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RESEARCH ARTICLE

The miticidal activity of silver nanoparticles towards phytophagous and predatory mites of citrus: efficacy and selectivity

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ABSTRACT

The biological activity of silver nanoparticles (SNP) has grabbed the attention of researchers in different fields, but this attention did not go beyond bioassays within laboratory or growth chambers. Few studies have investigated the miticidal activity of SNP, mostly against *Tetranychus urticae* Koch (Acari: Tetranychidae). Despite the promising preliminary results, field evaluation of miticidal activity towards both pest and non-target organisms are still lacking. SNP were chemically synthesized utilizing trisodium citrate in excess and then miticidal activity was tested against phytophagous and predatory mites in trifoliate orange (*Citrus trifoliata* L.). A commercial formulation of Bifenthrin was used as reference. In laboratory, SNP showed slightly higher miticidal activity, than bifenthrin with LC_{50} of 29.3, 43.9 and 27.4 mg/l in SNP and 43.3, 38.9 and 31.6 mg/l in bifenthrin with efficiency factor of 1.5, 0.9 and 1.2 for *P. oleivora*, *E. orientalis* and *B. obovatus*, respectively. In case of SNP, it showed lower toxicity than bifenthrin towards predatory mites with LC_{50} of 789.9 and 656.0 mg/l in SNP and 48.2 and 45.5 mg/l in bifenthrin for *P. oleivora*, *E. orientalis,* and *B. obovatus*, respectively, with safety factor of 14 to 16 times for *A. swirskii* and *P. plumifer*, respectively. While in the field, LC_{50} values of SNP were 25.4, 36.0 and 27.0 mg/l while bifenthrin values were 39.2, 39.9, 29.7 mg/l for *P. oleivora*, *E. orientalis* and *B. obovatus*, respectively. SNP showed highly selective toxicity (23 times at LC_{50}) towards phytophagous than predatory mites ($P = 0.0001$), whereas bifenthrin showed no selectivity ($P = 0.750$). Moreover, residues of SNP provided a 14-days prolonged activity against infesting mites. Exhibiting high selectivity towards the phytophagous mites, residues of SNP slightly affected the predatory ones. SNP showed comparable efficacy to bifenthrin for control of moving stages of *P. oleivora*, *E. orientalis* and *B. obovatus* mites and surpassed bifenthrin in ovicidal activity and saving associated predatory mites. SNP may be utilized for control of *P. oleivora*, *E. orientalis,* and *B. obovatus* mites in orange.

Keywords: Miticidal activity of SNP; Phytophagous mites; Predatory mites; Selectivity; Bifenthrin

INTRODUCTION

The citrus rust mite *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae) is a worldwide citrus key pest (Maoz et al., 2014). The citrus brown mite or red oriental mite *Eutetranychus orientalis* Klein (Acari: Tetranychidae) is a widely distributed pest of citrus and it causes leaf fall and branches' dieback, (Vela et al., 2017). The Tenuipalpidae flat mite, *Brevipalpus obovatus* Donnadieu (Acari: Tenuipalpidae), is a vector of citrus leprosis virus (Hao et al., 2016). The three mentioned phytophagous mites are being controlled using numerous chemical groups of acaricides including organochlorine, organophosphorus, carbamate pyrethroid, and growth regulators (Hardman et al., 2003; Maoz et al., 2014; Vela et al., 2017). Due to the evolved resistance of mites, these acaricides are becoming less effective, thus increasing control costs (Maoz et al., 2014). Therefore, higher rates of pesticides are applied that affect the environment, food quality, and decrease the population of natural enemies (Hardman et al., 2003). Safer, sustainable and more effective alternatives are looked-for. Nanotechnology can provide solutions for pests' resistance, increasing rates of pesticide usage, and offers simultaneous multi-target pests control that saves time and costs.

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Particles of silver within nanoscale diameter exhibited potent bioactivities against different organisms (Govindarajan et al., 2016; Pavela et al., 2017; Vrandecic et al., 2020). The biological activity of silver nanoparticles towards different agricultural and public health pests was extensively investigated. Insecticidal activity of SNP were tested against different insects (Benelli et al., 2018). Acaricidal activity of SNP were tested against cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Santhoshkumar et al., 2012; Avinash et al., 2017). Very limited reports have shed light on miticidal activity of silver nanoparticles (SNP) towards phytophagous mites and The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) received the most attention (Jalalizand et al., 2013; Pavela et al., 2017; Al-Azzazy et al., 2019).

So far, evaluation of SNP activity did not go beyond bioassays within laboratory or growth chambers. Trials investigating the efficacy of SNP against plant infesting mites in open field and attesting the activity towards non-target organisms are needed (Souza et al., 2019).

The current study aimed to achieve efficient synthesis of SNP using a handy procedure, evaluation of the synthesized SNP towards phytophagous mites in laboratory, evaluation of the control efficacy of SNP against real infestation of phytophagous mites in the field, comparing to the commercial acaricide bifenthrin. Special focus was given to the assessment of safety of SNP towards the associated predacious mites.

MATERIALS AND METHODS

Synthesis and characterization of silver nanoparticles

Silver NPs were synthesized using the procedures reported in a previous publication (Abdelsalam et al., 2019). Sodium citrate was used in excess to assure full reduction of Ag^{+} to Ag^{0} and stabilization of the produced particles. The development of greyish-brown turbidity evidenced the formation of SNP. Spectroscopic characterization of the synthesized SNP was performed to confirm the achievement of the required particles in terms of size and shape. UV/Vis spectrum (UV-1800, Shimadzu, Japan) showed a broad peak at (λ_{max}) of 450 nm, confirming the presence of SNP in the solution. Particle size was analyzed using Laser Particle Size Analyzer (S3500 Microtrac Bluewave, USA). Particle median size was 40 nm with a range of 25-95 nm (Fig. 1A). Scanning electron microscopy (JEOL SEM, USA) was also run to show, basically, the particle shape and its dimension as well. Particles were mostly spherical (Fig. 1B). Energy Dispersive X-Ray EDX Spectroscopic analysis was performed using EDX Spectrometer with AXS Flash Detector, Bruker, Germany). It showed two peaks at 2.6 and 3 Kev confirming the existence of the silver metal (Fig. 1C). Considering the data gained from the aforementioned analyses, the synthesis of 40 nm spherical silver nanoparticles was accomplished.

Miticidal and ovicidal activity of SNP in laboratory

Naturally infested orange fruits were brought from the Experimental Farm of Qassim University, Qassim, Saudi Arabia. fruits were infested with three phytophagous mites, citrus rust mite *Phyllocoptruta oleivora* Ashmead, Citrus brown mite *Eutetranychus orientalis* Klein and flat mite *Brevipalpus obovatus* Donnadieu and two predatory mites *viz Amblyseius swirskii* Athias-Henriot and *Phytoseius plumifer* (Canestrini & Fanzago) (Acari: Phytoseiidae). All mites were identified according to Meyer (1987); Chant and McMurtry (2003, 2005, 2007). Pre-treatment counts of moving individuals of all mites and laid eggs (for only phytophagous ones) were recorded before treatment. Serial concentrations of SNP i.e. 13.5, 27, 54, 108 and 216 µg/ml were freshly prepared

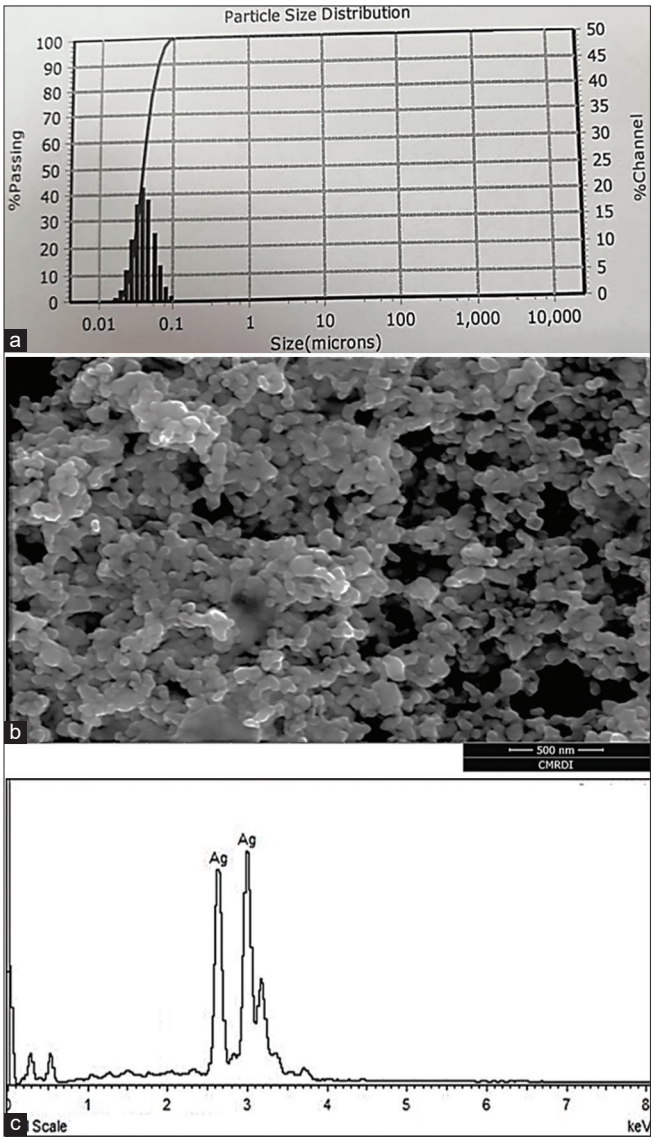

Fig 1. Scanning electron microscopy of the obtained nano particles.

and sprayed using hand atomizer. Bifenamin® formulation (10% EC, Bifenthrin, manufactured by Vapco, Jourdan, obtained from a local market) is a recommended acaricide, among other ones, by Saudi Ministry of Environment, Water and Agriculture for control of mites in different cultivations. Bifenamin® was employed in this study to compare mite control efficiency of SNP with a commercial miticide. Successive concentrations of bifenthrin (the commercial formulation, Bifenamine®) 8.0, 16.0, 33.0, 66.0 and 125.0 mg/l were prepared and sprayed using hand atomizer. Untreated control was sprayed with tap water. Ten replicates were employed for each concentration of SNP, bifenthrin and untreated control. Numbers of moving stages were recorded after three days of treatment. Hatched larvae were monitored for 7 days or until untreated control were fully hatched and hatched larvae were removed daily. Corrected mortality percent in each treatment were computed using equation (1) (Henderson and Tilton, 1955).

$$\text{Corrected Mortality } \% = \left(1 - \frac{Cb \times Ta}{Ca \times Tb}\right) \times 100 \quad (1)$$

Where: *Cb* is the number of mites or laid eggs in untreated control before spray, *Ca* is the number of mites or hatched larvae/protonymph in untreated control after spray, *Tb* is the number of mites or laid eggs in treatment before spray and *Ta* is the number of mites or hatched larvae/protonymph in treatment after spray. Dose- response lines were composed using average corrected mortality percent from equation (1) against applied concentration in mg/l.

Miticidal activity of SNP: Direct application on naturally infested orange trees

The experiment was executed in the orange field of Qassim University's Experimental Station (26°17'53"N 43°47'21"E), Qassim, Saudi Arabia, during orange fruiting season of November 2019. Orange trees (*Citrus trifoliata*) with natural mixed-mite infestation were selected for the experiment. Three phytophagous mites i.e. citrus rust mite *Phyllocoptruta oleivora* Ashmead, Citrus brown mite *Eutetranychus orientalis* Klein and flat mite *Brevipalpus obovatus* Donnadieu and two predatory mites *viz Amblyseius swirskii* Athias-Henriot and *Phytoseius plumifer* (Canestrini & Fanzago) (Acari: Phytoseiidae), were existent on orange fruits. Mites were identified as mentioned previously. Serial concentrations of spray solutions were sprayed on the selected trees' foliage using hand sprayer. Spray solution of each concentration was applied until dripping to achieve uniform and full coverage of the target tree with the spray solution. Bifenthrin, a recommended acaricide for control of mites in different cultivations, was employed in this study to compare mite control efficiency of SNP with a commercial miticide. The applied concentrations were 13.5, 27.0, 54.0, 108.0 and 216.0 mg/l for SNP and 8.0, 16.0, 33.0, 66.0 and 125.0 mg/l for bifenthrin. The highest concentration of bifenthrin (125.0 mg/l) is the recommended concentration. Each tree was treated with a single concentration of either bifenthrin or SNP solution. Untreated control was sprayed with only water. Ten orange fruits in each tree, representing all tree's levels and directions, were selected randomly and labeled. A pre-treatment count was made before spraying to determine the initial density of all the aforementioned mites' species. Post spraying, count of each mite on the labeled fruits was recorded in the field using a 10× hand lens. Counts of moving individuals or hatching of the laid eggs were recorded on orange fruits only. Counts of moving individuals were performed after 7 days after application for both phytophagous and predatory mites. Hatching of laid eggs were recorded in case of only phytophagous mites; where hatched larvae of *B. obovatus* and *E. orientalis* and protonymphs of *P. oleivora* were counted every day until the end of hatching in untreated control. Corrected mortality percent in each treatment were computed using equation (1). Dose- response lines were composed using average corrected mortality percent against applied concentration in mg/l.

Miticidal activity of SNP: Exposure to residues of SNP

In order to investigate the protection efficiency of NSP's residues, spray solutions of SNP (13.5, 27.0, 54.0, 108.0 and 216.0 mg/l) were applied to non-infested orange trees. A none-treated orange tree was designated as control. After spray solution was dried, artificial infestation was done by nailing a heavily infested leaf along a piece of fruit peel with rust blotch onto a non-infested sprayed orang fruit and left for 24 hours (Al-Azzazy, 2005). The infested transferred parts contain both the phytophagous and predatory mites. Numbers of moving individuals of phytophagous and predatory mites were recorded on artificially infested orange fruits after 1 (as initial population count), 7 and 14 days following artificial infestation. Calculations of corrected reduction percent of mite population in each treatment were carried out as previously reported.

Statistical analysis

Statistical tests were performed using IBM SPSS Statistics version 23. All data were initially investigated through the Shapiro-Wilk's test for normality (Shapiro and Wilk, 1965). Skewness, kurtosis Q-Q plots were used to demonstrate that data were normally distributed. Normally distributed and homogenous data were analyzed without transformation; otherwise, data were transformed to the normal logarithm. Thereafter, one way-ANOVA followed by Tukey's post hoc for separation of means were performed for statistical analysis within the same treatment either bifenthrin or SNP using corrected mortality percentage. Differences

of the means were considered statistically significant when $P \leq 0.05$.

Probit regression analysis was performed using average reduction percent from equation (1) versus normal logarithm of applied concentrations (Finney, 1971).

RESULTS

Miticidal and ovicidal activity of SNP in laboratory

Probit analysis of the acquired mortality versus the tested concentrations was performed for SNP and bifenthrin against tested phytophagous and predatory mites. Dose response relationship parameters are summarized in Tables 1, 2 and 3. All obtained values of Chi-square significance were higher than 0.2 and values of R^2 were higher than 0.94 proving goodness of the obtained regression lines.

SNP showed slightly higher miticidal activity, towards moving stages of phytophagous mites, than the activity of bifenthrin towards the same target mites. LC_{50} were 29.3, 43.9 and 27.4 mg/l in SNP treatment and 43.3, 38.9 and 31.6 mg/l in bifenthrin treatment (Table 1) for *P. oleivora*, *E. orientalis* and *B. obovatus*, respectively. Efficiency factor values were 1.5, 0.9 and 1.2 (Table 2). In case of predatory mites, SNP showed lower toxicity than bifenthrin. LC_{50} were 789.9 and 656.0 mg/l in SNP treatment and 48.2 and 45.5 mg/l in bifenthrin treatment for *A. swirskii* and *P. plumifer*, respectively. Safety factor of SNP was 14 to 16 times (Table 2). ANOVA test showed no significant difference ($p = 0.359$) between the effects of bifenthrin on all the tested mites, predatory and phytophagous. Confidence limits of LC_{50} and LC_{90} values were overlapped implying no significant difference is noted between toxicity lines of all tested mites and supporting the ANOVA test's results. This also suggest that bifenthrin is not selective towards phytophagous mites. For SNP, ANOVA test showed significant difference between mites treated with SNP ($p < 0.001$). Tukey's post-hoc test, separated the treated mites into two groups, one is for phytophagous mites and the other for predatory ones. Probit analysis showed that, confidence limits of LC_{50} and LC_{90} values were overlapped intra-phytophagous mites' treatment and intra-predatory mites' treatment but not overlapped between the two groups, supporting the results of ANOVA and Tukey's post-hoc tests. This also suggest that SNP is selective for phytophagous mites.

Ovicidal activity of SNP towards laid eggs of phytophagous mites were evaluated and toxicity parameters are summarized in Table 3. LC_{50} values were 95.5, 80.6, and 126.5 mg/l for *P. oleivora*, *E. orientalis* and *B. obovatus*, respectively (Table 3). ANOVA test showed that there was no significant difference ($P = 0.140$) between mite species. An overlapping of upper and lower limits at the confidence of 95 % (CL_{95}) was noted between the tested mites (Table 3). Hatchability lines of phytophagous mites indicated insignificant differences between mite species. Bifenthrin showed very limited ovicidal activity as the highest tested concentration affected less than 10 % of the laid eggs of the tested phytophagous mites.

Table 1: Toxicity parameters of bifenthrin towards phytophagous and predatory mites in laboratory.

	LC_{50} (mg/l)	CL_{95} [a]	LC_{90} (mg/l)	CL_{95}	slope	R^2	Chi[b]
Phytophagous mites							
P. oleivora	43.3	29.1-70.5	229.4	119.6-1035.4	1.79	0.958	0.770
E. orientalis	38.9	34.0-44.7	150.9	119.7-204.5	2.18	0.994	0.752
B. obovatus	31.6	26.8-37.3	178.3	132.2-268.9	1.70	0.988	0.615
Predatory mites							
A. swirskii	48.2	40.1-59.2	339.5	226.4-611.1	1.50	0.993	0.840
P. plumifer	45.5	36.5-58.4	515.5	297.9-1216.9	1.22	0.993	0.908

[a]95 % Confidence Limits, unoverlapped confidence limits in different mites imply that $LC_{50}$and LC_{90} considered significantly different. [b]Chi-square significance (*P* value). The value considered insignificant when *P*>0.05.

Table 2: Toxicity parameters of SNP towards phytophagous and predatory mites in laboratory, efficiency factor and safety factor.

	LC_{50} (mg/l)	CL_{95} [a]	LC_{90} (mg/l)	CL_{95}	slope	R^2	Chi[b]	
Phytophagous mites								EfficiencyFactor[c]
P. oleivora	29.3	24.8-33.9	123.6	99.9-163.6	2.16	0.982	0.328	1.5
E. orientalis	43.9	37.1-51.9	247.6	185.7-367.4	1.77	0.966	0.241	0.9
B. obovatus	27.4	22.4-32.4	145.1	113.4-202.9	1.84	0.986	0.572	1.2
Predatory mites								Safety Factor[d]
A. swirskii	789.9	401.3-3105.1	14868.5	3594.2-293577.1	1.06	0.976	0.892	16.4
P. plumifer	656.0	377.4-1823.3	654247	2214.3 53799.8	1.20	0.946	0.636	14.4

[a] 95 % Confidence Limits, unoverlapped confidence limits in different mites imply that LC_{50} and LC_{90} considered significantly different. [b] Chi-square significance (*P* value). The value considered insignificant when *P*>0.05. [c] Efficiency Factor=LC_{50} of Bifenthrin÷LC_{50} of SNP. [d]Safety Factor=LC_{50} of SNP÷LC_{50} of Bifenthrin.

Direct application on infested orange trees

Miticidal activity towards moving stages

Dose-response relationships for SNP and bifenthrin against phytophagous and predatory mites were carried out using mortality percent against concentration logarithm. Toxicity parameters were calculated and reported (Tables 4 and 5). Chi-square significance values were all higher than 0.05 and determination coefficient (R^2) values were more than 0.9, both implying the quality of representing lines.

Bifenthrin was toxic to both studied phytophagous and predatory mites, as shown from toxicity parameters. LC_{50} and LC_{90} values of bifenthrin were similar for all phytophagous and predatory tested mites (Table 4). LC_{50} ranged from 29.7 mg/l (*B. obovatus*) to 46.5 mg/l (*A. swirskii*). Probit analysis showed that bifenthrin's toxicity towards both phytophagous and predatory mites were not significantly different. Confidence limits for both LC_{50} and LC_{90} of all tested mites were overlapping, indicating similar toxicity of bifenthrin towards the three phytophagous mites as well as the predatory mites (Table 4). The same conclusion was also obtained from ANOVA analysis where no significant difference was observed between all mites (P= 0.750).

On the other hand, SNP showed considerable miticidal activity against the tested phytophagous mites. The calculated LC_{50} values of SNP (Table 5) were somewhat lower than the corresponding ones of bifenthrin (Table 4). Also, the obtained LC_{90} values of SNP were lower than those of bifenthrin except in the case of *E. orientalis* that might be attributed to lesser homogeneity of its population, which is also signified by the smaller slope value 1.39 (Table 5). According to probit analysis, the effect of SNP on the three phytophagous mites was significantly different than predacious mites (Table 5) and can be categorized into two groups. ANOVA test showed that the effect of SNP on mites was significantly different (P= 0.0001). Mites were sorted into three groups according to Tukey's test, two groups for phytophagous mites and one group for the predatory mites.

However, the effect of SNP and bifenthrin towards phytophagous mites were not significantly different. CL_{95} upper and lower bounds of both LC_{50} and LC_{90} do intersect for each phytophagous mite in both SNP and bifenthrin. In the same perspective, efficiency factor (EF) was calculated by dividing LC_{50} value of bifenthrin by SNP's LC_{50} value

Table 3: Ovicidal activity parameters of SNP towards laid eggs of phytophagous mites in laboratory.

	LC_{50}		LC_{90}		slope	R^2	Chi[b]
	(mg/l)	CL_{95}[a]	(mg/l)	CL_{95}			
P. oleivora	95.5	81.3-114.6	478.5	344.1-756.8	1.94	0.978	0.520
E. orientalis	80.6	68.1-97.2	478.5	335.5-789.4	1.72	0.973	0.388
B. obovatus	126.5	104.3-160.9	767.5	500.6-1430.9	1.68	0.973	0.334

[a]95 % Confidence Limits, unoverlapped confidence limits in different mites imply that LC_{50} and LC_{90} considered significantly different. [b]Chi-square significance (*P* value). The value considered insignificant when *P*>0.05.

Table 4: Toxicity parameters of bifenthrin towards phytophagous and predatory mites in field.

	LC_{50} (mg/l)	CL_{95}[a]	LC_{90} (mg/l)	CL_{95}	slope	R^2	Chi[b]
Phytophagous mites							
P. oleivora	39.2	33.1- 46.9	241.3	171.3- 390.7	1.63	0.986	0.579
E. orientalis	39.9	34.8- 46.0	159.0	125.3- 217.5	2.14	0.999	0.974
B. obovatus	29.7	24.5- 35.9	226.0	156.3- 384.5	1.45	0.988	0.728
Predatory mites							
A. swirskii	46.5	38.1- 58.4	410.1	256.6- 828.7	1.36	0.997	0.951
P. plumifer	41.6	34.1- 51.8	370.6	234.7- 734.2	1.35	0.989	0.803

[a] 95 % Confidence Limits, unoverlapped confidence limits in different mites imply that LC_{50} and LC_{90} considered significantly different. [b] Chi-square significance (*P* value). The value considered insignificant when *P* > 0.05.

Table 5: Toxicity parameters of SNP towards phytophagous and predatory mites in field, efficiency factor and safety factor.

	LC_{50} (mg/l)	CL_{95}[a]	LC_{90} (mg/l)	CL_{95}	slope	R^2	Chi[b]	
Phytophagous mites								EfficiencyFactor[c]
P. oleivora	25.4	20.5-30.4	144.0	111.7-204.0	1.72	0.982	0.481	1.5
E. orientalis	36.0	28.8-43.9	203.7	210.8-518.0	1.39	0.995	0.919	1.1
B. obovatus	27.0	21.6-32.6	167.9	117.7-295.6	1.67	0.951	0.182	1.1
Predatory mites								Safety Factor[d]
A. swirskii	747.2	405.0-2476.9	9041.0	2662.2-108553.7	1.34	0.919	0.396	16.1
P. plumifer	609.6	357.8-1624.5	6456.7	2204.9 50816.7	1.21	0.938	0.44	14.7

[a] 95 % Confidence Limits, unoverlapped confidence limits in different mites imply that $LC_{50}$and LC_{90}considered significantly different. [b] Chi-square significance (*P* value). The value considered insignificant when *P*>0.05. [c] Efficiency Factor=$LC_{50}$of Bifenthrin÷$LC_{50}$of SNP. [d]Safety Factor=$LC_{50}$of SNP÷$LC_{50}$of Bifenthrin.

for each phytophagous mite. EF was slightly higher than unity, indicating that SNP showed miticidal activity pretty similar to the activity of bifenthrin.

Differently, SNP showed a significant safety margin towards the associated predatory mites. Calculated LC_{50} values were 747.2 and 609.6 mg/l (SNP) in comparison to 46.5 and 41.6 (bifenthrin) for *A. swirskii* and *P. plumifer*, respectively. The same trend is noted for LC_{90} values. A safety factor (SF) was calculated by dividing LC_{50} value of SNP by LC_{50} value of bifenthrin. Calculated SF values were 16.1 and 14.7 for *A. swirskii* and *P. plumifer*, respectively. In other words, SNP is about 15 folds less toxic to the studied predatory mites than bifenthrin.

Ovicidal activity of SNP

The ovicidal activity of SNP was assessed. The effect of the highest tested concentration (125 mg/l) of bifenthrin did not exceed a 10 % reduction in the hatching of eggs of tested phytophagous mites. SNP exhibited ovicidal activity towards the tested phytophagous mites' eggs. LC_{50} values were 82.8, 88.8, and 121.8 mg/l for *P. oleivora*, *E. orientalis* and *B. obovatus*, respectively (Table 6). ANOVA test showed that there was no significant difference (P= 0.351) between mite species. An overlapping of upper and lower limits at the confidence of 95 % (CL_{95}) was noted between *P. oleivora* and *E. orientalis*, and between *E. orientalis* and *B. obovatus* (Table 6). Chi-square value was insignificant (P> 0.05) indicating the well fitness of regression lines. Hatchability lines of phytophagous mites indicated insignificant differences between mite species.

Miticidal activity towards moving stages after exposure to residues of SNP

Mites were exposed to dry residues of SNP to investigate the effect of SNP's residues on moving stages. Numbers of moving stages were checked after 7 and 14 days of artificial infestation. Although phytophagous mites were negatively affected by exposure to residues of SNP, predatory mites were less affected. Probit analysis showed insignificant differences within phytophagous mites after both one and two weeks. The effect of SNP on predatory mites together was significantly lower than phytophagous ones in both intervals. ANOVA analysis showed significant differences between mites (P= 0.0001 for week one) and (P= 0.0001 for week two). Tukey's HSD test separated mites into two groups with *E. orientalis* being in both groups for week one and into two groups for week two. Values of LC_{50} and LC_{90} generally decreased with time except in the case of *A. swirskii* where they increased, may be due to the decrease of mites in untreated control (Table 7). The effect of SNP lasted for 14 days, as the t-test revealed no significant difference between first- and second-week reduction percent for each mite (P> 0.05).

DISCUSSION

Synthesis of semi-spherical silver metal nanoparticles was accomplished using an easy and cost-effective traditional chemical method. Trisodium citrate was the sole reducing

Table 6: Ovicidal activity parameters of SNP towards laid eggs of phytophagous mites in field.

	LC_{50}		LC_{90}		slope	R^2	Chi[b]
	(mg/l)	CL_{95}[a]	(mg/l)	CL_{95}			
P. oleivora	82.8	71.8-96.6	345.5	264.5-494.7	2.12	0.994	0.856
E. orientalis	88.8	75.4-106.7	474.9	338.7-761.7	1.88	0.966	0.294
B. obovatus	121.8	100.4-154.9	770.7	499.5-1453.9	1.67	0.98	0.641

[a]95 % Confidence Limits, unoverlapped confidence limits in different mites imply that $LC_{50}$and LC_{90}considered significantly different. [b]Chi-square significance (*P* value). The value considered insignificant when *P*>0.05.

Table 7: Toxicity parameters of SNP towards phytophagous and predatory mites in field after exposure to SNP's residues for 7- and 14-days intervals.

	day	LC_{50} (mg/l)	CL_{95}[a]	LC_{90} (mg/l)	CL_{95}	slope	R^2	Chi[b]
Phytophagous mites								
P. oleivora	7	110.1	89.4-142.9	902.1	547.9-1913.4	1.43	0.992	0.856
	14	64.1	51.6-80.9	711.8	425.6-1580.4	1.23	0.998	0.987
E. orientalis	7	317.2	207.3-648.7	5170.1	1878.1-32456.6	1.04	0.957	0.628
	14	97.9	73.4-144.2	2255.8	950.8-10581.0	0.94	0.951	0.471
B. obovatus	7	155.9	94.5-483.4	932.6	351.6-22674.3	1.64	0.923	0.04
	14	109.9	87.8-145.8	1072.1	614.1-2546.8	1.36	0.943	0.17
Predatory mites								
A. swirskii	7	5512.1	-	1.7×10^5	-	0.7	0.771	0.289
	14	2.7×10^5	-	1.3×10^8	-	0.48	0.80	0.759
P. plumifer	7	2.1×10^7	-	2.4×10^{12}	-	0.26	0.977	0.989
	14	4543.8	-	1.1×10^5	-	1.08	0.895	0.612

[a]95 % Confidence Limits, unoverlapped confidence limits in different mites imply that LC_{50} and LC_{90} considered significantly different. [b]Chi-square significance (*P* value). The value considered insignificant when *P*>0.05.

and capping reagent. Trisodium citrate is not a hazardous or dangerous material according to European regulations (Regulation (EC) No. 1272/2008). The obtained results showed that the synthesized SNP possess miticidal and ovicidal activity against three phytophagous mites of orange. Bifenthrin, a well-known miticide recommended for control of several mite species in different cultivations, was used to scale the activity of SNP towards the tested mites in the field. Values of LC_{50} SNP and efficiency factor indicated comparable activity to bifenthrin. It is noteworthy to state that an inexpensive and easily synthesized zero-valent silver atom in nanoparticle form (SNP) showed about the same or slightly higher activity than a known commercial miticide (bifenthrin) towards three economic mite-pests of orange. The obtained results from laboratory experiments and field experiments were similar. Results and statistical analyses showed that bifenthrin was toxic to all tested mites, both phytophagous and predatory. It could not favor target over non-target organisms. Bifenthrin is toxic via ingestion and contact. Since that bifenthrin is a water-insoluble and non-systemic pesticide, its effect on mites would be merely via contact. The lipophilic bifenthrin molecules (Partition Coefficient 7.3) (Tomlin CDS, 2004) can easily solubilize in mite chitin and penetrate into the haemolymph.

On the contrary, SNP showed selective toxicity towards the undesired phytophagous mites while saving predatory mites. On average, SNP was 23 and 45 times toxic to phytophagous mites than the predators at LC_{50} and LC_{90} levels, respectively. Realistically, less than 25 percent of predatory mites were killed by the highest tested concentration, while the highest tested concentration of bifenthrin caused more than 70 % death in the mite population. An illustrative diagram of activity and selectivity of SNP and bifenthrin using viability percent versus applied concentrations for the tested phytophagous (*B. obovatus*) and predacious (*P. plumifer*) mites is shown in Fig. 2.

SNP is toxic as in particle form and/or as a source of Ag cations (McShan et al., 2014; Mabey et al., 2019). SNPs may work as "Trojan horses" to facilitate penetration of cell membrane then produce Ag^{+} ions causing damage to cellular systems (Benelli, 2018). SNP affect cell permeability (Navarro et al., 2008). SNP binds to sulfur-containing macromolecules and reduce glutathione levels (McShan et al., 2014). SNP impart function of enzymes, nucleic acid and mitochondria (Mabey et al., 2019).

Mode of action of SNP was investigated widely in insects (Benelli, 2018). SNP produced up and/or down-regulation of gens responsible for ribosomal protein gene (Nair et al., 2011), glutathione S-transferase (GST) (Nair and Choi, 2011), ecdysone receptor gene (Nair and Choi, 2012) and Mn superoxide dismutase (Nair et al., 2013) in *Chironomus riparius* midge. SNP caused accumulation of reactive oxygen species (ROS) and related apoptosis in *Drosophila melanogaster* (Mao et al., 2018). SNP decreased the activity of esterase enzymes (carboxylesterase and acetylcholinesterase), phosphatase and noticeable decrease

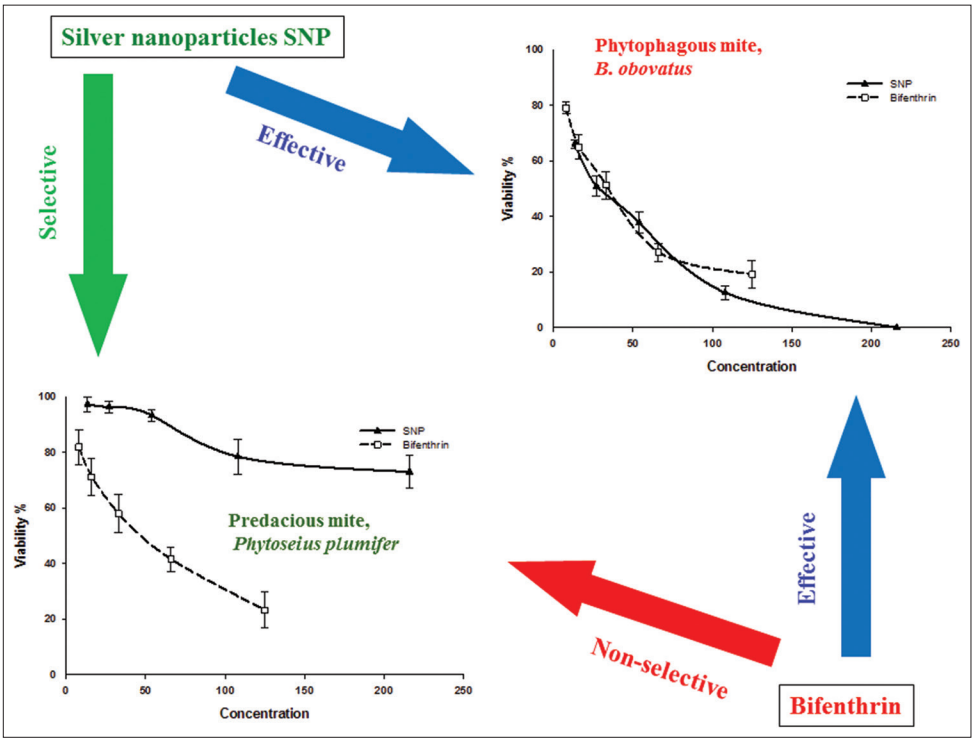

Fig 2. An illustrative diagram of activity and selectivity of SNP and bifenthrin using viability percent versus applied concentrations for the tested phytophagous (*B. obovatus*) and predacious (*P. plumifer*) mites.

in total protein levels in mosquitos (Fouad et al., 2018; Ga'al et al., 2018). Mechanisms of acaricidal activity of SNP were not investigated so far (Benelli et al., 2017; Benelli, 2018). Studies serving this purpose are crucial for understanding mode of action of SNP and help enhancing its activity.

SNP show systemic action as it can penetrate intercellular spaces and translocate through vascular tissues (Tripathi et al., 2017). However, SNP seems to affect phytophagous mites via ingestion when feeding on plant sap and via contact while crawling on sprayed leaves and fruits, or by direct reception of spray droplets. Higher activity of SNP towards phytophagous mites can be explained by the ability of SNP to penetrate their thin body wall, inter-skeletal membranes, or through body openings. Once inside, it can affect several bio-sites causing eventual death. SNP is neither water-soluble nor lipid-soluble. In the case of predatory mites, the exoskeleton is thicker and dorsal and ventral shields (Ignace et al., 2007) may prevent penetration of SNP into body tissues beneath them. In the case of the ingestion route, predacious mites feed only on alive individuals who most probably were not exposed to SNP spray droplets. Besides, the bodyweight of predacious mites is about 40 times higher than that of phytophagous ones that create weight difference selectivity. All the reasons above interlaced together to enhance the selectivity of SNP towards phytophagous over predacious mites. SNP showed significant ovicidal activity against the tested phytophagous mites. About 70 % of hatchability was reduced in *T. urtica* as affected by a 215 solution of SNP (Al-Azzazy et al., 2019). Madhiyazhagan et al. reported full suppression of egg hatching in *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitos using 30 ppm concentration of seaweed extract synthesized-SNP (Madhiyazhagan et al., 2015).

The protective effect of SNP lasted for 14 days after application, as proved by LC_{50} and statistical analyses in exposure to the SNP residue experiment. The after-spray hatched Individuals will suffer the control activity of SNP's residues. It is worth noting that predatory mites were not significantly affected by SNP residues after prolonged exposure for 14 days.

LC_{50} and LC_{90} values of SNP, in week one, were higher than the same ones in case of direct application on infested trees. Probit analysis indicated that the control efficiency of SNP in direct application on infested oranges was higher than exposure to SNP's residues means. Being in contact with spray solution *per se* seemed to be the most effective way against mites than to be exposed to the dry residues of SNP or ingesting plant sap containing SNP and the released silver ions. This way might allow a sufficient amount of intact SNP particles to infiltrate through exoskeleton or body openings and reach their site(s) of action. Condensed water vapor on plant surfaces or dew droplets may play a role in re-constituting and re-delivering of SNP to the moving stages of mites. More specialized studies are required to comprehend the mode of action and entry path of SNP. Benelli and his coworkers, extensively, studied the effect of plant extract-synthesized SNP on mosquitos (Benelli, 2016; Govindarajan et al., 2016; Murugan et al., 2016). Nonetheless, few studies have investigated the miticidal activity of SNP, of which, Jalalizand and colleagues reported moderate activity of a commercial SNP with a size of 18-34 nm towards *Tetranychus urticae* moving stages (Jalalizand et al., 2013).

Saponaria officinalis extract silver nanoparticles displayed miticidal and ovicidal actions against moving stages and eggs of *T. urticae* (Pavela et al., 2017). SNP affected nymphs (1200 mg/l) more than adults (6100 mg/l) while LC_{50} for eggs was 3100 mg/l. In the same study, SNP inhibited 50% of *Tetranychus urticae* oviposition at a concentration of 1400 mg per liter (Pavela et al., 2017).

Chemically synthesized SNP showed miticidal and ovicidal activities towards the moving stages and the eggs of *Aculops lycopersici* and *T. urticae* (Al-Azzazy et al., 2019). However, further studies are required to investigate the effect of SNP on the oviposition of the tested phytophagous mites.

CONCLUSION

SNP suspension was efficiently prepared by reducing silver nitrate and stabilizing the yielded SNP with trisodium citrate. The synthetic method respects the atom economy notion and environmental concerns. The miticidal activity of the furnished SNP was assessed in laboratory and real-life infested orange field. Infesting mites were *Phyllocoptruta oleivora*, *Eutetranychus orientalis* and *Brevipalpus obovatus* along two accompanying predatory mites *Amblyseius swirskii* and *Phytoseius plumifer*. The activity of SNP against infesting mites was comparable to the known miticide bifenthrin, but unlike bifenthrin, their toxicity to predacious mites was minimal. Indeed, high selectivity towards phytophagous versus predatory mites was remarkable. Residues of SNP protected towards phytophagous mites for 14 days, with no cumulative effect on predators. No signs of phytotoxicity were witnessed for the experimented concentrations of SNP. From pest control perspective, SNP can be used for control of the studied phytophagous mites as long as other safety and environmental concerns are not breached.

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Conflict of interest

Sherif B Abdel Ghani, Mahmoud M Al-Azzazy and Luigi Lucini declare that they have no conflict of interests.

Author contribution

Sherif Abdel Ghani: conceptualization, methodology, statistical analyses and preparation of the manuscript; Mahmoud Al-Azzazy: conceptualization, methodology and data tabulation; Luigi Lucini: revision and preparation of the manuscript.

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