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Original Article

Field evaluation of the efficacy of copper nanoparticles against mites associated with orange trees

Avaliação de campo da eficácia de nanopartículas de cobre contra ácaros associados às laranjeiras

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Abstract

Phytophagous mites are dangerous pests, causing economic losses to the world's crops. Nanotechnology is a promising field for pests and disease management, and enhancement of agricultural productivity. The aim of the study was to evaluation of the effectiveness of copper nanoparticles (CuNP) against all stages of eriophyid mite, the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) and tetranychid mite, the citrus brown mite *Eutetranychus orientalis* (Klein) (Acari: Tetranychidae), and Tenuipalpid mite, the false spider mite, *Brevipalpus obovatus* Donnadieu (Acari: Tenuipalpidae). This includes its impacts on predacious mites, *Amblyseius swirskii* Athias-Henriot and *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae), on orange trees under field conditions. Five different concentrations of copper nanoparticles (40, 80, 160, 240, and 320 ppm), as well as the control (well water) were examined. The obtained results indicated that the mortality rate of both phytophagous and predacious mites was associated with an increase in the concentrations of copper nanoparticles. Copper nanoparticles were significantly effective in killing *P. oleivora*, *E. orientalis* and *B. obovatus* with minimal effects on *A. swirskii* and *E. scutalis*. The mortality percentage was 15.24, 20.32, 46.32, 78.97 and 86.37% for *P. oleivora*, 6.87, 9.86, 28.91, 56.30 and 77.52% for *E. orientalis* and 8.38, 23.50, 48.83, 68.80 and 84.08% for *B. obovatus* while the mortality percentage was 0.00, 0.56, 5.83, 9.91 and 15.19% for *A. swirskii* and 0.44, 3.96, 6.93, 8.63 and 21.39% for *E. scutalis* one week after exposure to 40, 80, 160, 240 and 320 ppm of copper nanoparticles, respectively. Moreover, the results showed that copper nanoparticles caused a reduction in the percentage of eggs hatching. The percentages of larvae hatching from eggs were 96.29, 80.00, 64.13, 45.66 and 32.17% for *P. oleivora*, 97.38, 83.28, 69.41, 48.01 and 35.29 for *E. orientalis* and 96.60, 76.92, 56.38, 40.55 and 33.28% for *B. obovatus* one week after exposure to copper nanoparticles at 40, 80, 160, 240 and 320 ppm respectively, compared with the control (well water). According to the results, the use of copper nanoparticles significant effect on reducing the population of phytophagous mites associated with orange trees, with low detrimental effects on predatory mites.

Keywords: miticidal activity of CuNP, phytophagous mites, predatory mites, selectivity.

Resumo

Os ácaros fitófagos são pragas perigosas, causando prejuízos econômicos às lavouras mundiais. A nanotecnologia é um campo promissor para o manejo de pragas e doenças, aumentando a produtividade agrícola. O objetivo do presente estudo foi avaliar a eficácia de nanopartículas de cobre (CuNP) em laranjeiras em condições de campo, contra todos os estágios das seguintes espécies: o ácaro eriofídeo, também chamado de ácaro da falsa ferrugem dos citros, ou Phyllocoptruta oleivora (Ashmead) (Acari: Eriophyidae); o ácaro tetraniquídeo, também chamado de ácaro marrom dos citros, ou Eutetranychus orientalis (Klein) (Acari: Tetranychidae); e o ácaro Tenuipalpidae, também chamado de falso ácaro, ou Brevipalpus obovatus (Donnadieu) (Acari: Tenuipalpidae). Isso inclui seus impactos sobre ácaros predadores, como o Amblyseius swirskii Athias-Henriot e o Euseius scutalis (Athias-Henriot) (Acari: Phytoseiidae). Foram examinadas cinco diferentes concentrações de nanopartículas de cobre (40, 80, 160, 240 e 320 ppm), assim como o controle (água de poço). Os resultados obtidos indicaram que a taxa de mortalidade de ácaros fitófagos e predadores esteve associada ao aumento das concentrações de nanopartículas de cobre. As nanopartículas de cobre foram significativamente eficazes em matar o P. oleivora, o E. orientalis e o B. obovatus, com efeitos mínimos sobre o A. swirskii e o E. scutalis. As porcentagens de mortalidade foram: 15,24, 20,32, 46,32, 78,97 e 86,37% para P. oleivora; 6,87, 9,86, 28,91, 56,30 e 77,52% para E. orientalis; 8,38, 23,50, 48,83, 68,80 e 84,08% para B. obovatus; 0,00, 0,56, 5,83, 9,91 e 15,19% para A. swirskii; e 0,44, 3,96, 6,93, 8,63 e 21,39% para E. scutalis, uma semana após a exposição a 40, 80, 160, 240 e 320 ppm de nanopartículas de cobre, respectivamente.

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Além disso, os resultados mostraram que as nanopartículas de cobre causaram uma redução na porcentagem de eclosão dos ovos. As porcentagens de larvas eclodindo dos ovos foram: 96,29, 80,00, 64,13, 45,66 e 32,17% para P. oleivora; 97,38, 83,28, 69,41, 48,01 e 35,29 para E. orientalis; e 96,60, 76,92, 56,38, 40,58 e 33,29 para B. obovatus, uma semana após a exposição às nanopartículas de cobre a 40, 80, 160, 240 e 320 ppm, respectivamente, em comparação com o controle (água de poço). De acordo com os resultados, o uso de nanopartículas de cobre teve efeito significativo na redução da população de ácaros fitófagos associados às laranjeiras, com baixo efeito prejudicial aos ácaros predadores.

Palavras-chave: atividade miticida do CuNP, ácaros fitófagos, ácaros predadores, seletividade.

1. Introduction

Phytophagous mites are a major problem for crop production, and the reduction in crop yield is the worst outcome of these pests. Several species of Eriophyidae, Tetranychidae and Tenuipalpidae are major pests causing economically high yield losses in many crop species worldwide (Jeppson et al., 1975; Zhang, 2003). Therefore, the integrated management of these pests is essential to improve world crop production. The citrus rust mite, *Phyllocoptruta oleivora* (Eriophyidae), *Eutetranychus orientalis* (Tetranychidae) and *Brevipalpus obovatus* (Tenuipalpidae) are important pests of citrus in many countries (Childers, 1994; Rodrigues and Childers, 2013; Garzia and Lillo, 2018). *Phyllocoptruta oleivora* is a cosmopolitan key pest of all citrus varieties (Childers, 1994). This mite infests leaves, green twigs, stems and most significantly the fruit of all citrus varieties. *P. oleivora* inflicts significant economic injury by leading to decrease weight and fruit size and can reduce yield by 75–100% if not managed (Gerson and Vacante, 2011). *Eutetranychus orientalis* also attacks citrus in many countries around the world, especially on lemon (Walter et al., 1995; Gerson, 2003). It can produce heavy infestations on fruits and leaves, causing leaf wilting and drop following chlorophyll depletion, the death of upper branches and fruit drop (Walter et al., 1995; Gerson, 2003). *Brevipalpus obovatus* is considered a serious agricultural pest on more than 450 hosts including citrus (Childers et al., 2003). This mite injects its toxic saliva into new shoots, leaves, twigs, fruits and tissues of its host plants. In addition, *B. obovatus* inject several species of plant viruses into the tissues of host plants (Childers et al., 2003; Kubo et al., 2011). Management of phytophagous mites is still mainly dependent on conventional acaricides, which has led to environmental pollution, risk of pest resistance, human exposure to toxic acaricides, harmful side effects and persistence of chemical residues in consumed fruits. Moreover, the negative effects of these chemicals on the natural environment as well as their impacts on natural enemies have motivated scientists to seek safe effective acaricides for pest management (Al-Azzazy et al., 2019). Nanotechnology is a young and innovative branch and is currently employed in most human-related fields; including agricultural applications, environmental, chemical, biological and biomedical applications (Kumar et al., 2021). Due to nanoparticles unique traits, e.g., high penetrability, very reduced size and enlarged surface area, they exhibit novel characteristics on the various bulk particles. These unique traits can also render the particles toxic to cells and organisms. Nanoparticles can be utilized in various forms for effective phytophagous mites' management as a combined product of metal and other material that has been proven to be toxic against a given category of pests. With the advancement in nanotechnology, copper nanoparticles showed activity towards various arthropods, and common bacterial and fungal infections (Kiaune and Singhasemanon, 2011; Atwa et al., 2017; Rai et al., 2018; Dorri et al., 2018). Additionally, various nanoparticles have shown efficacy in controlling plant pests and diseases, such as gold nanoparticles (Au) (Huang et al., 2007), nano-emulsions (Wang et al., 2007), nano-silica (Barik et al., 2008), titanium oxide, aluminum oxide (Goswami et al., 2010), nanostructured alumina (NSA) (Stadler et al., 2012), metallic oxides (Kitherian, 2016), silver nanoparticles (Al-Azzazy et al., 2019), iron nanoparticles (Wang et al., 2019), nanoforms of carbon (Ramezani et al., 2019) and Zinc Oxide nanoparticles (Pittarate et al., 2021). Notwithstanding, very few studies have been conducted under field conditions of nanomaterial and pest management and a lot more are expected in the forthcoming. Hence, the important issue in this study was the evaluation of the efficacy of copper nanoparticles against mites associated with orange trees and its impacts on non-target natural enemies under field conditions.

2. Materials and Methods

2.1 Synthesis of copper nanoparticles

Synthesis of Copper nanoparticles was accomplished by reducing Cu^{+2} of copper sulfate using an aqueous solution of $NaBH_4$ (Liu et al., 2012). All used solutions were cooled (15 C°) and dissolved oxygen was removed using nitrogen gas prior preparation step. 90 ml of 10 mM solution of sodium borohydride (Loba, India) was added slowly to 100 ml of 5 mM solution of $CuSO_4$ (Merck, Germany). The mixture was stirred until a dark yellow color was appeared. 10 ml of 100 mM solution of trisodium citrate (BioRad, USA) was added dropwise to the mixture and left to stir for 20 min. Development of CuNP was monitored by UV/Vis spectrum (UV-1800, Shimadzu, Japan) showing a peak at (λ_{max}) of 580 nm, confirming the formation of CuNP in the solution. The obtained particles were in the size range of 20 to 100 nm (Liu et al., 2010).

2.2 Evaluation of the miticidal activity of CuNP in the field

The field experiments were carried out at the Agricultural Research Station of Qassim University, Al-Mulida district, Saudi Arabia. Six groups, each consisting of three orange shrubs (*Citrus sinensis* L.) (5-year-old) of similar size, vigor and shape with a history of *P. oleivora*, *E. orientalis* and *B. obovatus* infestations were selected for the study. The experimental design comprised complete randomized individual trees (replicates). Three replicates (three shrubs) were employed for each concentration of (CuNP) and untreated control. Five different concentrations of copper nanoparticles (CuNP) i.e., 40, 80, 160, 240 and 320 ppm were freshly prepared from the stock solution and sprayed directly on orange shrubs using a five-liters volume hand atomizer for testing all moving stages and eggs of *P. oleivora*, *E. orientalis* and *B. obovatus* and compared with the control, as well as their side effects on the predacious mites *A. swirskii* and *E. scutalis*. The untreated control was sprayed with well water. Twenty-five leaves were picked at random from all directions, kept in polyethylene bags and inspected under the stereoscopic microscope in the laboratory. Pre-spray count was made for all replicates to determine the initial distribution and density of the eriophyid, tetranychid and Tenuipalpid mite species and their predators. Observations were made 1, 3 days and 7 days post-treatment.

2.3 Effect of copper nanoparticles (CuNP) on egg hatchability in laboratory

The effects of copper nanoparticles (CuNP) on egg hatchability of *P. oleivora*, *E. orientalis* and *B. obovatus* were determined on detached orange leaf discs in Petri dishes (5 cm in width × 2 cm high) The leaf discs were positioned upside down on a cotton kept soaked with water, as defined by El-Banhawy (1977). 4 mL of water are added to each Petri dish daily to prevent desiccation. Phytophagous mites were collected from the orange orchard that had received no pesticide application in the Agricultural Research Station of Qassim University, Al-Mulida district, Saudi Arabia. A few days before the starting the test, and to obtain same aged cohorts of eggs for egg experiments, 180 gravid females of *E. orientalis* and *B. obovatus* were randomly selected from heavily infected leaves and held separately (10 females) on each disk to obtain eggs. Due to the difficulty of differentiating between males and females of *P. oleivora*, 20 mature individuals were placed on each disk to obtain eggs. There were three replicates for each concentration tested, in addition to the control. After one day, females were removed from the discs and the eggs deposited were treated with five different concentrations of (CuNP) (40, 80, 160, 240 and 320 pp) and a control treatment with well water was sprayed as an untreated control. Trials were carried out in the laboratory with 12:12 h L: D at 31 ± 2°C and 55 ± 4% RH. Observations were conducted every 12 hours for a week to check on the hatching rate.

2.4 Statistical analysis

The percentage reduction in the average populations of *P. oleivora*, *E. orientalis* and *B. obovatus* and of the predatory mites' *A. swirskii* and *E. scutalis* were calculated using the equation of Henderson and Tilton (1955). Where: n = Where, n is the number of phytophagous mites or predatory mites, T= treated, C = control.

The average percentage of the number of larvae hatching from eggs of *P. oleivora*, *E. orientalis* and *B. obovatus* were calculated as follows (Equation 1):

$$Percentage\ hatchability = \frac{number\ of\ eggs\ hatched}{total\ number\ of\ eggs} \times 100 \quad (1)$$

The mortalities of the phytophagous mites and the predatory mite were calculated manually by direct observation. Thereafter, the obtained data for all variables were statistically analyzed using a One-way analysis of variance (ANOVA).

3. Results

The obtained results indicated that all stages of *P. oleivora*, *E. orientalis* and *B. obovatus*, including eggs, were susceptible to the tested copper nanoparticles (CuNP) while showing little effect on the mortality of the associated predatory mites, *A. swirskii* and *E. scutalis*. The mortality percentage was 15.24, 20.32, 46.32, 78.97 and 86.37% for *P. oleivora*, 6.87, 9.86, 28.91, 56.30 and 77.52% for *E. orientalis* and 8.38, 23.50, 48.83, 68.80 and 84.08% for *B. obovatus*, whereas the mortality percentage was 0.00, 0.56, 5.83, 9.91 and15.19% for *A. swirskii* and 0.44, 3.96, 6.93, 8.36 and 21.39% for *E. scutalis* one week after exposure to 40, 80, 160, 240 and 320 ppm of (CuNP), respectively, compared with the control (well water) (Tables 1-2). The maximum mortality percentage was 86.37% for *P. oleivora*, 77.52% for *E. orientalis* and 84.08% for *B. obovatus*, all obtained at 320 ppm of copper nanoparticles three days after exposure, whereas with other concentrations, the mortality increased with time. The five concentrations of copper nanoparticles were also found to be less toxic to *A. swirskii* and *E. scutalis* compared with *P. oleivora*, *E. orientalis* and *B. obovatus* one week after application.

The mortality percentage of *A. swirskii* and *E. scutalis* resulting from copper nanoparticles applied at concentrations of 40, 80, 160, 240 and 320 ppm did not exceed a low toxicity of 25% (referring to the International Organization for Biological and Integrated Control (IOBC) classification on the toxicity to non-target organisms). Generally, the applied copper nanoparticles showed lower toxicity and appeared to be selective to both predacious mite species *A. swirskii* and *E. scutalis* under field conditions. In addition, the obtained results indicate that copper nanoparticles are the most promising control agent against *P. oleivora*, *E. orientalis* and *B. obovatus* on orange trees (86.37, 77.52 and 84.08%). *P. oleivora* and *E. orientalis* seems to be more sensitive to copper nanoparticles than *B. obovatus*. However, it was slightly toxic by contact with *A. swirskii* and *E. scutalis* (15.19% and 21.39%) (Table 3).

Clearly, Copper nanoparticles not only kill *P. oleivora*, *E. orientalis* and *B. obovatus* but can also induce malformation of the eggs. Moreover, the obtained results showed that copper nanoparticles cause a reduction in the percentage of eggs hatching. The percentages of larvae hatching from eggs were 96.26, 80.00, 64.13, 45.66 and 32.17% for *P. oleivora*, 97.38,83.28,69.41, 48.01 and 35.29 for *E. orientalis* and 96.60, 76.92, 56.38, 40.55 and 33.28% for *B. obovatus* at 40, 80, 160, 240 and 320 ppm respectively, of copper nanoparticles 7 days after treatment compared with the control (well water) (Tables 4-5).

Table 1. Effect of copper nanoparticles on the citrus rust mite, *P. oleivora* and the citrus brown mite *E. orientalis* infested orange trees under field conditions.

No. of mites/leaf							
P. oleivora				***E. orientalis***			
Concentration (ppm)	**Pre-spray count**	**Average post-spray count***	**Reduction %****	**Concentration (ppm)**	**Pre-spray count**	**Average post-spray count***	**Reduction %****
Control	66.00	71.00	0.00 [a]	Control	18.33	19.00	0.00 [a]
40	68.00	62.00	15.24 [b]	40	23.66	18.66	6.87 [a]
80	70.00	60.00	20.32 [b]	80	21.00	19.66	9.68 [a]
160	71.33	41.00	46.32 [c]	160	**19.00**	14.00	28.91 [b]
240	84.00	19.00	78.97 [d]	240	17.66	8.00	56.30 [c]
320	75.00	11.00	86.37 [d]	320	20.00	4.66	77.52 [d]

*Counts made 1, 2, 3 days, and one-week post-treatment. **Mortality values calculated with the Henderson-Tilton's equation. Different letters in the vertical columns denote significant differences, (F-test, $P < 0.05$).

Table 2. Effect of copper nanoparticles on the false spider mite, *B. obovatus* infested orange trees under field conditions.

No. of mites/leaf			
Concentrations (ppm)	**Pre-spray count**	**Average post-spray count***	**Reduction %****
Control	14.66	15.00	0.00 [a]
40	16.00	15.00	8.38 [a]
80	15.33	12.00	23.50 [b]
160	14.00	7.33	48.83 [c]
240	15.66	5.00	68.80 [d]
320	16.33	2.66	84.08 [e]

*Counts made 1, 2, 3 days, and one-week post-treatment. **Mortality values calculated with Henderson-Tilton's equation. Different letters in the vertical columns denote significant differences, (F-test, $P < 0.05$).

Table 3. Corrected percentage mortality of the predatory phytoseiid mites, *A. swirskii* and *E. scutalis* associated with orange trees with five concentrations of copper nanoparticles under field conditions.

No. of predatory mites/leaf							
A. swirskii				***E. scutalis***			
Concentrations (ppm)	**Pre-spray count**	**Average post-spray count***	**Reduction %****	**Concentration (ppm)**	**Pre-spray count**	**Average post-spray count***	**Reduction %****
Control	6.00	5.66	0.00 [a]	Control	8.00	8.33	0.00 [a]
40	6.00	5.66	0.00 [a]	40	9.00	9.33	0.44 [a]
80	5.33	5.00	0.56 [a]	80	8.33	8.33	3.96 [a]
160	6.00	5.33	5.83 [a]	160	11.00	10.66	6.93 [a]
240	6.66	5.66	9.91 [a]	240	7.00	6.66	8.63 [a]
320	5.00	4.00	15.19 [b]	320	7.33	6.00	21.39 [b]

*Counts made 1, 2, 3 days, and one-week post-treatment. **Mortality values calculated with Henderson-Tilton's equation. Different letters in the vertical columns denote significant differences, (F-test, $P < 0.05$).

Table 4. Number of larvae hatching from eggs of the citrus rust mite, *P. oleivora* and the citrus brown mite *E. orientalis* treated with copper nanoparticles and under laboratory conditions.

Concentrations (ppm)	No. of eggs and larvae /leaf						
	Phyllocoptruta oleivora			*Eutetranychus orientalis*			
	No. of eggs pre-spray count	Average number of larvae post-spray count*	Hatching (%)**	Concentration (ppm)	No. of eggs pre-spray count	Average number of larvae post-spray count*	Hatching (%)**
Control	26.00	26.00	100.00 ᵃ	Control	12.00	12.00	100.00 ᵃ
40	27.00	26.00	96.29 ᵃ	40	13.00	12.66	97.38 ᵃ
80	30.00	26.00	80.00 ᵇ	80	14.00	11.66	83.28 ᵇ
160	35.33	22.66	64.13 ᶜ	160	12.00	8.33	69.41 ᶜ
240	30.66	14.00	45.66 ᵈ	240	16.66	8.00	48.01 ᵈ
320	29.00	9.33	32.17 ᵉ	320	17.00	6.00	35.29 ᵉ

*Counts made one-week post treatment. **Hatching percentage calculated with percentage hatchability equation. Different letters in the vertical columns denote significant differences, (F-test, P < 0.05).

Table 5. The number of larvae hatching from eggs of the false spider mite, *B. obovatus* treated with copper nanoparticles under laboratory conditions.

Concentrations (ppm)	No. of eggs and larvae /leaf		
	Copper nanoparticles		
	No. of eggs pre-spray count	Average number of larvae post-spray count*	Hatching (%)**
Control	10.00	10.00	100.00 ᵃ
40	11.00	10.66	96.90 ᵃ
80	13.00	10.00	76.92 ᵇ
160	13.00	7.33	56.38 ᶜ
240	12.33	5.00	40.55 ᵈ
320	14.00	4.66	33.28 ᵉ

*Counts made one-week post treatment. **Hatching percentage calculated with percentage hatchability equation. Different letters in the vertical columns denote significant differences, (F-test, P < 0.05).

4. Discussion

The use of nanotechnology for phytophagous mites' management is a potential biotic alternative to chemical acaricides (Al-Azzazy et al., 2019). Various formulations of nanomaterials have been used as acaricides, fungicides or insecticides for safe, effective and successful pest management (Rai et al., 2018; Korghond et al., 2021). Quite a few studies have examined the efficacy of copper nanoparticles towards phytophagous mites and predatory mites. Our results are an additional example of the effectiveness of metal pesticides against phytophagous mites. In this study, the acute toxicity of copper nanoparticles to all stages of *P. oleivora*, *E. orientalis* and *B. obovatus*, including eggs and predatory mites *A. swirskii* and *E. scutalis* were investigated. Our results showed that the tested CuNP had contact toxicity against all stages of *P. oleivora*, *E. orientalis* and *B. obovatus*, including eggs with little negative effects on their predatory mites. In a previous study, Dorri et al. (2018) investigated the toxicity effect of the CuO nanoparticles and nanocapsule on the two-spotted spider mite *Tetranychus urticae* Koch. They reported that copper nanoformulations have a significant impact on the reducing population of *T. urticae*. In addition, Atwa et al. (2017) stated higher larval mortality of the Egyptian armyworm *Spodoptera littoralis* that fed on a diet incorporated with CuO nanoparticles when compared to the control. As a metal, it is thought that copper nanoparticles (CuNP) act by combining with the –SH groups of key enzymes and impair vital biological systems (Allaker and Memarzadeh, 2014). Results from many studies comparing the toxicity of different metal-containing nanoparticles have shown copper-containing nanoparticles to be highly toxicity when compared with other nanoparticles (Lanone et al., 2009; Sun et al., 2012).

The toxicity of Cu NPs seems to be involved rapid disruption of the cell membrane integrity by the metal release at the cell membrane surface, resulting in membrane damage (Karlsson et al., 2013). In addition to that, the release of copper ions within e cells leads to high levels of toxicity (Cronholm et al., 2013). The present results showed a significant difference between phytophagous mites and predatory mites ($P < 0.001$), indicating lower toxicity of CuNP towards non-target predatory mites. We hypothesize that the copper nanoparticles studied might affect phytophagous mites via contact poison while crawling on sprayed leaves, via digestive or by direct reception of spray droplets. The higher activity of copper nanoparticles towards phytophagous mites can be explained by the ability of copper nanoparticles to penetrate their thin body wall, inter-skeletal membranes or through body openings. In the case of predatory mites, the thicker exoskeleton with shields on ventral and dorsal sides (Duso and Fontana, 2002; Zannou et al., 2007) may prevent penetration of copper nanoparticles into body tissues. in addition to that, predacious mites feed only on active phytophagous mites and it is Improbable to feed on intoxicated individuals. Thus, decreasing the dose of copper nanoparticles received by predacious mites. In a related study, silver nanoparticles (AgNP) showed miticidal activity against eggs and moving stages of eriophyid mite *Aculops lycopersici* (Massee) and tetranychid mite *T. urticae*, with low detrimental effects on predatory mites, *Neosiulus cucumeris Oudemans* and *Euseius scutalis* (Athias-Henriot) (Al-Azzazy et al., 2019).

Any potential biopesticide should infect all stages of the target pest, namely, the mobile and quiescent stages in addition to the feeding and non-feeding stages (Zhang et al., 2017). In the current study, when *P. oleivora*, *E. orientalis* and *B. obovatus* eggs were treated with copper nanoparticles before hatching, the results showed a varied percentage of hatchability in response to different concentrations. The results revealed that the eggs were infected with (CuNP) even at lower concentrations. In all treatments, significant differences were observed between the percentages of eggs hatched in (CuNP) treatments and the control, except the first concentration of 40 ppm, which is consistent with a previous study on the influence of silver nanoparticles against different mite species (Al-Azzazy et al., 2019). In a similar study, silver nanoparticles (AgNP) showed miticidal activity toward eggs, nymphs, and adults of spider mite, *T. urticae* (Pavela et al., 2017).

5. Conclusion

Phyllocoptruta oleivora, *E. orientalis* and *B. obovatus* are major pests causing high-yield losses in orange trees. Copper nanoparticles were found to be an effective acaricide against these pests under field conditions at low concentrations. In summary, the application of copper nanoparticles will be useful for reducing the risks of chemical acaricides. Our findings showed that copper nanoparticles have a safety profile when compared with other pesticides as they spared a reasonable number of predacious mites. Hence, copper nanoparticles would be an eco-friendly component, for controlling mites, in an Integrated Pest Management (IPM) strategy. These field obtained results comprise broad research prospects including a study to gain a fundamental understanding of the interaction between nanoscale materials and mites and the development of nano pesticide formulations using nanoparticles as active acaricide agents.

References

AL-AZZAZY, M.M., GHANI, S.B.A. and ALHEWAIRINI, S.S., 2019 [viewed 23 January 2023]. Field evaluation of the efficacy of silver nanoparticles (AgNP) against mites associated with tomato plants in greenhouses. *Pakistan Journal of Agricultural Sciences* [online], vol. 56, no. 1, pp. 283-288. Available from: https://www.pakjas.com.pk/TableOfContents

ALLAKER, R.P. and MEMARZADEH, K., 2014. Nanoparticles and the control of oral infections. *International Journal of Antimicrobial Agents*, vol. 43, no. 2, pp. 95-104. http://dx.doi.org/10.1016/j.ijantimicag.2013.11.002. PMid:24388116.

ATWA, A.A., SALAH, N.A., KHAFAGI, W.E. and AL-GHAMDI, A.A., 2017. Insecticidal effects of pure and silver doped copper oxide nanosheets on *Spodoptera littoralis* (Lepidoptera: noctuidae). *Canadian Entomologist*, vol. 149, no. 5, pp. 677-690. http://dx.doi.org/10.4039/tce.2017.36.

BARIK, T.K., SAHU, B. and SWAIN, V., 2008. Nano-silica: from medicine to pest control. *Parasitology Research*, vol. 103, no. 2, pp. 253-258. http://dx.doi.org/10.1007/s00436-008-0975-7. PMid:18438740.

CHILDERS, C.C., 1994. Biological control of phytophagous mites on Florida citrus utilizing predatory arthropods. In: D. ROSEN, F.D. BENNETT and J.L. CAPINERA, eds. *Pest management in the subtopics, biological control: a Florida perspective*. Andover: Intercept Ltd., pp. 255-288.

CHILDERS, C.C., RODRIGUES, J.C.V. and WELBOURN, W.C., 2003. Host plants of *Brevipalpus californicus, B. obovatus*, and *B. phoenicis* (Acari:Tenuipalpidae) and their potential involvement in the spread of viral diseases vectored by these mites. *Experimental & Applied Acarology*, vol. 30, no. 1-3, pp. 29-105. http://dx.doi.org/10.1023/B:APPA.0000006544.10072.01. PMid:14756412.

CRONHOLM, P., KARLSSON, H.L., HEDBERG, J., LOWE, T.A., WINNBERG, L., ELIHN, K., WALLINDER, I.O. and MÖLLER, L., 2013. Intracellular uptake and toxicity of Ag- and CuO nanoparticles–a comparison between nanoparticles and their corresponding metal ion. *Small*, vol. 9, no. 7, pp. 970-982. http://dx.doi.org/10.1002/smll.201201069. PMid:23296910.

DORRI, H.R., KHAGHANI, S., MOGHADAM, A., GHANBARI, D. and BIHAMTA, M.R., 2018. The effect of copper nanocapsules on the control of two spotted spider mite (*Tetranychus urticae*). *Journal of Nanostructures*, vol. 8, no. 3, pp. 316-324. http://dx.doi.org/10.22052/JNS.2018.03.012.

DUSO, C. and FONTANA, P., 2002 [viewed 23 January 2023]. On the identity of *Phytoseius plumifer* (Canestrini & Fanzago) (Acari: phytoseiidae). *Acarologia* [online], vol. 2, no. 2, pp. 127-136. Available from: https://www1.montpellier.inra.fr/CBGP/acarologia/article.php?id=98

EL-BANHAWY, M.E., 1977. Effect of photoperiod, light intensity and temperature on the development and reproduction of the predacious mite *Amblyseius brazilli* (Mesostigmata, Phytoseiidae). *Revista Brasileira de Biologia*, vol. 37, no. 3, pp. 579-583.

GARZIA, G.T. and LILLO, E., 2018. Geographic distribution of *Phyllocoptruta oleivora* in the Mediterranean Basin, with particular emphasis on Italy. *Systematic and Applied Acarology*, vol. 23, no. 6, pp. 1021-1023. http://dx.doi.org/10.11158/saa.23.6.1.

GERSON, U. and VACANTE, V., 2011. *Integrated control of citrus pests in the Mediterranean region*. Sharjah: Bentham Science Publishers. Acari, pp. 88-108. https://doi.org/10.2174/97816080529431120101.

GERSON, U., 2003. Acarine pests of citrus: overview and non-chemical control. *Systematic and Applied Acarology*, vol. 8, pp. 3-12. http://dx.doi.org/10.11158/saa.8.1.1.

GOSWAMI, A., ROY, I., SENGUPTA, S. and DEBNATH, N., 2010. Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. *Thin Solid Films*, vol. 519, no. 3, pp. 1252-1257. http://dx.doi.org/10.1016/j.tsf.2010.08.079.

HENDERSON, C.F. and TILTON, E.W., 1955. Test with acaricides against the brown wheat mite. *Journal of Economic Entomology*, vol. 48, no. 2, pp. 157-161. http://dx.doi.org/10.1093/jee/48.2.157.

HUANG, J., LI, Q., SUN, D., LU, Y., SU, Y., YANG, X., WANG, H., WANG, Y., SHAO, W., HE, N., HONG, J. and CHEN, C., 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology*, vol. 18, no. 10, p. 105104. http://dx.doi.org/10.1088/0957-4484/18/10/105104.

JEPPSON, L.R., KEIFER, H.H. and BAKER, E.W., 1975. *Mites injurious to economic plants*. Berkeley: University of California Press. http://dx.doi.org/10.1525/9780520335431.

KARLSSON, H.L., CRONHOLM, P., HEDBERG, Y., TORNBERG, M., BATTICE, L., SVEDHEM, S. and WALLINDER, I.O., 2013. Cell membrane damage and protein interaction induced by copper containing nanoparticles: importance of the metal release process. *Toxicology*, vol. 313, no. 1, pp. 59-69. http://dx.doi.org/10.1016/j.tox.2013.07.012. PMid:23891735.

KIAUNE, L. and SINGHASEMANON, N. (2011). Pesticidal Copper (I) Oxide: environmental fate and aquatic toxicity. In: D. Whitacre, ed. *Reviews of environmental contamination and toxicology.* New York: Springer, vol. 213.. PMid:21541846.

KITHERIAN, S., 2016. Nano and bio-nanoparticles for insect control. *Research Journal of Nanoscience and Nanotechnology*, vol. 7, no. 1, pp. 1-9. http://dx.doi.org/10.3923/rjnn.2017.1.9.

KORGHOND, G.T., SAHEBZADEH, N., ALLAHYARI, H. and RAMROODI, S., 2021. Acute toxicity and sublethal effects of metal oxide nanoparticles against the bulb mite. *Systematic and Applied Acarology*, vol. 26, no. 4, pp. 788-800. http://dx.doi.org/10.11158/saa.26.4.9.

KUBO, K.S., NOVELLI, V.M., BASTIANEL, M., LOCALI-FABRIS, E.C., ANTONIOLI-LUIZON, R., MACHADO, M.A. and FREITAS-ASTÚA, J., 2011. Detection of Brevipalpus-transmitted viruses in their mite vectors by RT-PCR. *Experimental & Applied Acarology*, vol. 54, no. 1, pp. 33-39. http://dx.doi.org/10.1007/s10493-011-9425-9. PMid:21279538.

KUMAR, A., CHOUDHARY, A., KAUR, H., MEHTA, S. and HUSEN, A., 2021. Metal-based nanoparticles, sensors, and their multifaceted application in food packaging. *Journal of Nanobiotechnology*, vol. 19, no. 1, p. 256. http://dx.doi.org/10.1186/s12951-021-00996-0. PMid:34446005.

LANONE, S., ROGERIEUX, F., GEYS, J., DUPONT, A., MAILLOT-BMARECHAL, E., BOCZKOWSKI, J., LACROIX, G. and HOET, P., 2009. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. *Particle and Fibre Toxicology*, vol. 6, no. 1, p. 14. http://dx.doi.org/10.1186/1743-8977-6-14. PMid:19405955.

LIU, Q., ZHOU, D., NISHIO, K., ICHINO, R. and OKIDO, M., 2010. Effect of reaction driving force on copper nanoparticle preparation by aqueous solution reduction method. *Materials Transactions*, vol. 51, no. 8, pp. 1386-1389. http://dx.doi.org/10.2320/matertrans.M2010067.

LIU, Q., ZHOU, D., YAMAMOTO, Y., ICHINO, R. and OKIDO, M., 2012. Preparation of Cu nanoparticles with NaBH4 by aqueous reduction method. *Transactions of Nonferrous Metals Society of China*, vol. 22, no. 1, pp. 117-123. http://dx.doi.org/10.1016/S1003-6326(11)61149-7.

PITTARATE, S., RAJULA, J., RAHMAN, A., VIVEKANANDHAN, P., THUNGRABEAB, M., MEKCHAY, S. and KRUTMUANG, P., 2021. Insecticidal effect of zinc oxide nanoparticles against *Spodoptera frugiperda* under laboratory conditions. *Insects*, vol. 12, no. 11, p. 1017. http://dx.doi.org/10.3390/insects12111017. PMid:34821816.

RAI, M., INGLE, A.P., PANDIT, R., PARALIKAR, P., SHENDE, S., GUPTA, I., BISWAS, J.K. and SILVA, S.S., 2018. Copper and copper nanoparticles: role in management of insect-pests and pathogenic microbes. *Nanotechnology Reviews*, vol. 7, no. 4, pp. 303-315. http://dx.doi.org/10.1515/ntrev-2018-0031.

RAMEZANI, M., RAMEZANI, F. and GERAMI, M., 2019. Nanoparticles in pest incidences and plant disease control. In: D. PANPATTE and Y. JHALA, eds. *Nanotechnology for agriculture: crop production & protection*. Singapore: Springer, pp. 233-272. http://dx.doi.org/10.1007/978-981-32-9374-8_12.

RODRIGUES, J.C.V. and CHILDERS, C.C., 2013. Brevipalpus mites (Acari: Tenuipalpidae): vectors of invasive, non-systematic cytoplasmic and nuclear viruses in plants. *Experimental & Applied Acarology*, vol. 59, no. 1-2, pp. 165-175. http://dx.doi.org/10.1007/s10493-012-9632-z. PMid:23203501.

STADLER, T., BUTELER, M., WEAVER, D. and SOFIE, S., 2012. Comparative toxicity of nanostructured alumina and a commercial inert dust for Sitophilus oryzae (L.) and *Rhyzopertha dominica* (F.) at varying ambient humidity levels. *Journal of Stored Products Research*, vol. 48, pp. 81-90. http://dx.doi.org/10.1016/j.jspr.2011.09.004.

SUN, T., YAN, Y., ZHAO, Y., GUO, F. and JIANG, C., 2012. Copper oxide nanoparticles induce autophagic cell death in A549 cells. *PLoS One*, vol. 7, no. 8, p. e43442. http://dx.doi.org/10.1371/journal.pone.0043442. PMid:22916263.

WALTER, D.E., HALLIDAY, R.B. and SMITH, D., 1995. The oriental red mite, *Eutetranychus orientalis* (Klein) (Acarina: Tetranychidae), in Australia. *Australian Journal of Entomology*, vol. 34, no. 4, pp. 307-308. http://dx.doi.org/10.1111/j.1440-6055.1995.tb01345.x.

WANG, L., LI, X., ZHANG, G., DONG, J. and EASTOE, J., 2007. Oil-in-water nanoemulsions for pesticide formulations. *Journal of Colloid and Interface Science*, vol. 314, no. 1, pp. 230-235. http://dx.doi.org/10.1016/j.jcis.2007.04.079. PMid:17612555.

WANG, X., XU, J., WANG, X., QIU, B., CUTHBERTSON, A.G.S., DU, C., WU, J. and ALI, S., 2019. *Isaria fumosorosea*-based zero-valent iron nanoparticles affect the growth and survival of sweet potato whitefly, *Bemisia tabaci* (Gennadius). *Pest Management Science*, vol. 75, no. 8, pp. 2174-2181. http://dx.doi.org/10.1002/ps.5340. PMid:30653825.

ZANNOU, I.D., MORAES, G.J., UECKERMANN, E.A., OLIVERIA, A.R., YANINEK, J.S. and HANNA, R., 2007. Phytoseiid mites of the subtribe Amblyseiina (Acari: Phytoseiidae: Amblyseiini) from Sub-Saharan Africa. *Zootaxa*, vol. 1550, no. 1, pp. 1-47. http://dx.doi.org/10.11646/zootaxa.1550.1.1.

ZHANG, C., ALI, S., MUSA, P.D., WANG, X.-M. and QIU, B.-L., 2017. Evaluation of the pathogenicity of *Aschersonia aleyrodis* on *Bemisia tabaci* in the laboratory and greenhouse. *Biocontrol Science and Technology*, vol. 27, no. 2, pp. 210-221. http://dx.doi.org/10.1080/09583157.2016.1274878.

ZHANG, Z.-Q., 2003. *Mites of greenhouses: identification, biology and control.* Wallingford: CABI Publishing. Part II: pest mites, pp. 54-61 http://dx.doi.org/10.1079/9780851995908.0000.