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Toxicities of synthesized green iron oxide nanoparticles on *Trialeurodes vaporariorum* and their effects on antioxidant enzymes and lipid peroxidation

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Abstract. In this study, lethal concentrations of iron-oxide nanoparticles (FeONPs) synthesized from plants (alfalfa, basil, eucalyptus, cinnamon) were investigated for their effects on mortality, antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferases (GST)) and lipid peroxidation (LPO) to develop an effective and inexpensive method for controlling the economic losses caused by *Trialeurodes vaporariorum*. The characterization of prepared FeONPs with plant extracts was performed using an ultraviolet-visible spectrophotometer (UV-Vis), Fourier transforms infrared spectroscopy (FTIR), and field emission scanning electron microscopy (FESEM) to examine the size, elemental composition, and morphology. The UV-Vis results of the green synthesized FeONP showed a peak at 256 nm, confirming the synthesis of FeONPs. An FTIR detected strong absorption peaks at 3420, 3446, 3421, and 3446 cm^{-1} (hydroxyl group); 2925 and 2926 cm^{-1} (C–H group); 1618, 1636, 1647, and 1716 cm^{-1} (C=O group), at approximately 1030, 1034, 1044, and 1048 cm^{-1} (C–O group). Bioassay tests have been conducted with adults of *T. vaporariorum* in plastic leaf cages treated with different concentrations of FeONPs. SPSS 21 software (IBM, New York, US) with a confidence interval (CI) of 95% and Probit analysis was employed to determine lethal concentrations (LC_{10}, LC_{25}, LC_{30}, and LC_{50}) of synthesized FeONPs. The results demonstrated that basil, alfalfa, eucalyptus and cinnamon FeONPs effectively killed 50% of *T.vaporariorum* adults, at concentrations of 4.876, 16.935, 10.584 and 11.948 mg L^{-1}, respectively. Significant increases in LPO, GST, GPX, CAT, and SOD activities were observed in *T.vaporariorum* adults exposed to the lethal concentrations of different FeONPs. Moreover, the findings suggested that exposure to FeONPs induced oxidative stress in *T. vaporariorum* adults and may decline their longevity in greenhouses.

Keywords: Biomarker, Enzymatic antioxidants, Nanotoxicology, Oxidative stress, Reactive oxygen species

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Introduction

Over 250 plant species are identified as *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) hosts. This pest, also known as the greenhouse whitefly (GHWF), primarily attacks ornamental and vegetable crops grown in greenhouses. As a vector for certain plant viruses, GHWF can reduce the longevity and productivity of its hosts, resulting in significant economic loss. Through phloem-feeding, the transmission of pathogenic plant viruses, and the formation of sticky honeydew for producing sooty mold, whitefly species can cause billions of

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dollars in economic damage (Liang *et al.*, 2007). The widespread use of pesticides to eradicate GHWF is associated with phytotoxicity, chemical insecticide resistance, and pesticide residues on vegetables (Liang *et al.*, 2007).

Considering the demand for reducing pesticide use and producing residue-free food, alternative control strategies, such as novel pest management technologies and biological control, are required. In recent years, nanotechnology has been emphasized in pest management, and it is believed that nanotechnology will revolutionize agriculture in the near future. A new range of insecticides, namely green synthesis of nanoparticles (NPs) which are environmentally safe, have been recently investigated for controlling agricultural pests and human disease vectors. These insecticides can be utilized to develop pesticides, insecticides, and insect repellents based on their physical properties. It was hypothesized that the imbalance between free radicals and antioxidants in insects is responsible for the toxicity of pesticides such as metal NPs (Goswami *et al.*, 2010; Parisi *et al.*, 2015).

Although various physicochemical techniques have been introduced to develop NPs, synthesis via environmentally biogenic and nontoxic techniques is intriguing, especially for invasive applications. Biogenic synthesis is associated with less environmental damage compared to some physicochemical production methods. This method can also be applied to form large quantities of contamination-free NPs with well-defined morphology and size. In the past few decades, the use of plants or whole plant tissues and extracts to reduce metal salts to NPs has received considerable attention due to its ease of application (Mittal *et al.*, 2013). In developing NPs, it is typically simpler to use plant extracts than whole plant extracts or plant tissues. In fact, the synthesis of NPs through the use of plant extracts is becoming an increasingly intriguing technique. Processes for producing NPs using plant extracts are easily scalable and may be less expensive than methods based on relatively costly microbial processes and whole plants. Extracts can serve as both stabilizing and reducing agents in NP synthesis. In addition, the extracted source affects the properties of NPs because different types of extracts contain various combinations and concentrations of organic reducing agents (Mittal *et al.*, 2013).

Some evidence indicates that insecticide exposure is associated with the formation of superoxide-hydrogen peroxide (H_2O_2) and changes in the concentration of antioxidant enzymes in animals (Yu *et al.*, 2011). The pro-oxidative effects of xenobiotics in insects are ameliorated by antioxidant enzymes, which can protect them against damage caused by reactive oxygen species (ROS) (Büyükgüzel, 2009). Due to the presence of unpaired valence shell electrons, ROS are recognized as highly reactive molecules that interact with essential macromolecules (e.g., proteins, DNA, and lipids), particularly those within the cell membrane, and cause alternations in physiological processes. Insect antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferases (GST), and catalase (CAT). GST exhibits different substrate properties, among antioxidant enzymes, contributing to xenobiotic detoxification (Vontas *et al.*, 2001). SOD, which catalyzes the dismutation of superoxide radical dismutation to H_2O_2 and oxygen, appears to be the most important response to dietary prooxidants. Additionally, CAT converts H_2O_2 to oxygen and water (Felton & Summers, 1995).

Green synthesized metal NPs have recently been used are applied to control agricultural pests; however, their mode of action in insects is poorly understood. In fact, there is no information regarding the effect of green synthesized metal NPs on the physiology of insects. Consequently, this study assessed the impact of green-synthesized iron oxide nanoparticles (FeONPs) on parameters such as oxidative stress that may affect the physiology of GHWF. It was assumed that antioxidative enzymes are the most important biomarkers for assessing insect tissue damage caused by NP treatment (Yasur & Pathipati, 2015).

Materials and methods

Green synthesis of FeONPs

Fresh leaves of alfalfa (*Medicago sativa*), basil (*Ocimum basilicum*), and eucalyptus (*Eucalyptus microtheca*) were collected. The barks of cinnamon (*Cinnamomum zeylanicum*) were purchased from the local market. The voucher specimens were stored in the entomology laboratory (Faculty of Agriculture, University of Zabol).

Spring-Fresh alfalfa, basil, eucalyptus plants (leaves), and cinnamon bark (purchased randomly from the local market) were repeatedly washed and cut. Subsequently, 20 g of chopped plants were boiled for 5-15 min at 60°C in 200 mL of ultra-pure water before being filtered (Whatman filter paper no.1, 125 mm diameter). Each plant's filtered extract was used separately to synthesize iron oxide nanoparticles (Wang *et al*., 2014).

FeONPs derived from plant extracts (alfalfa: A-FeONPs, basil: B-FeONPs, eucalyptus: E-FeONPs, cinnamon: C-FeONPs) were synthesized by adding 0.10 M $FeSO_4$ $7H_2O$ (as a precursor) to 10 mL of each extract, vigorously shaking, and boiling at 50°C. The solutions' transformation from light yellow to black indicated the formation of FeONPs. The residue was obtained 24 hours later using a centrifuge and then dried at 40°C in an oven (12 h). After the purified FeONPs were dried, they were stored at 4°C for future experiments (Devatha *et al*., 2016).

Characterization of prepared FeONPs with plant extracts was done. 1 mL of each FeONP was diluted with distilled water (2 mL); then, the UV-Vis spectrum of the solution was measured between wavelength 200 to 730 nm in a spectrophotometer (Jenway 5405, USA), with a resolution of 1 nm. The dried FeONPs were analyzed using the potassium bromide pellet method (1:100 ratio) and the spectrum was recorded using Fourier transform infrared (FTIR) spectroscopy (Bruker optics Ft Tensor, 27, Germany). Field emission scanning electron microscopy (FESEM) (Hitachi S4160, Japan) was employed to investigate the size, elemental composition, and morphology of the FeONPs.

Insect rearing and toxicological experiments

The pest-infested greenhouse tomato (*Lycopersicom esculentum* Mill) leaves were sampled for adults of *T.vaporariorum*. Before being used in experiments, adults were raised on young green beans (*Phaseolus vulgaris* L.) in a laboratory greenhouse for at least five generations (25 ± 1 °C, 50-60% RH and a 16:8 h light/dark photoperiod, Khooshe-Bast *et al*., 2016).

Preliminary tests were conducted with 1 to 30 mg L^{-1} concentration ranges of the synthesized FeONPs to identify concentrations with 10-90% mortality. Then, 100 adults of the same age of *T. vaporariorum* (for each concentration and a replication) were transferred to Petri dishes (1.5 cm height × 8 cm diameter) with filter paper-covered bottoms, where the desired concentrations of 5 mL were sprayed on them using a handy sprayer first. Before spraying, all prepared concentrations were sonicated (10 minutes) to prevent nanoparticle precipitation. After completion, the insects were transferred to leaf cages affixed to green bean leaves. After 24 h, the number of dead insects was tallied, and mortality was calculated after three replications. Distilled water (5 mL) was a control (Khooshe-Bast *et al*., 2016).

Biochemical experiments

This experiment was conducted with LC_{10}, LC_{25}, LC_{30}, and LC_{50} concentrations of green synthesized nanoparticles to determine their lethal effects. 100 *T. vaporariorum* adults were added to a cage containing a bean seedling. Then, 5 mL of different concentrations of FeONPs (which caused 10, 25, 30, and 50 percent of mortalities in preliminary experiments) were y handy-sprayed separately on the bean seedling beneath the cage. Three replicates of each concentration and FeONPs were conducted. The adults were confined in environmental control chambers with greenhouse conditions (25 ± 1 °C, 50-60% RH and a 16:8 h light/dark photoperiod). Alive adults of *T. vaporariorum* were collected to rearing conditions and following FeNPs treatment, followed by a 24-h exposure period. The crude enzyme was extracted by homogenizing insects in 100 mM phosphate buffer (pH7) and centrifuging at 10,000 g (4 °C, 10 min). Supernatants were used as the source of enzymes and stored at -20°C.

To measure malondialdehyde (MDA), live insects from each treatment were homogenized in 6 mL of hexane. The homogenates were agitated and allowed to stand for 24 hours before being filtered to remove residue and rinse the filter system with three portions of 0.5 mL of hexane. Supernatants were used to determine of malondialdehyde (MDA) content (Olmedo *et al*., 2015). The MDA level as an index of lipid peroxidation (LPO) was expressed as nmol mg-1 of protein. The MDA content was evaluated using the methodology described by Cervera *et al*. (2003). The absorbance of the supernatant was measured at 535 nm and corrected for non-specific absorbance at 600 nm. All the experiments were conducted in triplicate.

To determine catalase (CAT) activity, 50 µL of the extracted enzyme from treated *T. vaporariorum* was combined with 450 µL distilled water and 500 µL of 30 mM H_2O_2. The hydrolysis of H_2O_2 was measured at 240 nm over 3 min. CAT activity was expressed regarding mmol mg^{-1} of H_2O_2, using an extinction coefficient of 0.0394 $mM^{-1}cm^{-1}$. A blank without homogenate was used to control the nonenzymatic hydrolysis of peroxide in phosphate buffer (Aebi, 1984).

Glutathione S-transferases (GST) activity was determined using 1-chloro-2,4-dinitrobenzene and reduced glutathione as substrates according to the method described by Habig *et al.* (1974). The variation in absorbance was continuously measured for 5 min at 340 $nm^{-1}min^{-1}mg^{-1}$ protein.

Glutathione peroxidase (GPX) activity was determined according to the method in Lukasik and Golawska (2007). The absorbance was recorded at 340nm for 3min against the control. GPX activity was quantified as nmol NADPH $min^{-1}mg^{-1}$ protein utilizing an extinction coefficient of 0.00373 mM^{-1} cm^{-1}.Polyphenol oxidase (PPO) activity was determined using the method described by Ma *et al.* (2008). The absorbance was measured at 420 nm^{-1} min^{-1} mg^{-1} protein. The activity of superoxide¬dismutase (SOD) was measured per the method by Li *et al.* (1994). The reaction mixture was incubated at 25°C (15 min). The absorbance was measured at 560 nm^{-1} min^{-1} mg^{-1} protein. Peroxidase activity (POD) was measured according to the method described by Li *et al.* (1994). The absorbance was measured at 430 nm^{-1} min^{-1} mg^{-1} protein. The protein concentration in *T. vaporariorum* tissues was determined using the method outlined by Lowry *et al.* (1951). 20 µl of the sample was added to 100 µl of reagent and incubated for 30 min prior to reading the absorbance at 545 nm.

Statistical analysis

SPSS 21 software (IBM, New York, US) with confidence limits of 95 % and probit analysis was used to determine lethal concentrations (LC_{10}, LC_{25}, LC_{30}, and LC_{50}) of synthesized FeONPs. The Percentage of mortalities was adjusted using Abbott's formula (1925). The means of enzyme activities were compared using a one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests with a significance level of $p<0.05$.

Results

The colloidal FeONP solution's UV-Vis spectrograph was evaluated concerning time. The green synthesis of FeONPs was confirmed by observing a peak at 256 nm in the absorption spectrum of extracts at different wavelengths (Fig 1). Biomolecules that could account for the reduction of "Fe" ions and act as potential capping agents of reduced FeONPs, thereby contributing to NP stabilization, were identified via FTIR analysis. Fig 2 displays the FTIR data for cinnamon (A), alfalfa (B), basil (C), and eucalyptus (D) extracts. As evident, strong absorption peaks at 3420, 3446, 3421, and 3446cm^{-1}, are attributed to the stretching of the hydroxyl group (–OH) from the free H2O or alcohol group. The presence of a C–H group can potentially produce absorption peaks at approximately 2925 and 2926cm^{-1} representating of the C=O group. The peaks at approximately 1618, 1636, 1647, and 1716cm^{-1}. Additionally, the C–O group in C–CCOOR is capable of producing the intense band at approximately 1030, 1034, 1044, and 1048cm^{-1}. An example of a field emission scanning electron microscopy (FESEM) image for synthesized FeONPs with basil leaf extract is shown in Fig 3.

Table 1 displays the insecticidal activities of various green-synthesized FeONPs against *T. vaporariorum*. The results revealed that the FeONPs synthesized with basil leaf extract (B-FeONPs) could effectively kill 50% and 25 % of T. *vaporariorum* at concentrations of 4.876 and 2.697 mg L^{-1}, which were below the LC_{50} and LC_{25} of E-FeONPs, C-FeONPs, and A-FeONPs, respectively (Table 1). The bioassay results demonstrated that FeONPs synthesized from alfalfa (A-FeONPs) had a lesser impact on *T. vaporariorum*. Adult *T. vaporariorum* contained 0.72 nmol mg^{-1} protein of MDA in the control group. There was a proportional increase (p =0.002, F-value=7.19) in LPO when *T. vaporariorum* adults were exposed to FeONPs at varying concentrations (concentrations causing different levels of mortality) (Fig 4). The effects of different concentrations of FeONPs on the activity of antioxidant enzymes (GST, GPX, CAT, and SOD) in *T. vaporariorum* adults are shown in Table 2.

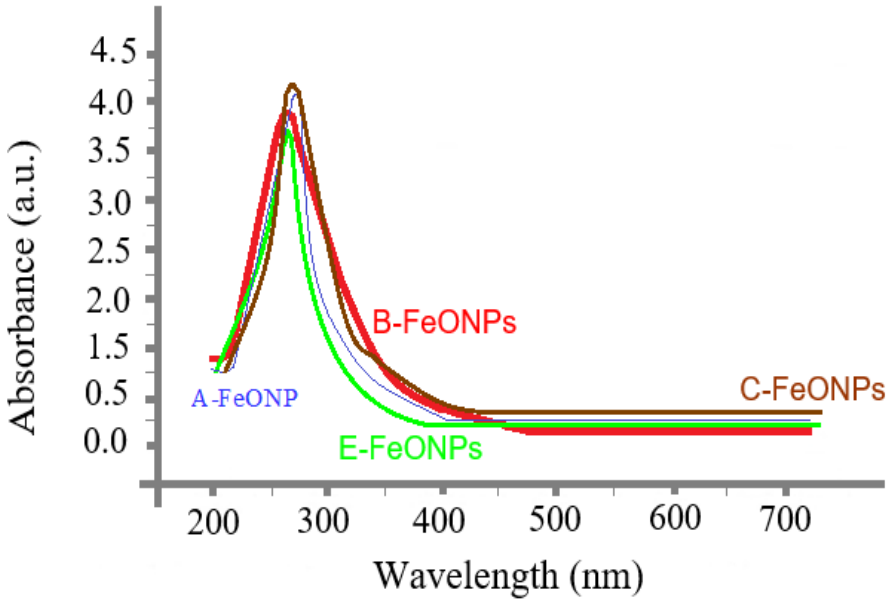

Fig 1. UV-vis spectrograph of FeONPs

A significant increase in the level of these enzymes was observed in *T. vaporariorum* adults exposed to concentrations 1, 2, 3, and 4 (which showed 10, 25, 30, and 50% mortalities on the GHWF in the bioassay tests) of different FeONPs in comparison with the control insects (p<0.05, F-value=4.36). The enzyme activities increased in a dose-dependent manner in different FeONPs-fed GHWF adults (p=0.0001, F-value=8.22).

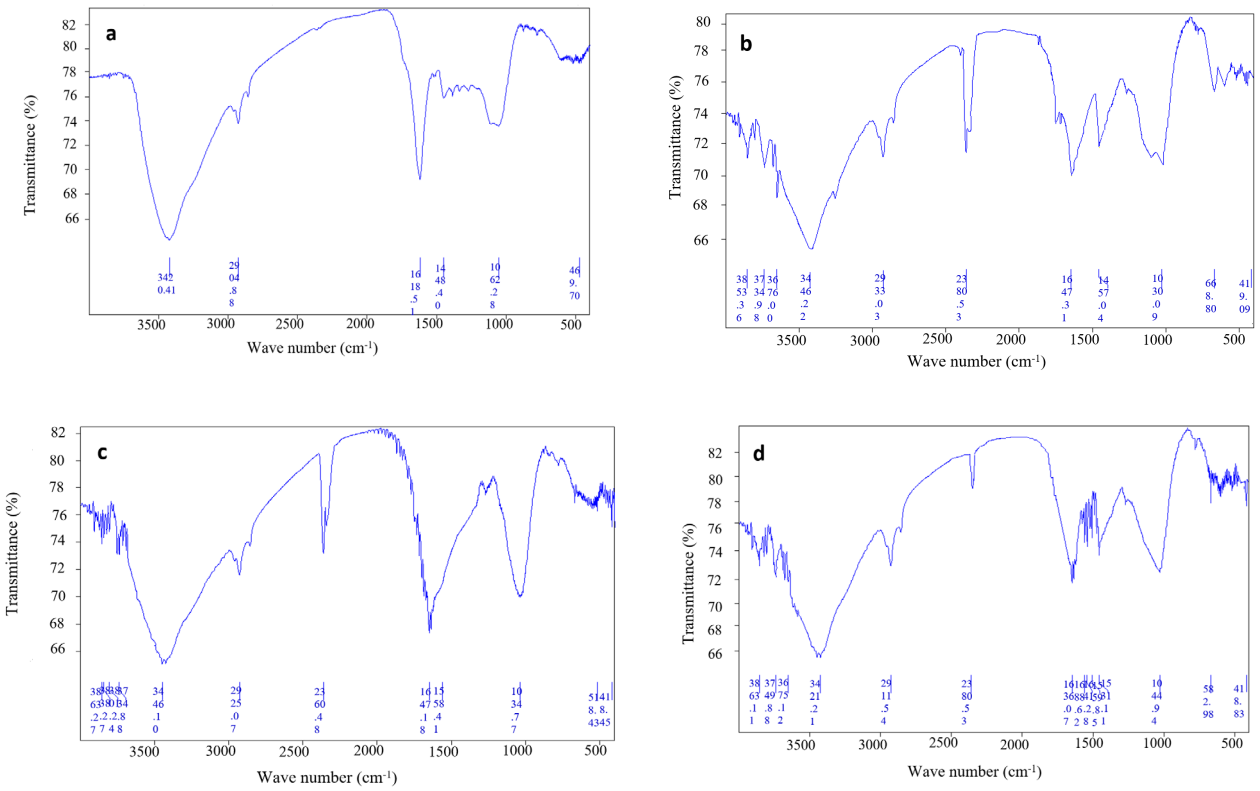

Fig 2. FTIR of FeONPs synthesized using different leaf extract. (A): Cinnamon, (B): Alfalfa, (C): Basil, (D): Eucalyptus

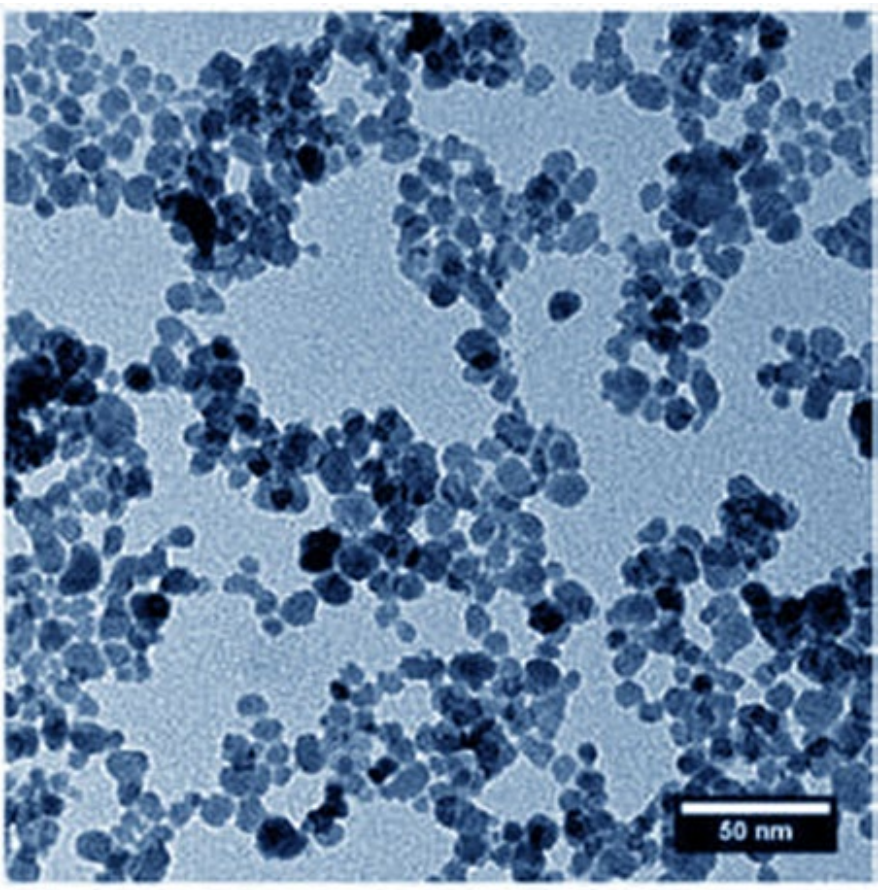

Fig 3. Field emission scanning electron microscopy (FESEM) images obtained for FeONPs synthesized using basil leaf extract

In our study, when *T. vaporariorum* was treated with varying concentrations of B-FeONPs (1.583, 2.697, 3.077, and 4.876 mg L^{-1}), antioxidant enzyme activities were higher (p=0.0001, F-value=42.51) than other FeONPs (Table 2). When GHWF was treated with various concentrations of A-FeONPs (2.797, 6.564, 8.106, 16.935mg L^{-1}), the lowest activities of all studied antioxidant enzymes studied were observed when GHWF were treated with different. Following exposure to the highest concentrations of each FeONP, significant increases in the antioxidant enzyme's activity were observed compared to the control group (p=0.0001, F-value=37.05). Changes in the antioxidant enzyme activities (GST, GPX, CAT, and SOD) following FeONP application indicate that *T. vaporariorum* was exposed to FeONPs, which induced oxidative stress.

Table 1. Mean values of lethal concentrations of synthesized FeONPs using leaf extract on *Trialeurodes vaporariorum*

	Treatment			
	C-FeONPs	**E-FeONPs**	**A-FeONPs**	**B-FeONPs**
intercept	-1.742±0.096	-1.565±0.088	-2.014±0.113	-1.805±0.094
LC_{10} (95% CL)	1.926 mg L^{-1} (1.600-2.2095)	1.532 mg L^{-1} (1.225-1.801)	2.797 mg L^{-1} (2.421-3.137)	1.583 mg L^{-1} (1.396-1.753)
LC_{25} (95% CL)	4.572 mg L^{-1} (4.126-5.165)	3.827 mg L^{-1} (3.461-4.263)	6.564 mg L^{-1} (5.693-8.025)	2.697 mg L^{-1} (2.513-2.872)
LC_{30} (95% CL)	5.662 mg L^{-1} (5.028-6.609)	4.800 mg L^{-1} (4.306-5.480)	8.106 mg L^{-1} (6.827-10.428)	3.077 mg L^{-1} (2.891-3.261)
LC_{50} (95% CL)	11.948 mg L^{-1} (9.627-16.300)	10.584 mg L^{-1} (8.657-14.076)	16.935 mg L^{-1} (12.654-26.510)	4.876 mg L^{-1} (4.576-5.241)
Slope±se	1.617±0.160	1.527±0.149	1.639±0.186	2.623±0.158
X2 (df)	2.123 (4)	2.146 (4)	4.451 (4)	4.795 (4)

LC_{10}, LC_{25}, $LC_{3}0$ and LC_{50}: lethal concentration that kills 10, 25, 30 and 50 % of *T. vaporariorum* after exposure to FeONPs. Values in parentheses indicate 95 % confidence limits (CL). df and se refer to degrees of freedom and standard error, respectively. The synthesized FeONPs using alfalfa, basil, eucalyptus, and cinnamon are summarized as A-FeONPs, B-FeONPs, E-FeONPs, and C-FeONPs, respectively.

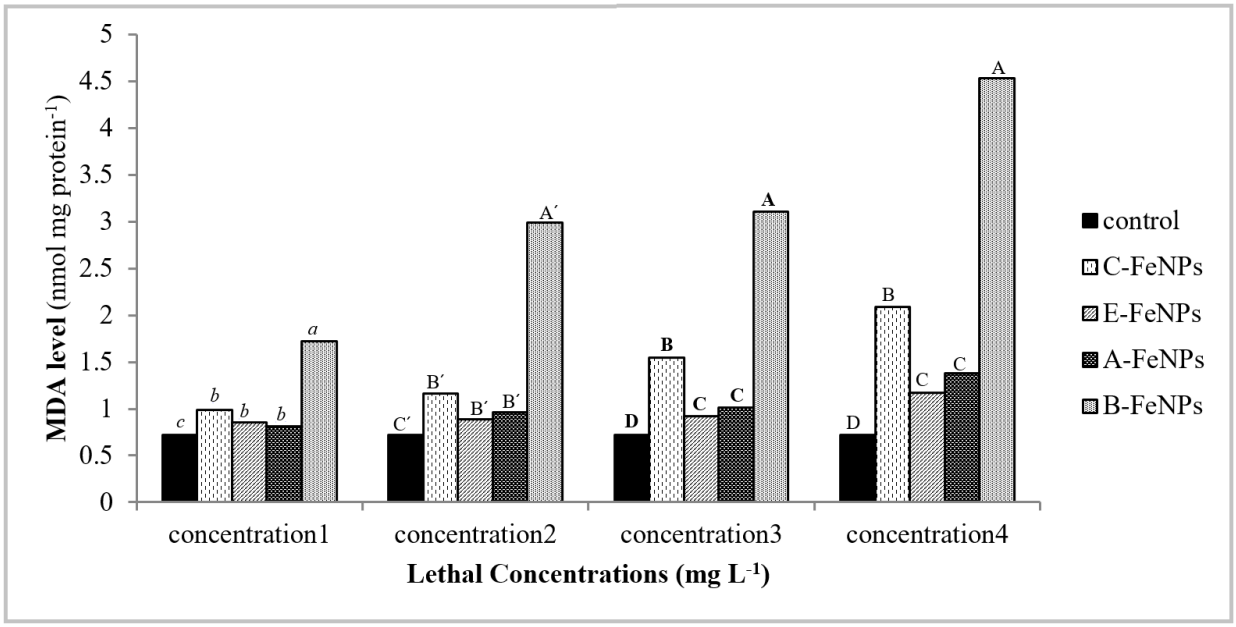

Fig. 4. Effect of different concentrations of green synthesized FeONPs on MDA levels in *T. vaporariorum*. Concentrations 1, 2, 3, and 4 "A-FeONPs"; "B-FeONPs"; "E-FeONPs"; and "C-FeONPs" were "2.797, 6.564, 8.106, and 16.935"; "1.583, 2.697, 3.077, and 4.876"; "1.532, 3.827, 4.800, and 10.584", and "1.926, 4.572, 5.662, and 11.948" mg L^{-1}, respectively. Different letters indicate significant differences between concentrations (Tukey test, $p<0.05$).

Glutathione-dependent enzymes, including GST and GPx, showed substantially improved activity in *T. vaporariorum* treated with different concentrations of FeONPs. The highest levels of GST were observed with 4.876, 1.583, and 2.697 mg L^{-1} of B-FeONPs, while GPx activity showed a significant increase with 4.572 and 1.926 mg L^{-1} of C-FeONPs (Table 2). The findings confirmed that even low concentrations of B-FeONPs and C-FeONPs could increase the activity of glutathione-dependent enzymes (p=0.0001, F-value=15.08).

The highest activity of CAT (p=0.0001, F-value=9.14) and SOD (p=0.0001, F-value=8.11) was found in the *T. vaporariorum* treated with 4.876 mg L^{-1} of B-FeONPs (Table 2). The increased activity of CAT suggested H_2O_2-generating chemicals. Following FeONP treatment, neither polyphenol oxidase nor peroxidase was detected in *T. vaporariorum*. In this study, *T. vaporariorum* treated with varying concentrations of FeONPs exhibited no polyphenol oxidase or peroxidase in this study. This could be related to these enzymes' low concentration, making it difficult to assess their activities or absence. Future research should focus on improving insect extraction and determining if these enzymes are present in *T. vaporariorum*.

Discussion

It is well-established that spherical-shaped green FeONPs have an estimated size of 50 nm. Generally, insects' cuticular layers vary in insects varies in thickness between 100 and 300 µm depending on their developmental stage and body region. Hegazy *et al.* (1990) demonstrated that the cuticle of *T. vaporariorum* is composed of a thin epicuticle and a thick procuticle. The cuticular pore canals are potential transport routes for uncontrolled water penetration and solutes, including pesticides. We hypothesize that the green FeONPs synthesized in this study will penetrate the GHWF cuticle; however, electronic micrographs are required to observe this phenomenon in their bodies.

Recent research has focused on developing effective green synthesis methods for metal NPs to introduce a green method for forming well-characterized NPs. In this regard, producing metal NPs by various organisms is one of the most notable techniques. Plants appear to be the best candidates for large-scale NP biosynthesis, they have the most significant potential for accumulating and detoxifying heavy metals; consequently, they may also be used to photosynthesize metal NPs (Iravani, 2011; Virkutyte & Varma, 2013). Recent research has focused on green chemistry-based methods for synthesizing of FeONPs, given the importance of these compounds in various remediation technologies (Shah *et al.*, 2014, 2015), due to their distinctive physicochemical properties, such as superparamagnetism. FeONPs have numerous applications, particularly in biomedical fields.

Table 2. The effect of green synthesized FeONPs using leaf extract on antioxidant enzyme activities of *Trialeurodes vaporariorum*. The values are Mean±SEM.

Antioxidant enzyme activities		Treatment			
		C-FeONPs	E-FeONPs	A-FeONPs	B-FeONPs
	Concentration (mg L^{-1})				
GST (nmol min^{-1} mg^{-1} protein)	control	0.56±0.03 d	0.42±0.01 c	0.25±0.4 b	0.77±0.02 c
	concentration 1	0.67±0.01 c	0.61±0.02 a	0.33±0.1 a	0.95±0.01 a
	concentration 2	0.71±0.02 b	0.59±0.01 a	0.30±0.2 a	0.94±0.01 a
	concentration 3	0.78±0.01 b	0.55±0.02 b	0.30±0.1 a	0.81±0.03 b
	concentration 4	0.85±0.02 a	0.62±0.03 a	0.30±0.2 a	0.98±0.02 a
GPX (nmol min^{-1} mg^{-1} protein)	control	0.63±0.03 bc	0.39±0.01 b	0.31±0.10 bc	0.41±0.12 c
	concentration 1	0.81±0.01 a	0.45±0.02 ab	0.45±0.11 a	0.62±0.03 a
	concentration 2	0.85±0.01 a	0.41±0.01 b	0.38±0.08 b	0.55±0.10 b
	concentration 3	0.69±0.01b	0.40±0.01 b	0.37±0.09 b	0.53±0.07 b
	concentration 4	0.71±0.02 b	0.47±0.02 a	0.42±0.07 a	0.53±0.10 b
CAT (nmol min^{-1} mg^{-1} protein)	control	114±1.79 d	118±1.22 c	100±2.18 c	128±2.04 d
	concentration 1	120±4.11 c	121±4.63 c	117±1.96 b	145±1.68 c
	concentration 2	133±3.22 b	130±2.02 b	122±2.32 b	164±3.08 b
	concentration 3	141±2.11 a	136±5.09 b	119±2.23 b	171±1.11 b
	concentration 4	145±1.22 a	140±2.04 a	131±1.52 a	185±2.15 a
SOD (nmol min^{-1} mg^{-1} protein)	control	0.35±0.41 c	0.41±0.12 c	0.32±0.02 c	0.67±0.09 c
	concentration 1	0.49±0.09 b	0.55±0.10 a	0.48±0.11 a	0.85±0.03 ab
	concentration 2	0.47±0.15 b	0.49±0.07 b	0.35±0.14 c	0.91±0.04 a
	concentration 3	0.54±0.21 a	0.57±0.08 a	0.46±0.01 a	0.89±0.06 ab
	concentration 4	0.54±0.08 a	0. 44±0.09 b	0.40±0.13 b	0.94±0.01 a

Green synthesized FeONPs using alfalfa, basil, eucalyptus, and cinnamon are summarized as A-FeONPs, B-FeONPs,: E-FeONPs, and C-FeONPs, respectively. Concentrations 1, 2, 3, and 4 "A-FeONPs"; "B-FeONPs"; "E-FeONPs"; and "C-FeONPs" were "2.797, 6.564, 8.106, and 16.935"; "1.583, 2.697, 3.077, and 4.876"; "1.532, 3.827, 4.800, and 10.584", and "1.926, 4.572, 5.662, and 11.948" mg L^{-1}, respectively.
Means of enzyme activities followed by the same letter in each treatment and column are not significantly different according to Tukey honestly significant difference (HSD) (Tukey-HSD) tests at $p<0.05$.

Even though numerous FeONPs applications have been evaluated, little is known about their potential toxicity. Magnetite (Fe_3O_4), maghemite (γ--Fe_2O_3), and hematite (α-Fe_2O_3) are the most frequently used iron oxides for biomedical and technical applications (Valdiglesias *et al.*, 2016). Green-synthesized FeONPs are useful for drug/gene delivery (Liu *et al.*, 2012; Shen *et al.*, 2018), antibacterial performance, cell labeling, labeling, nanosensors, and contaminant remediation (Devatha *et al.*, 2016, 2018). However, additional toxicological research on FeONPs is necessary, and evaluation criteria for toxicity should be outlined.

Our findings validated the efficiency of green FeONPs in controlling GHWF, particularly B-FeONPs. Very few studies exist on the use of FeONPs in pest management programs. Devatha *et al.* (2018) demonstrated that plant polyphenols can bind to FeONPs and suppress oxidative stress, caused by ROS production. To confirm this mechanism, analyzing the use of plant secondary metabolites in synthesizing FeONPs is necessary. Various *in vitro* studies have demonstrated the antioxidant properties of *Ocimum* species extract (Hakkim *et al.*, 2008). In addition, the insecticidal activity of the major active constituents of basil oil was studied on three species of tephritid fruit flies, and it was evidenced that if these metabolites were combined with attractants, they could be used as a botanical (Chang *et al.*, 2009).

In this study, *E. microtheca* leaves synthesized another FeONP. Among the 101 compounds found in the essential oil of *E. microtheca*, α-phellandrene, aromadendrene, α-pinene, globulol, ledene, P-cymene, and β-pinene were the most abundant. The antioxidant properties of *Eucalyptus* species in human health were demonstrated. The oil of *Eucalyptus* has been placed in the category of substances generally regarded as safe and classified as nontoxic to human health. In addition, the insecticidal activity of *Eucalyptus* species' essential oil is well documented by researchers (Batish *et al.*, 2008; Russo *et al.*, 2018; Shahriari *et al.*, 2019).

Brodowska *et al.* (2016) demonstrated that cinnamon extract is a potent radical scavenger. Cinnamon extract and its essential oil are valuable tools for managing insect pests (Viteri Jumbo *et al.*, 2018). Its main

secondary metabolite is recognized as (E)-cinnamaldehyde, which is known as a potential insecticide against pests (Zaio *et al.*, 2018).The saponin and flavonoid classes are the most intriguing secondary metabolites of alfalfa. Saponins and flavonoids inhibit insect growth and reproduction through repellent or deterrent properties, cytotoxicity, and molting. Furthermore, its insecticidal properties have been documented (Singh & Kaur, 2018).

Despite the significant pesticidal activities of alfalfa, basil, eucalyptus, and cinnamon against agricultural pests, based on our literature review we found no published research on the effects of green synthesized FeONPs from the mentioned plants on pests. Environmental stressors, including pesticides, toxic metals, and volatile organic compounds, were demonstrated to promote excess ROS production and exhibit genotoxic effects (Doganlar & Doganlar, 2015). Due to their ease of preparation and manipulation, NPs have received much attention in pest management programs to reduce the effects of conventional pesticides on the environment and non-target organisms. Different chemical and physical methods are extensively applied in producing metal and metal oxide NPs. Nonetheless, highly reactive and toxic reducing agents are necessary, although they have undesirable effects on the environment, plants, and animals (Saif *et al.*, 2016). Furthermore, due to several limitations in physical and chemical methods for NP synthesis (such as high reactivity in nature, the tendency to form aggregates, inapplicability in biomedical applications, loss of magnetism, and dispersibility when exposed to air), the development of eco-friendly protocols for synthesis processes have been emphasized (Herlekar *et al.*, 2014).

Green synthesis of metallic NPs by various plant parts has been identified as the most economical, simple, and reproducible method (Kharissova *et al.*, 2013). FeONPs possess significant advantages among nanoparticles, which can combat environmental pollution (Mittal *et al.*, 2013; Shah *et al.*, 2015; Saif *et al.*, 2016). Although various studies have evaluated the toxic effects of exposure to metal NPs, the underlying mechanisms for the toxic activities of NPs remain unknown. Our literature review shows that ROS generation and oxidative stress are the most frequently reported mechanisms in NP toxicity. ROS is one class of effectors implicated in insect innate immunity (Valdiglesias *et al.*, 2015). MDA is typically described as a secondary LPO product and a quantitatively dominant aldehyde that forms creating Schiff bases with the amines of proteins, nucleic acids, and phospholipids, causing cellular biomolecule damage. LPO is described as a cellular injury mechanism and indicator of oxidative stress in animal and plant tissues and cells (Büyükgüzel & Kalender, 2009).

In our study, LPO, showed a significant increase in *T. vaporariorum* when treated with green synthesized FeONPs especially B-FeONPs ($p<0.05$). In general, disruption disturbances in the electron transport chain at any site can form H_2O_2 and ROS, therefore, the high MDA level in B-FeONPs-fed GHWF adults may indicate peroxidizing membrane lipids. Similarly, previous research has reported that the toxicity of numerous xenobiotics, such as pesticides, is related to ROS production (Sohn *et al.*, 2004). The reason why B-FeONPs increased the level of MDA more than other FeONPs, may be attributable to the quantity of secondary metabolites in basil, which is recommended for further investigation.

The increase in MDA content during treatment compared with the control represented the FeONP success in the flux induction of free radicals and an increase in LPO content and mortality in *T. vaporariorum*. Similar results were demonstrated by Sohn *et al.* (2004); Dubovskii *et al.* (2005); Hyršl *et al.* (2007); Dubovskiy *et al.* (2008); Büyükgüzel & Kalender, (2009); Aslanturk *et al.* (2011); Yu *et al.* (2011); Büyükgüzel *et al.* (2013); Büyükgüzel (2014); Kayis *et al.* (2015); Xin *et al.* (2017); Nareshkumar *et al.* (2017); Gavrilović *et al.* (2017); and Rahimi *et al.* (2018). The activities of antioxidant enzymes and MDA levels are known to change due to oxidative stress, which may serve as biomarkers in insects (Emre *et al.*, 2013). Antioxidant systems, consisting of enzymatic and nonenzymatic compounds, play a significant role in mitigating ROS toxicity. Enzymatic antioxidant compounds, such as CAT, SOD, and GPX, can shield tissues from ROS (Abdelsalam *et al.*, 2016). Our results showed that the effects of different plant FeONPs on the antioxidant activity of GST, GPX, CAT, and SOD enzymes were significantly increased. Increased levels of antioxidant enzymes such as SOD, CAT, and GST, indicate that the organisms adapt to oxidative stress.

Kafel *et al.* (2012) showed that metal ions are associated with changes in the activity of detoxifying the antioxidant enzymes in insects. Nevertheless, potentially toxic H_2O_2 is produced during detoxification, where POD and CAT remove peroxides and SOD directly dismutase superoxide anions (Ahmad & Pardini, 1990).

Generally, antioxidant defense components can play a protective role in an organism via ROS scavenging and triggering oxidative stress. In a study by Büyükgüzel & Kalender (2009), exposure to streptomycin (an antibiotic insecticide) changed oxidative and antioxidative responses, as indicated by the increase in the level of MDA, SOD, and GPx, along with the simultaneous CAT and GST depletion in the larval midgut *Galleria mellonella* tissues. Through the conjugation of reactive species and detoxification of LPO products, GST significantly contributes to the inhibition of oxidative damage (Singh *et al.*, 2001). In a similar study, GST activity increased in the midgut of gypsy moth larvae fed an artificial diet in response to phenolic glycosides (Hemming & Lindroth, 2000).

Consistent with the present findings, Feng *et al.* (1993) and Büyükgüzel & Kalender, (2009) demonstrated that the induced GST activity of insects should potentiate them to metabolize chemicals more effectively and make them highly tolerant to insecticides. It was hypothesized that GST could protect if FeONPs, were metabolized to their compounds (e.g., metal ions) and secondary metabolites (e.g., polysaccharides, polyphenols, triterpenoid, saponins, and flavonoids) and caused oxidative stress.

GPx can catalyze the glutathione-mediated decrease in hydrogen peroxides and lipid hydroperoxides for xenobiotic detoxification. Contrary to our results, Büyükgüzel & Kalender (2009) reported an increased GPx activity and decreased GST at several streptomycin levels. Similarly, in a study by Hyršl *et al.* (2007), treatment with boric acid led to a significant decline in GST activity in hemolymphs of *G. mellonella* larvae. Moreover, Aslanturk *et al.* (2011) observed similar results to our findings. They demonstrated that GPx activity improved significantly in the midgut tissues of methidathion-treated *Lymantria dispar* (Lepidoptera, Lymantriidae) larvae.

SOD and CAT are involved in stepwise oxygen reduction (Krishnan & Kodrik, 2006). Our results confirmed that enhanced SOD activity in treated *T. vaporariorum* should elevate H_2O_2 concentration and increase CAT activity. In addition, improvements in SOD and CAT activities can promote ROS eradication (Wu *et al.*, 2011a, b). In another study, Aslanturk *et al.* (2011) examined the effects of methidathion on antioxidant enzymes in the midgut tissues of *L.dispar*. A meaningful increase was observed in SOD, CAT, and GPx activity at LC_{50} concentration of methidathion-treated larvae versus the controls. According to their results, methidathion leads to the progression of oxidative stress. Similar to our results, SOD activity significantly increased when *G. mellonella* larvae were exposed to streptomycin, eicosanoids, and Malathion; moreover, POD activity increased in simultaneous treatments with streptomycin and eicosanoids (Büyükgüzel & Kalender, 2009; Büyükgüzel *et al.*, 2010).

Recently, Sezer & Ozalp (2015) determined the effects of pyriproxyfen on the activity of SOD and CAT in *G. mellonella*. The larvae, exposed to different pyriproxyfen concentrations, showed a significant increase in CAT and SOD activities. Pyriproxyfen exposure could induce oxidative stress. In this regard, Hyršl *et al.* (2007) reported an increase in SOD activity among insects receiving low doses of boric acid, while high doses caused a reduction in SOD activity of larval hemolymph from *G. mellonella*; the SOD activity significantly increased, whereas CAT activity reduced in the larval fat body. In addition to these general responses, oxidative stress significantly increased the activities of SOD and CAT. Changes in the activity of antioxidant enzymes in the hemolymph are a biomarker of oxidative stress in *G.mellonella*. Similarly, Doganlar & Doganlar, (2015) evaluated the effects of a mixture of pesticides in *Drosophila melanogaster* to determine the effects of antioxidant enzymes. According to their study, this mixture induced oxidative stress. Similar to our findings, SOD and CAT activities increased in a study on a grasshopper species following treatment with pesticides (Wu *et al.*, 2011a, b). Likewise, SOD, CAT, and POD activities have increased in *Nilaparvata lugens* after treatment with chlorpyrifos (Ling & Zhang, 2013).

According to several studies, the most important defensive response to oxidative stress, induced by pesticides, is increased SOD activity (Kayis *et al.*, 2012). The increase in CAT activity may be attributed to the formation of H_2O_2 by SOD and superoxide anion radicals. Aslanturk *et al.* (2011) showed increased CAT activity of *L. dispar* due to pesticide-induced stress. Gupta *et al.* (2005) and Bamidele *et al.* (2013) evidenced

similar results, using dichlorvos in *D. melanogaster* and *Rynchophorous phoenicis*, respectively. In the study by Aslanturk *et al*. (2011), SOD, CAT, and GPx activities significantly changed in the midgut tissues of *L.dispar* following the use of LC_{50} of methidathion. Therefore, methidathion produced oxidative damage in *L.dispar*, which might be attributed to ROS production. Moreover, the SOD activity increased significantly due to exposure to methidathion, suggesting the stimulation of SOD by scavenging superoxide radicals to protect larvae against methidathion-mediated stress. In addition, their research revealed a significant increase in CAT activity in the midgut tissues of *L.dispar* in response to methidathion-induced oxidative stress. CAT, regulated by H_2O_2 concentration, is suitable for lowering elevated H_2O_2 levels. It was concluded that increased SOD activity might cause an increase in H_2O_2 and CAT activity (Aslanturk *et al*., 2011).

Based on previous studies, CAT can prevent oxidative stress and increase the lifespan of insects (Orr & Sohal, 1994). Similarly, Abdelsalam *et al*. (2016) showed that spinosad induces antioxidative responses of CAT, SOD, and GST in *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae). Contrary to our findings, spinosad inhibited the midgut's CAT, SOD, and GST activities. However, no major changes were observed in the activity of these enzymes. Surprisingly, treatment with bioinsecticides, (e.g., plant extracts and hematoporphyrin, resulted in SOD inhibition in insect species (Kolawole & Kolawole, 2014). Conversely, spinosad treatment did not affect GST activity in insects (Wang *et al*., 2006; Al-Daheri & Al-Deeb, 2012). Similar to the current study, Yasur & Pathipati, (2015) evaluated changes in the antioxidant enzymes of treated *Spodoptera litura* and *Achaea janata* with silver nanoparticles. The findings showed that SOD, CAT, and POD activities changed in larval bodies due to nanoparticle treatment, suggesting that larval exposure to NPs triggers oxidative stress, countered by antioxidant enzymes. According to the current findings, changes in CAT, POD, and SOD activity in the larval body following silver treatment indicate an efficient ROS scavenging system.

Generally, pesticides produce ROS and cause oxidative stress, as well as changes in radical scavenging enzymes. ROS can cause LPO, protein, and enzyme oxidation, and glutathione depletion in insects. Antioxidant compounds alleviate the oxidative challenge and more importantly antioxidant enzymes, such as CAT, SOD, GPx, and GST, which develop a defense complex against ROS (Felton & Summers, 1995).

Conclusion

This study was conducted to determine the effects of green-synthesized FeONPs using different plant extracts, (alfalfa, basil, eucalyptus, and cinnamon) on *T. vaporariorum* adults. Our findings confirmed that FeONPs could regulate GHWFs efficiently. The results showed that the increased GST, GPx, CAT, and SOD activity could contribute to free radical scavenging in *T. vaporariorum*, leading to oxidative stress induced by green synthesized FeONPs. Our findings showed that MDA content increased in treatments, which may indicate the inadequacy of defense mechanisms in cells and tissues. The increased activity of most tested antioxidant enzymes suggests that they play an essential role in preventing green-synthesized FeONP toxicity, which can cause oxidative stress in *T. vaporariorum*. FeONPs could induce cytotoxic effects due to ROS formation and release of iron ions. In addition, toxic ROS accumulation in living organisms, resulting from an inefficient antioxidant system, can produce genotoxic effects and cause gene expression in antioxidant enzymes; however, additional research is required in this area.

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سمیت نانوذرات اکسید آهن سبز سنتز شده بر *Trialeurodes vaporariorum* و اثرات آنها بر آنزیم‌های آنتی اکسیدانی و پراکسیداسیون لیپیدی

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چکیده

در این مطالعه، اثرات کشندگی غلظت‌های نانوذرات اکسیدآهن (FeONPs) سنتز شده از گیاهان (یونجه، ریحان، اکالیپتوس، دارچین) بر مرگ‌ومیر، فعالیت آنزیم‌های آنتی‌اکسیدانی (سوپراکسید دیسموتاز (SOD)، کاتالاز (CAT) گلوتاتیون پراکسیداز (GPx)، گلوتاتیون S–ترانسفرازها (GST) و پراکسیداسیون لیپیدی (LPO) برای ارائه روشی موثر و ارزان جهت کنترل خسارات اقتصادی ناشی از *Trialeurodes vaporariorum* بررسی شد. خصوصیات FeONPهای آماده شده با عصاره‌های گیاهی توسط اسپکتروفتومتر مرئی– فرابنفش (UV-Vis)، طیف سنجی مادون قرمز تبدیل فوریه (FTIR) و میکروسکوپ الکترونی روبشی نشر میدانی (FESEM) برای بررسی اندازه، ترکیب عنصری و شکل شناسی انجام شد. نتایج UV-Vis از FeONP سنتز شده گیاهی با نشان دادن پیک در ۲۵۶ نانومتر، سنتز نانوذرات را تایید کرد. FTIR پیک‌های جذب قوی را در ۳۴۲۰، ۳۴۴۶، ۳۴۲۱ و ۳۴۴۶ cm^{-1} (گروه هیدروکسیل)، ۲۹۲۵ و ۲۹۲۶ cm^{-1} (گروه C-H)، ۱۶۱۸، ۱۶۳۶، ۱۶۴۷، ۱۷۱۶ cm^{-1} (گروه C=O) و ۱۰۳۰، ۱۰۳۴، ۱۰۴۴ و ۱۰۴۸ cm^{-1} (گروه C-O) شناسایی کرد. آزمایش‌های زیست سنجی با حشرات بالغ *T.vaporariorum* در قفس‌های برگیتیمارشده با غلظت‌های مختلف FeONPs انجام شد. نرم افزار نسخه SPSS 21 (IBM, New York, US) با فاصله اطمینان 95 (CI)٪ و تجزیه و تحلیل Probit برای تعیین غلظت کشنده (LC_{10}، LC_{25}، LC_{30} و LC_{50}) FeONPها استفاده شد. نتایج نشان داد که FeONPهای ریحان، یونجه، اکالیپتوس و دارچین به ترتیب در غلظت‌های ۴/۸۷۶، ۱۶/۹۳۵، ۱۰/۵۸۴ و ۱۱/۹۴۸ $mg\ L^{-1}$ به طور موثر ۵۰ درصد از حشرات بالغ *T.vaporariorum* را از بین بردند. افزایش قابل توجهی در فعالیت LPO، GST، GPX، CAT و SOD در حشرات بالغی که در معرض غلظت‌های کشنده FeONPها قرار داشتند، مشاهده شد. علاوه بر این، یافته‌ها نشان داد که قرار گرفتن در معرض FeONPها، باعث ایجاد استرس اکسیداتیو در حشرات بالغ *T.vaporariorum* شده و ممکن است طول عمر آنها را در گلخانه کاهش دهد.

کلمات کلیدی: نشانگر زیستی، آنتی اکسیدانهای آنزیمی، نانوسم شناسی، تنش اکسیداتیو، گونه‌های اکسیژن فعال

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