was repeated five times.
The plants were placed in a growth chamber (L16:D8, 25.0±1.0°C). The numbers of adults and nymphs on the plants were determined using a microscope at 48 h after application. The eggs were left to develop until the birth of the nymphs (for approximately 10 days); those eggs from which no nymphs had hatched were considered dead.

Following Pavela et al. (2015c), the effect of the treatments on the inhibition of oviposition was determined. Five mite females (3-4 days old) were transferred using a fine brush onto each of the cut bean leaf discs sized 1 cm^{-2}. The leaf discs were obtained from those bean leaves that had been treated identically as described above and after drying of the spray, using a cork borer. The cut discs with the females were placed in Petri dishes with an agar bottom. Females were removed after 48 h and the laid eggs were counted. Subsequently, the number of eggs was determined for individual concentrations, and the lethal concentration causing oviposition inhibition by 50% or

90% compared with the control was estimated using Probit analysis. Petri dishes were placed in a growth chamber (L16:D8, 25°C). The experiment was repeated five times.

2.4. Data analysis

Experimental tests demonstrated that more than 20% of the controlled mortality was discharged and repeated. When the controlled mortality reached 1–20%, the observed mortality was corrected by Abbott (1925). Probit analysis of mortality and antioviposition data was conducted to estimate the LC_{50} and LC_{90} values and related 95% confidence limits for each tested product (Finney, 1971; Benelli, 2017).

3. Results

3.1. Characterization of Ag nanoparticles

The concentrated aqueous extract contained, on average, 61.7g.L^{-1} of dissolved substances. It had a light yellow colour. On the contrary, the synthesized Ag nanoparticles extract contained 7.7g.L^{-1} of dissolved substances and had a brown-yellow colour. Figure 1 showed the UV-vis spectra of the *S. officinalis* extract reacting with silver nitrate 1mM over time. A main absorbance peak at 490nm is clearly visible after 120 and 180 min from the reaction. ESEM showed the effective synthesis of Ag nanoparticles, which are mostly spherical or cubical, with a mean size ranging from 10 to 20nm (Figure 2a). EDAX confirmed the presence of metallic Ag in the tested nanocomposite (Figure 2b). FTIR spectroscopy showed peaks at 428, 771, 1068, 1645,

237 2357, 2931 and 3807cm^{-1} (Figure 3). Results from XRD analysis (Figure 4) showed the
238 Bragg's reflections corresponding to (200), (202), (311) and (402) planes, which
239 characterizes the face-centred-cubic structure of Ag nanoparticles.
240
241 3.2. Efficacy of *S. officinalis* root extract and Ag nanoparticles on mites
242
243 Both the *S. officinalis* extract and Ag nanoparticles showed a very good
244 acaricidal efficacy. However, a significant difference was observed both in efficacies on
245 individual development instars of *T. urticae*, and between the tested products. Estimated
246 lethal concentrations for acute toxicity for adults, nymphs and eggs are shown in Table
247 1. As a general trend, the highest sensitivity was seen in nymphs. However, Ag
248 nanoparticles were significantly effective, with LC_{50} estimated as 1.2g.L^{-1} (LC_{90}=2.8
249 g.L^{-1}) , i.e. significantly less compared with the extract alone ($LC_{50(90)}$=7.8 (11.9)g.L^{-1}).
250 The adults of *T. urticae* showed the lowest sensitivity, with LC_{50} estimated as 6.1 and
251 19.9g.L^{-1} for Ag nanoparticles and for the aqueous extract, respectively. Both treatments
252 also showed ovicidal activity, with LC_{50} estimated as 3.1 and 13.8g.L^{-1} for the AgNP
253 and aqueous extract, respectively.
254 Extract spray residues also caused significant inhibition of oviposition in
255 females of *T. urticae* (Table 2). AgNP showed a significantly higher inhibition of
256 oviposition, with the effective concentration $EC_{50\,(90)}$ estimated as 1.4 (8.6)g.L^{-1}, i.e. a
257 concentration significantly lower compared with the extract alone (EC_{50}=6.1 and
258 EC_{90}=30.1g.L^{-1}). No phytotoxicity of both treatments was observed in short-term tests.
259
260 **4. Discussion**

Results showed that the use of extracts obtained by simple maceration of *S. officinalis* roots in water is very promising in plant protection against phytophagous mites such as *T. urticae*. Nymphs were the most sensitive instar to the root aqueous extract, with LC_{90} estimated as 11.9g.L^{-1}, and the lowest sensitivity was shown by adults (LC_{90}=36.1g.L^{-1}). Also, Ag nanoparticle synthesis could be easily achieved, and this boosted the acaricidal effect. For example, for the most sensitive developmental stage (nymphs), LC_{50} was reduced more than 6 times and LC_{90} more than 4 times for the Ag nanoparticles compared with the aqueous extract alone.

The extract of *S. officinalis* also caused significant inhibition of oviposition in the females of *T. urticae*. However, the synthesized Ag nanoparticles showed a significantly higher efficacy. Comparing the EC_{90} values, the effective concentration of 30.1g.L^{-1} was estimated for the aqueous extract, while it was only 8.6g.L^{-1} for the AgNP extract, i.e. 3.5 times less. Similarly, a significant increase of larvicidal efficacy was previously demonstrated in several researches focusing on larvae of important mosquito vectors, including *Anopheles stephensi, An. subpictus, Aedes aegypti, Ae. albopictus* and *Culex quinquefasciatus* (Benelli, 2016a; Govindarajan et al., 2016; Murugan et al., 2016a,b), even if extremely scarce information are available about the extract mechanisms of toxicity of Ag nanoparticles against mosquito larvae, as well as other arthropods of economic importance. Nanoparticle toxicity on different arthropod species may be related to the ability of nanoparticles to penetrate through the exoskeleton. In the intracellular space, nanoparticles can bind to sulphur from proteins or to phosphorus from DNA, leading to the rapid denaturation of organelles and enzymes. Therefore, a decrease in membrane permeability and disturbance in proton motive force may led to

285 the loss of cellular function and cell death (Benelli 2016b). Further research on the
286 possible mechanisms at the basis of differential toxicity when testing the *S. officinalis*
287 root extract and nanoparticles on the different mite instars is needed.
288 Notably, the extracts from the roots of *S. officinalis* contain especially saponins.
289 Our FTIR results showed peaks related to saponin absorptions of OH, C = O, C-H, and
290 C = C (Almutairi and Ali, 2015), indicating that these molecules may play a role as
291 reducing and stabilizing agents for the fabrication of crystalline Ag nanoparticles with
292 mean size of 10-20nm (Govindarjan et al.2016; Rajaganesh et al., 2016). Saponins
293 contained in the *S. officinalis* roots are employed for a wide array of applications in
294 food industry (Bedigian, 2004; Korkmaz and Özçelik, 2011; Mureşan et al., 2013) and
295 cosmetics (Kucukkurt et al., 2011). In addition, the effects of triterpene glycosides
296 (saponins), recently extracted from *S. officinalis* radices, on the cellular and humoral
297 innate immunity factors were studied (Kuznetsova et al., 2014). Tests showed a positive
298 impact on natural immunity given that triterpene glycosides stimulated the phagocytic,
299 bactericidal, and adhesion activities of polymorphonuclear leukocytes (Kuznetsova et
300 al., 2014). Saponins promoted the maturation of human peripheral blood dendritic cells,
301 which was proven by a high expression of the terminal differentiation marker and bone-
302 stimulating molecule on the cell membrane. Moreover, no acute or chronic toxicity, or
303 growth and tissue abnormalities, were found for the extract from the roots of *S.*
304 *officinalis* administered orally to mice (Yudina et al., 2007). It was also found that
305 plants rich in triterpenoid saponins are a diet-dependent potential factor that has an
306 important role in modulation of rumen fermentation processes (Szczechowiak et al.,
307 2013). These findings provide evidence of the health safety of *S. officinalis* extracts,

308 including their potential residues, which may occur on vegetables treated using this
309 botanical pesticide.
310
311 **5. Conclusions**
312
313 Nanotechnology has the potential for applications in many areas of human
314 activity (Govindarajan et al., 2016; Murugan et al., 2016a,b) including plant protection
315 as shown in this study. Green nanosynthesis provides advancement over chemical and
316 physical method, as it is cost-effective, environment friendly, and easily scaled up for
317 large-scale synthesis (Govindarajan et al. 2016; Benelli, 2016). To conclude, it can be
318 noted that Ag nanoparticles were prepared employing the root extract of *S. officinalis* At
319 the same time, it was showed that this synthesis can be used to boost the acaricidal and
320 antiovipositional activity of the saponin complex contained in the extract against all
321 developmental stages of an important polyphagous pest, *T. urticae*. Given that the
322 aqueous extract alone from the roots of *S. officinalis* has been used in the food and
323 cosmetic industries, it can be considered as safe for application in plant protection. In a
324 nutshell, Ag nanoparticles synthesized here are highly promising, as they showed high
325 biological efficacy and, at the same time, the production method is relatively simple and
326 inexpensive.
327 However, it is clear to us that the development of this young field of science has
328 only begun, at least regarding applications of synthesized Ag nanoparticles in plant
329 protection. Further tests will thus be needed including toxicological tests or the study of
330 Ag nanoparticle effects on phytotoxicity of the treated plants, which would demonstrate

the safety of the newly synthesized Ag nanoparticles in Integrated Pest Management strategies.

Acknowledgements

Financial support for this work has been provided by the Technology Agency of the CR (Project no. TA04020103). G. Benelli is sponsored by PRAF 2012-2015 PROAPI.

Conflicts of interest

The authors declare no conflicts of interest.

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Figure 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).

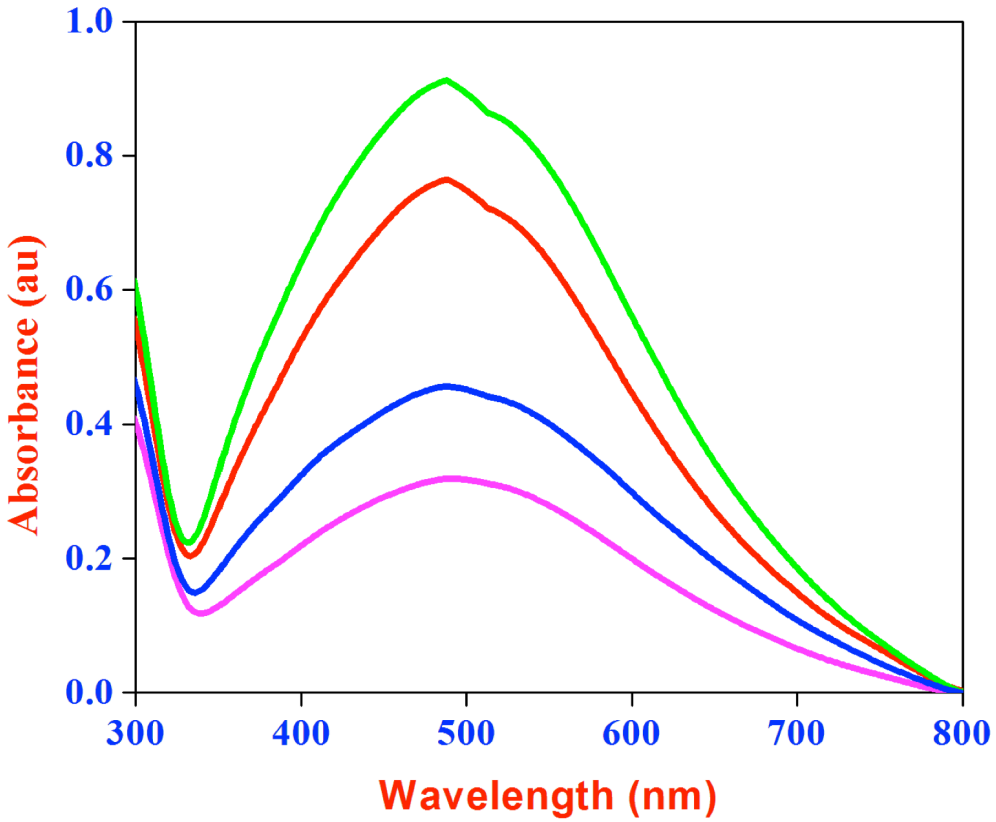
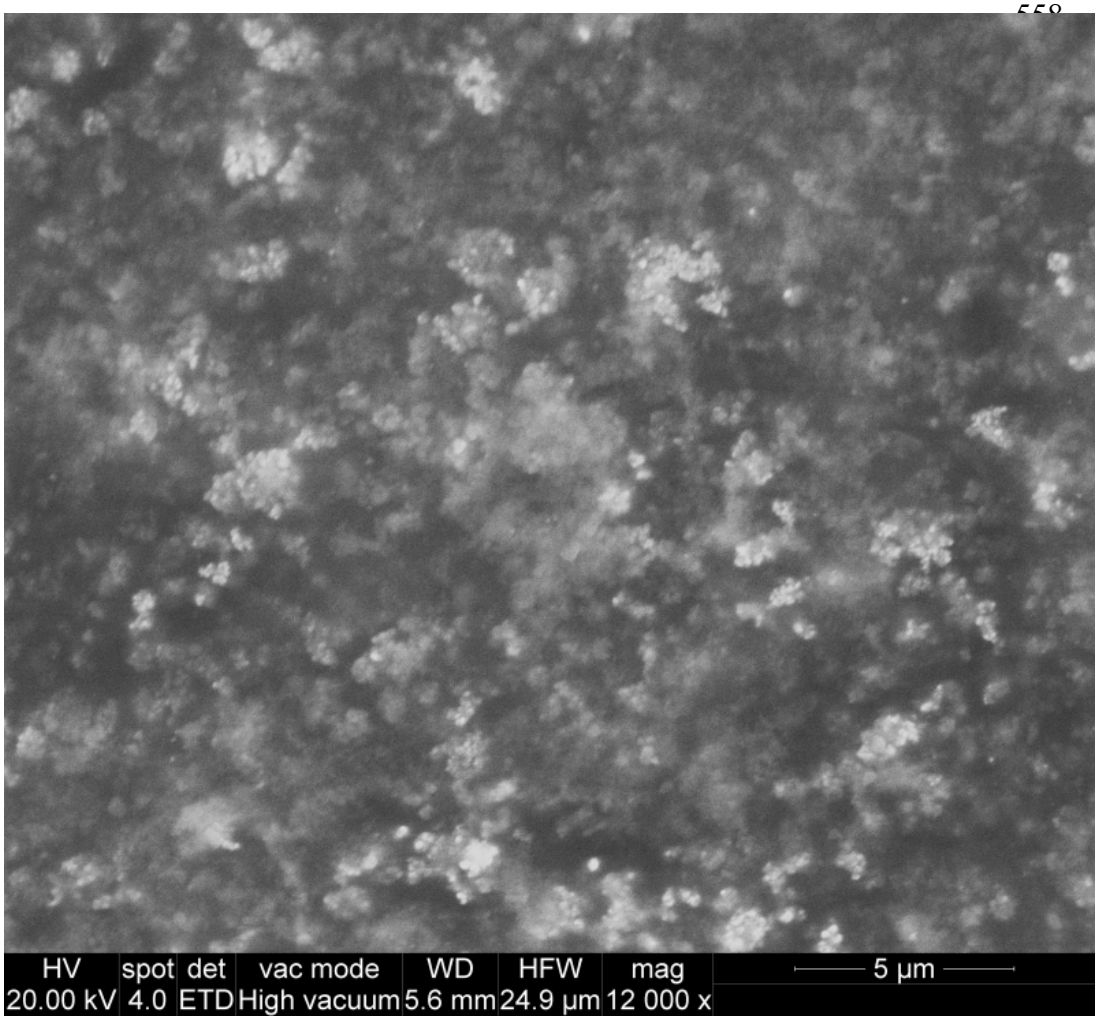


Figure 2. (a) ESEM of Ag nanoparticles biosynthesized using the *Saponaria officinalis* aqueous extract, and **(b)** EDAX spectrum showing the chemical composition of nanoparticles (Ag 44.49%, O 55.51%).

(a)



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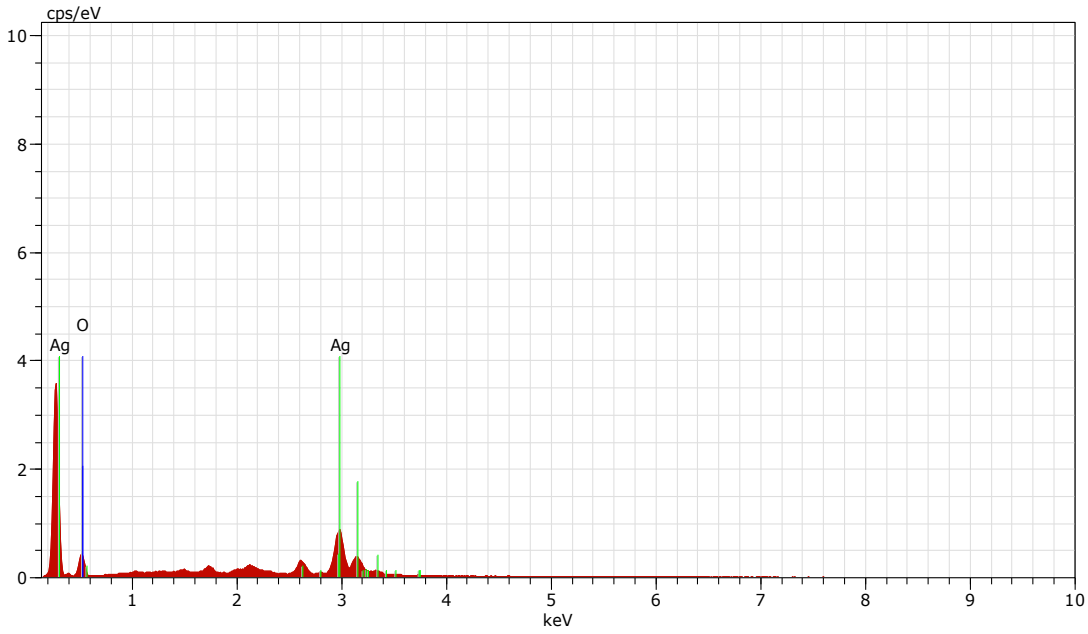
575 **(b)**



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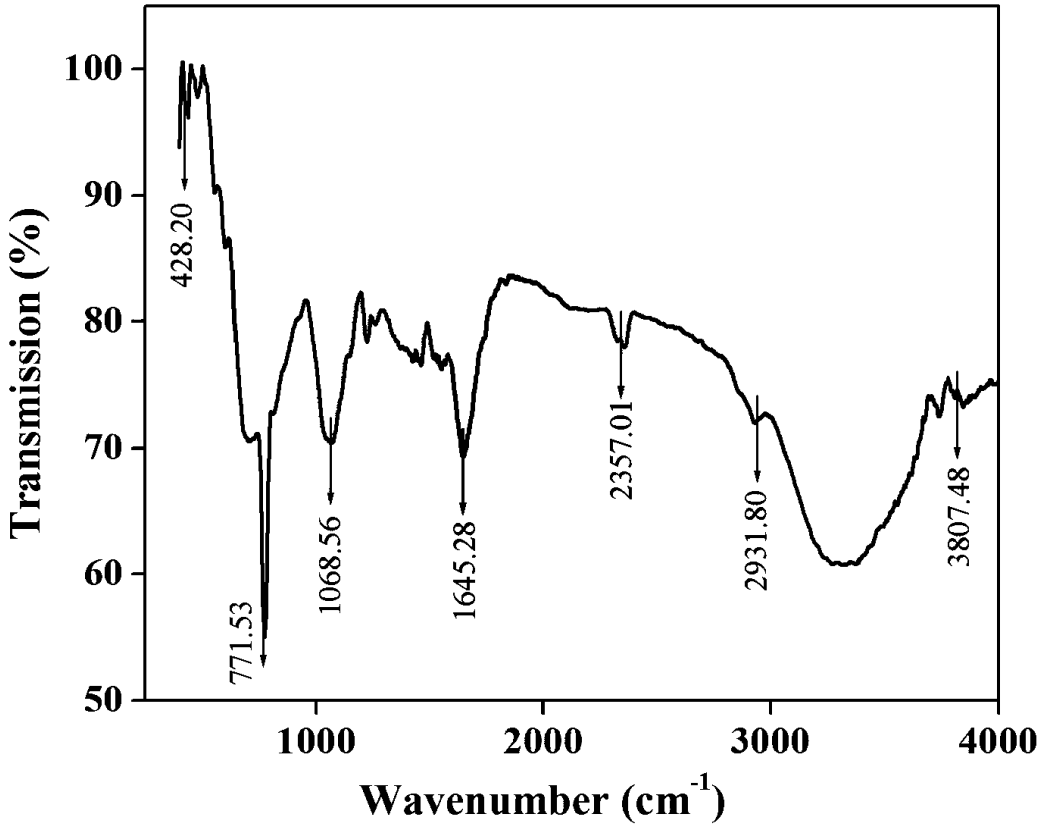
578 **Figure 3.** FTIR spectrum of Ag nanoparticles green synthesized using the *Saponaria*

579 *officinalis* aqueous roots extract.



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592 **Figure 4.** XRD pattern of Ag nanoparticles biosynthesized using the *Saponaria*

593 *officinalis* aqueous extract.

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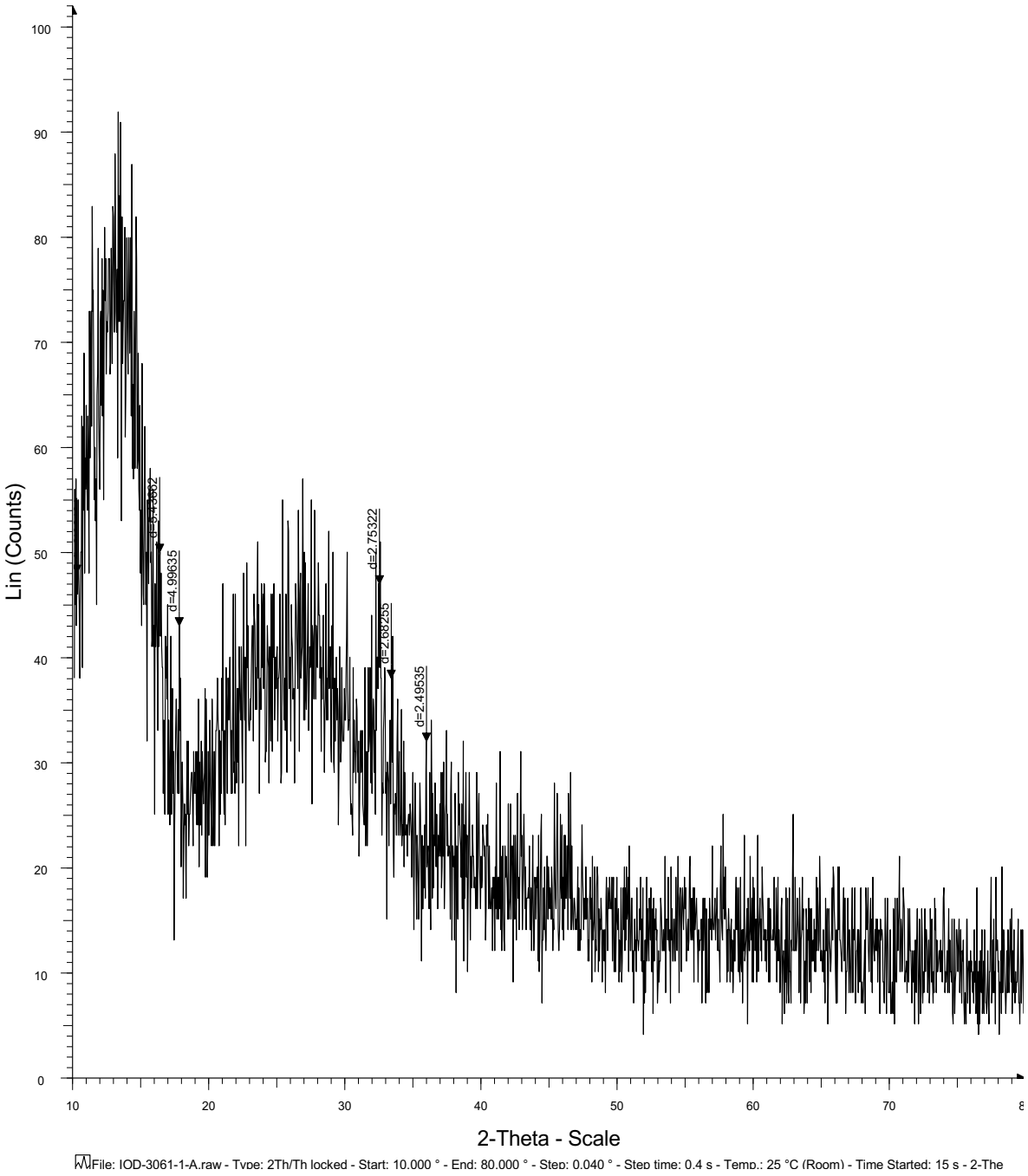

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Table 1. Acute toxicity of *Saponaria officinalis* root crude extract and green synthesized Ag nanoparticles tested on *Tetranychus urticae* mites.

	Crude root extract					Ag nanoparticles				
Target	LC_{50} [a]	CI_{95}	LC_{90} [a]	CI_{95}	Chi [b]	LC_{50} [a]	CI_{95}	LC_{90} [a]	CI_{95}	Chi [b]
Adults	19.9	17.6-22.3	36.1	31.9-45.5	0.517	6.1	4.5-8.9	15.4	12.8-22.9	0.013
Nymphs	7.8	7.2-8.3	11.9	10.9-13.6	0.213	1.2	1.0-1.3	2.8	2.5-3.5	0.055
Eggs	13.8	12.4-14.6	18.8	17.7-21.2	0.032	3.1	2.5-3.5	13.1	8.6-21.8	0.511

[a] Concentration LC_{50} (LC_{90}) in $g.L^{-1}$ causing 50% (90%) mortality of *T. urticae* adults, nymphs and eggs

[b] Chi-square value, not significant (P=0.05)

CI_{95} = 95% confidence intervals, extract activity is considered significantly different when the 95% CI fail to overlap

607 **Table 2.** Effect of *Saponaria officinalis* root crude extract and green synthesized Ag
608 nanoparticles tested as oviposition inhibitors on *Tetranychus urticae* mites.
609

Dose	Crude root extract					Ag nanoparticles				
($g.L^{-1}$)	Eggs/Female ± SD[a] (n)	Inhibition (%) ± SD[b]	EC_{50} (CI_{95})[c]	EC_{90} (CI_{95})[c]	Chi[d]	Eggs/Female ± SD[a] (n)	Inhibition (%) ± SD[b]	EC_{50} (CI_{95})[c]	EC_{90} (CI_{95})[c]	Chi[d]
30.0	0.3±0.1	94.1±3.8	6.1 (5.2-7.4)	30.1 (24.4-41.8)	3.524	ND	-	1.4 (1.2-3.5)	8.6 (8.2-12.8)	0.323
20.0	0.6±0.3	88.4±2.9				ND	-			
15.0	1.3±0.4	76.5±5.5				ND	-			
10.0	2.8±1.2	55.6±4.8				ND	-			
7.7	ND	-				0.1±0.1	97.9±3.9			
5.0	3.9±0.7	43.1±5.2				1.6±0.3	71.9±5.2			
2.0	5.2±1.1	32.7±2.8				3.5±0.8	47.4±3.2			
1.0	8.9±1.8	4.8±3.2				3.9±0.9	43.1±3.1			

0.5	9.2±1.7	3.1±0.1				5.2±1.1	30.7±2.8			
Control	9.8±1.2	-	-	-	-	9.8±1.2	-	-	-	-

610

611 [a] The average number of eggs laid per female ± standard deviation (SD)

612 [b] Mean inhibition of oviposition in comparison with the control ± standard deviation

613 [c] Effective concentration EC_{50} (EC_{90}) in $g.L^{-1}$ causing 50% (90%) inhibition of egg

614 laying by *T. urticae* females, compared with untreated control

615 [d] Chi-square value, not significant (P=0.05)

616 CI_{95} = 95% confidence intervals, extract activity is considered significantly different

617 when the 95% CI fail to overlap

618 ND = not determined

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