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# Lethal and Sublethal Effects of Commercial and Nano-Encapsulated Deltamethrin and Matrine against *Habrobracon hebetor* (Hymenoptera: Braconidae)

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## ABSTRACT

**In this study, deltamethrin and matrine were encapsulated with Polyethylene Glycol (PEG) and Chitosan (Cs), respectively, and their toxicity were investigated against *Habrabracon hebetor* Say using the contact method. According to the Scanning Electron Microscopy (SEM), spherical nanoparticles for both formulations were observed. The average hydrodynamic nanoparticle diameters for deltamethrin and matrine were 65 and 70.5 nm. The $LC_{50}$ values were 254.48, 334.90, 760.31 and 1,021 mg $L^{-1}$ in PEG-encapsulated deltamethrin, commercial deltamethrin, Cs-encapsulated matrine, and commercial matrine, respectively. Exposing to the $LC_{30}$ of the commercial and nano-encapsulated deltamethrin significantly prolonged the total pre-adult period. Furthermore, the sublethal exposure to the PEG-based nanoformulation of deltamethrin and commercial deltamethrin resulted in significant reduction of the intrinsic rate of natural increase ($r_m$) (0.159 and 0.168 $d^{-1}$, respectively). The same trend was observed for the Gross Reproductive Rate (*GRR*), net Reproductive rate ($R_0$), and finite rate of increase ($\lambda$) of the parsitoid. Our findings indicate that the negative side effects of commercial and nano-based formulations of deltamethrin on *H. hebetor* should be considered in IPM programs.**

**Keywords:** Chitosan, Integrated pest management, Nano-insecticides, Parasitoid, Polyethylene glycol.

## INTRODUCTION

Biological and chemical controls are two essential techniques in integrated pest management (IPM) programs that may be simultaneously used to control insect pests in fields or greenhouses (Heibatian *et al*., 2018; Wu *et al*., 2019). Integrating pesticides with biocontrol agents usually requires critical information about the impact and selectivity of the pesticides on natural enemies including predators and parasitoids (Manjunath, 2022). The parasitoid *Habrabracon hebetor* Say is one of the important species of Braconidae, used for controlling lepidopterous pests (Ghimire and Phillips, 2010). Chemical control is widely used throughout the world for reducing pest populations to prevent crop losses; however, the large-scale utilization of pesticides against agricultural pests has turned out to cause serious problems for either the health of humans or the environment, especially by contamination of air, soil, and underground water (Gill and Grag, 2014; Ochoa and Maestroni, 2018).

In recent years, a growing interest has been found in developing nano-based

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formulations of pesticides to decrease the hazardous impacts of the conventional pesticides (Shao *et al*., 2022). Nano-pesticides provide not only the successful and long-term control of pests, but reduce the essential dosage of pesticides, frequency of use, and their environmental risks (Memarizade *et al*., 2014; Agathokleous *et al*., 2020). Polyethylene Glycol (PEG) is a synthetic and biocompatible polymer synthesized by ring-opening polymerization of ethylene oxide. For facilitating sustained release of active ingredients of the water-insoluble pesticides, a semisynthetic polymer of Hydroxypropyl Methyl-Cellulose (HPMC) is also used. It forms a strong viscous gel around the particles in contact with aqueous media (Karavas *et al*., 2006). Chitosan (Cs) is a naturally occurring polysaccharide obtained by de-acetylation of chitin from different sources such as fungi, crustaceans, and insects under alkaline conditions (Younes and Rinaudo, 2015). The Cs can readily form spherical nano-capsules by adding a polyanionic salt of Tripolyphosphate (TPP) (Dutta *et al*., 2004; Mason *et al*., 2006; Ahmadi *et al*., 2018b).

Despite the reported enhanced bioactivity of nano-pesticides against mites or insect pests (Gonzalez *et al*., 2015; Ahmadi *et al*., 2018a, b; Ahmadi *et al*., 2020; Ebadollahi *et al*., 2022), the impacts of nano-based formulation of pesticides should be evaluated toward natural enemies to guarantee their safety use (Preetha *et al*., 2018; Yan *et al*., 2022). The nano-formulation of insecticides may exhibit higher toxicity to natural enemies, as reported by Sun *et al.* (2020) for nano-formulated abamectin on *Adalia bipunctata* L. larvae, or show no adverse impacts on them, as reported by Wu *et al.* (2024) for nano-pesticides based on a cationic Star Polymer (SPc) against *Picromerus lewisi* Scott. According to our unpublished data, both insecticides showed partially less toxicity to *H. hebetor* compared to different insecticides that had been used. Therefore, the current research was aimed to evaluate the probable toxicity of nano-formulations of deltamethrin and matrine insecticides along with their commercial analogues against different growth stages of *H. hebetor*. Furthermore, the sublethal concentrations of commercial and nano-formulations of both insecticides on the development, reproduction, and life table parameters of *H. hebetor* were also assessed.

## MATERIALS AND METHODS

All the experiments were conducted during 2022 in the laboratory of the Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Iran.

### Insects' Rearing

The colony of *H. hebetor* was obtained from a mass-rearing insectarium belonging to the Agriculture Organization in Khoda Afarin County, East Azerbaijan Province, Iran. Adults of *H. hebetor* were placed in pairs (5 pairs) inside Petri dishes 9 cm in diameter. Inside each Petri dish, 20 last instar larvae of *Ephestia kuehniella* were placed as hosts for parasitizing. A narrow strip of paper covered with a thin layer of honey was used as a food source for adult parasitoids. After 24 hours, the adults were removed from the Petri dishes and the parasitized larvae were kept in a growth chamber at 26±1°C, 60±5% RH, and 16L: 8D photoperiod until the emergence of the adult parasitoids.

The colony of *E. kuehniella* was obtained from a colony maintained in the insectarium of the Agriculture Organization in Khoda Afarin County, Iran. About 0.2 g of the moth's eggs (< 24 hours-old) were placed in plastic containers (32×22×9.5 cm) with 2 kg of wheat flour and 0.5 kg of wheat bran. After the adult emergence, they were kept in the growth chamber for mating and oviposition. The produced eggs were daily collected from the sheets and used for colony rearing.

### Materials

The commercial formulation of deltamethrin (Decis® 2.5% EC, Ariashimi Com., Iran) and matrine (Rui Agro® 0.6% SL, Hangzhou Ruigiang Com., China) were used in the current study. Polyethylene glycol-400 (PEG-400) (density 1.128 g $cm^{-3}$, MW 380 –420 g $mol^{-1}$), Mydroxypropyl Methylcellulose (HPMC) (Molecular weight (MW) 150 000, HPMC-K100M), and Chitosan (Cs) with a viscosity-average molecular weight of $(5.2 \pm 0.4) \times 10^5$ and a degree of de-acetylation larger than 90% were purchased from Sigma-Aldrich (St Louis, MO). All of the other chemicals used in this research were also purchased from Sigma-Aldrich.

### Nanoparticles

PEG-400, acetone, HPMC, distilled water and surfactant were used to prepare nanodeltamethrin. Initially, 0.5% a.i. (w/v) of deltamathrin was added to 12 mL PEG-400, and 2 mL acetone (organic phase). Then, 0.2 g of HPMC was dissolved in 20 mL of distilled water and 2 mL surfactant (aqueous phase). After that, organic phase was slowly dropped into the aqueous phase and stirred for 30 minutes at 4,000 rpm. The obtained coarse emulsion was diluted with distilled water (30 cc), then, converted into a nano-emulsion through subjecting to ultrasonic emulsification using a 20 kHz Sonicator (BANDELIN Sonopuls) for 10 minutes. For the preparation of matrine nanoparticles (with water-soluble substances) (Kowah *et al*. 2023), chitosan, acetic acid solution, and tripolyphosphate (TPP) were used. First, chitosan (0.1 g) was dissolved in acetic acid solution (50 mL) (1% v/v in water) by stirring at room temperature for about 30 min at 4000 rpm. Then, the quantity of 0.5% a.i. (w/v) of matrine was added and allowed to dissolve completely. The TPP solution was separately made by dissolving TPP (0.08 g) in distilled water (5 mL) and, later, it was gradually dropped into to the previous solution. The solution was then stirred for almost 60 minutes at 500 rpm to gain a homogeneous solution (Ahmadi *et al*., 2018a, b).

The size and morphology of PEG-deltamethrin and Cs-matrine nanoparticles were assessed by Scanning Electron Microscopy (SEM) (VEGAII, XMU, Czech Republic) at the Central Laboratory, University of Tabriz, Tabriz, Iran. The mean particle size was analyzed by Dynamic Light Scattering (DLS) via a Zetasizer Photon Correlation Spectroscopy (PCS) instrument (Malvern Instruments Limited, UK) at the Central Laboratory, University of Tabriz, Tabriz, Iran. The DLS were replicated three times (Ahmadi *et al*., 2020; Taktak *et al*., 2021). Dried samples were imaged by SEM (Ahmadi *et al*., 2018a). Nanoparticles (5 mL) of PEG-encapsulated deltamethrin and Cs-encapsulated matrine were simply separated from the liquid phase by centrifugation for 20 minutes at 8,000 rpm. The supernatants were assessed for deltamethrin or matrine by UV spectroscopy. The solubility of the PEG-deltamethrin and Cs-matrine nanoparticles was compared to those of deltamethrin and matrine using UV absorbance (UV-Vis Spectroscopy, Unico, UV-2802, USA) at $\lambda_{max}$= 290 nm. First, 1 mg of the active ingredient of the examined encapsulated formulations was dissolved in distilled water (1,000 µL) and stirred for 30 min at normal temperature. Then, the absorption amount of deltamethrin or matrine in the supernatants at 200 µL, 25˚C was determined at 0, 0.5, 24, 48, 72, and 96 hours.

### Lethal Effects of the Chemicals on *H. hebetor*

The lethal effects of the commercial and nano-formulated insecticides on the adults of *H. hebetor* were evaluated by contact method. By using a micropipette, 3 mL of each concentration (12.5, 9.94, 7.905, 6.287, and 5 mg ai $L^{-1}$ for commercial deltamethrin, 9.5, 7.652, 6.15, 4.965, and 4 mg ai $L^{-1}$ for PEG-encapsulated delthamethrin, 12, 8.485, 6, 4.242, and 3 mg ai $L^{-1}$ for commercial matrine, and 9, 6.467, 4.647, 3.339, and 2.4 mg ai $L^{-1}$ for Cs-encapsulated matrine) was poured into the McCartney glass bottles (28 mL) and swirled well to ensure a complete coating, with excess liquid removed. In the control, distilled water plus Tween-80® (Merck, Darmstadt, Germany) was used. The bottles were completely dry for 2 h in the laboratory. Then, 20 newly emerged adults (< 24 hours-old) were anesthetized by $CO_2$ and placed in each bottle and the aluminum caps of bottles were screwed onto the bottles. The wasps were supplied with honey as a food source on narrow strips of paper (5×10 mm). All the bottles were kept in the growth chamber at 26±1°C, 60±5% RH, and 16:8 hours (L:D). The mortality of *H. hebetor* adults in each bottle was recorded 24 hours after the initial exposure to the different concentrations of insecticides. Each insecticide's bioassay test was replicated three times. The recommended field concentrations (https://www.ppo.ir/fa-IR/ppo/5186/) of deltamethrin, nano-deltamethrin, matrine and nano-matrine were 500, 500, 1,000, and 1,000 mg $L^{-1}$ based on the formulated substance, respectively.

## Sublethal Effects Study

To evaluate the sublethal effects of the tested insecticides, 20 pairs of adults of *H. hebetor* were placed in Petri dishes (9 cm diameter) with holes (5 cm diameter) in the lids covered by the fine-mesh net for ventilation to parasitize 100 last instar larvae of *E. kuehniella*. Honey was offered to the wasps on narrow strips of paper (5×10 mm). After 24 hours, the adults were removed and 40 parasitized larvae were kept in a growth chamber at 26±1°C, 60±5% RH, and 16L: 8D photoperiod. Four days later, when one-day old larvae of *H. hebetor* appeared, they were sprayed using a Potter spray tower (Burcard Scientific®) with 5 mL of $LC_{30}$ values of the commercial formulation (6.36 and 4.02 mg ai $L^{-1}$ of deltahmetrin and matrine, respectively) and nano-based formulation of insecticides (5.00 and 3.21 mg ai $L^{-1}$ of PEG-encapsulated delthametrin and Cs-encapsulated matrine, respectively). The larvae in the control were treated with distilled water plus Tween-80®. The treated larvae were transferred to 9 cm diameter Petri dishes and kept in a growth chamber until the emergence of the adult wasps. For each treatment, 55 pairs of males and females of *H. hebetor* (24 hours old) were randomly selected and transferred to Petri dishes (9 cm diameter). Each pair of wasps in each Petri dish was provided with three *E. kuehniella* larvae for oviposition and fed with honey on a thin strip of paper. The host larvae in new Petri dishes were offered to the wasps every 24 hours to determine their daily reproduction. The survival, oviposition period, longevity, and fecundity of the parasitoid were monitored daily and recorded until the death of the last individual.

## Data Analysis

The Encapsulation Efficiency (EE) was evaluated according to the following formula (Ahmadi *et al*., 2022):

$$\mathrm{EE\%} = \frac{\mathrm{ATI} - \mathrm{AFI}}{\mathrm{ATI}} \times 100$$

Where ATI means amount of total insecticide; AFI means amount of free insecticide.

The bioassay data were analyzed by SAS program (SAS Institute, 2002). Mortality data from the exposure of adult female insects to recommended field concentrations were analyzed by a one-way Analysis Of Variance (ANOVA) using the SAS Institute (2002). The life table parameters were estimated with the TWOSEX–MSChart computer program (Chi, 2022). Differences between the life table parameters of *H. hebetor* were examined with the bootstrap procedure (with 100,000 times resampling for estimating the variances and SE of the data).

## RESULTS

### Characterization of PEG-deltamethrin and Cs-matrine Nanoparticles

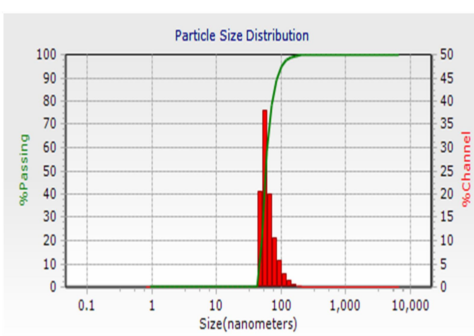
The shape and mean size of the nanoparticles of PEG-based nano-formulation of deltamethrin and Cs-based nano-formulation of matrine were investigated by SEM and DLS techniques, respectively. SEM analysis revealed that the nanoparticles of PEG-deltamethrin were spherical, with a mean size of 100±10 nm (Figure 1-a). The same morphology (spherical shape) was detected in nanoparticles of Cs-matrine. However, their average diameter was not distinguishable due to the aggregation during the drying process (Figure 1-b). Based on DLS analysis, the average hydrodynamic diameter of 65 and 70.5 nm with a Polydispersity Index (PDI) of 195.0 and 16.40 was obtained for PEG-deltamethrin and Cs-matrine nanoparticles, respectively (Figures 2-a and -b). In comparison to the DLS result, the diameter of PEG-deltamethrin nanoparticles from the SEM result was obtained larger than 70.5 nm (about 100±10 nm). This phenomenon can be attributed to coating of the produced deltamethrin nanoparticles by the PEG ingredient during the drying process.

### Encapsulation Efficiency

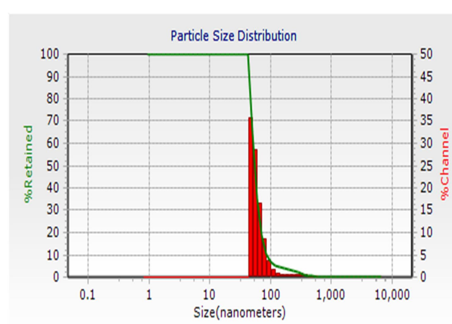
The encapsulation efficiency (EE%) was measured by UV-Vis spectroscopy using a standard graph for PEG-encapsulated deltamethrin ($y$= 0.1597$x$-0.0266, $R^2$= 0.9895) and Cs-encapsulated matrine ($y$= 0.0815$x$-0.0086, $R^2$= 0.9886) at 290 nm (Figure 3). The concentrations of deltamethrin and matrine in the supernatant were obtained via the standard curve. Once the insecticide loading efficiencies in nanoparticles were determined, deltamethrin and matrine were found in 89.13+0.50% and 91.87+0.63% of the nanoparticles. This result suggested that the nanoparticles of PEG and Cs were promising vehicles for encapsulation of the tested insecticides.

### Water Dispersion of the Tested Chemicals

It was revealed that PEG-deltamethrin and Cs-matrine nanoparticles in the absence of organic solvents dissolved more efficiently in water than their commercial forms. After about an hour, the concentration of commercial formulations of deltamethrin and matrine dissolved in water were 26.95 and 26.41 mg ai mL$^{-1}$, respectively (Figure 4). Furthermore, the solubility of PEG-deltamethrin and Cs-matrine nanoparticles were 47.50 and 47.35 mg ai mL$^{-1}$ (Figure 4). The results indicated an increase in the rate

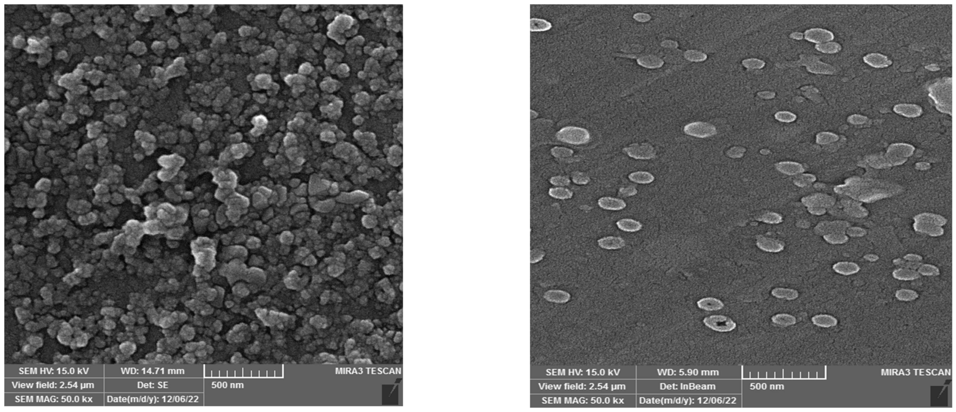


(a)



(b)

**Figure 1.** Dynamic Light Scattering (DLS) measurement of particle size distribution of (a) PEG (Polyethylene Glycol)-deltamethrin, and (b) Cs (Chitosan)-matrine nanoparticles.




(a) (b)

**Figure 2.** Scanning Electron Microscopy (SEM) micrographs of (a) PEG (Polyethylene Glycol)-deltamethrin, and (b) Cs (Chitosan)-matrine nanoparticles.

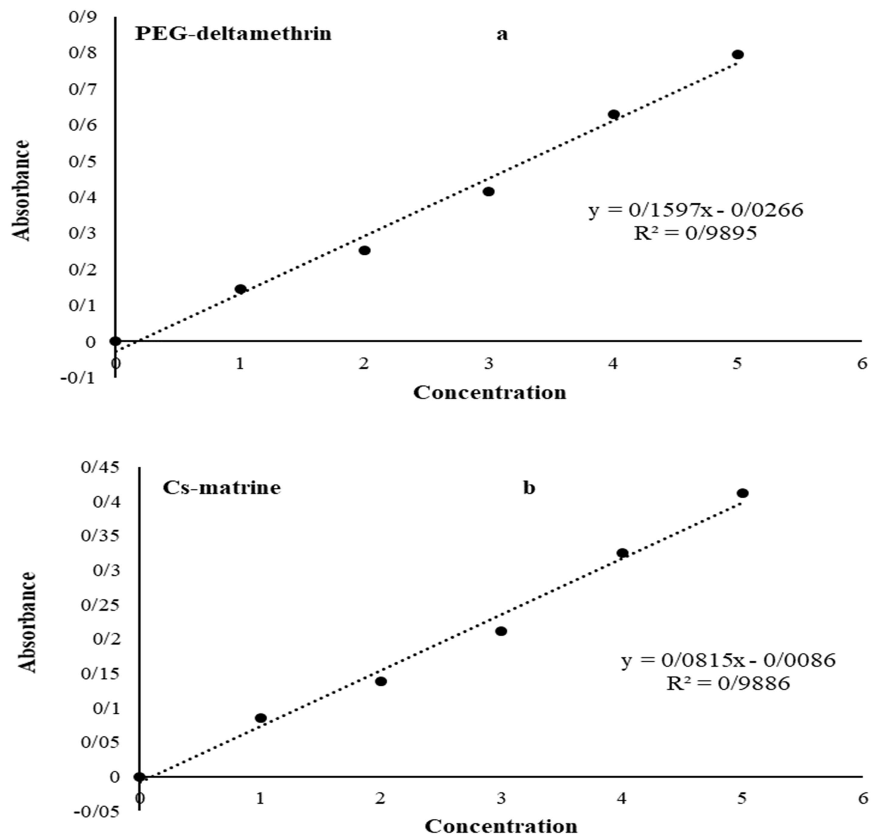



**Figure 3.** The Encapsulation Efficiency (EE%) calculated by UV-Vis spectroscopy using a standard graph for (a) Nano-encapsulated deltamethrin, and (b) Matrine based on PEG (Polyethylene Glycol) and Cs (Chitosan), respectively.

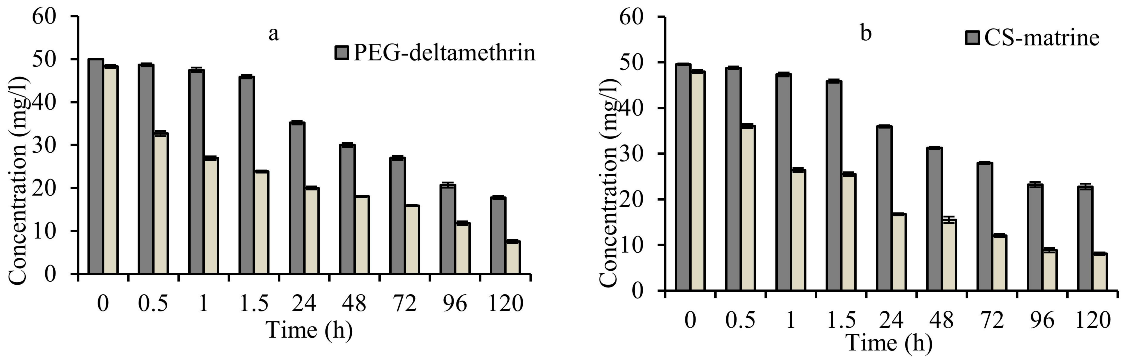



**Figure 4.** Differences in water solubility of (a) PEG (Polyethylene Glycol)-encapsulated deltamethrin and commercial deltamethrin, and (b) Cs (Chitosan)-encapsulated matrine and commercial matrine.

**Table 1.** Toxicity of commercial and nano-encapsulated deltamethrin and matrine based on PEG and Cs (mg $L^{-1}$) against the adults of *Habrabracon hebetor*.[a]

| Treatments | $\chi^2$ | Slope ±SE | Lethal concentrations (mg ai $L^{-1}$) | | |
|---|---|---|---|---|---|
| | | | $LC_{30}$ (95% FL) | $LC_{50}$ (95% FL) | $LC_{90}$ (95% FL) |
| Commercial deltamethrin | 48.44 | 4.40 ± 0.63 | 6.36 (5.53–7.00) | 8.37 (7.67-9.16) | 16.36 (13.81-21.86) |
| PEG-deltamethrin | 59.53 | 3.46 ± 0.45 | 5.00 (4.43–5.45) | 6.36 (5.88–6.87) | 11.41 (9.92–14.36) |
| Commercial matrine | 49.03 | 2.88 ± 0.41 | 4.02 (3.21–4.68) | 6.12 (5.35–7.00) | 17.08 (13.31–26.07) |
| Cs-matrine | 59.53 | 3.46 ± 0.45 | 3.21 (2.68–3.65) | 4.56 (4.05–5.09) | 10.71 (8.83–14.52) |

[a] Lethal concentration and 95% Fiducial Limits (FL) were estimated using logistic regression (SAS Institute, 2002). *PEG: Polyethylene Glycol, Cs: Chitosan.

**Table 2.** Effect of field concentrations of the tested insecticides on adult female insects of *H. hebetor.*

| Insecticides | Recommended field concentration (mg $L^{-1}$) | Mortality rate | IOBC classification[a] |
|---|---|---|---|
| Commercial deltamethrin | 500 | 76.66±3.33 b | slightly harmful |
| PEG-deltamethrin | 500 | 100± 0.0 a | Harmful |
| Commercial matrine | 1000 | 42.67±2.86 d | slightly harmful |
| Cs-matrine | 1000 | 65.33±3.09 c | slightly harmful |
| Control | Distilled water | 1.33 ±0.87 e | - |

[a] IOBC (International Organization for Biological Control) classification: (1) Harmless (Mortality< 30%), (2) Slightly harmful (> 30 and < 79%), and (3) Moderately harmful (> 80 and < 99%), and 4) Harmful (> 99%) (Hassan, 1994; Biondi *et al.* 2012).

**Table 3.** The developmental times and survival (mean±SE) of *Habrabracon hebetor* exposed to $LC_{30}$ of commercial and nano-encapsulated deltamethrin and matrine based on PEG* and Cs*.[a]

| Treatments | Incubation period (Day) | Larval period (Day) | Pupal period (Day) | Total pre-adult period (Day) | Pre-adult survival (%) |
|---|---|---|---|---|---|
| Commercial deltamethrin | 2.36±0.07 a | 4.44±0.1 a | 8.11±0.10 b | 14.91±0.23 a | 0.80±0.05 a |
| PEG-deltametrin | 2.25±0.07 a | 4.52±0.08 a | 8.45±0.09 a | 15.17±0.21 a | 0.76±0.06 a |
| Commercial matrine | 1.59±0.07 c | 3.81±0.08 c | 7.52±0.09 c | 12.98±0.19 c | 0.80±0.05 a |
| Cs-matrine | 1.86±0.09 b | 4.15±0.09 b | 7.86±0.09 b | 13.90±0.25 b | 0.76±0.06 a |
| Control | 1.43±0.07 c | 3.38±0.07 d | 7.02±0.01 d | 11.78±0.18 d | 0.84±0.05 a |

[a] Means followed by different letters in each column are significantly different (P< 0.05, paired bootstrap test). *PEG: Polyethylene Glycol, *Cs: Chitosan.

**Table 4.** The oviposition period, longevity, and fecundity (mean±SE) of *Habrabracon hebetor* exposed to $LC_{30}$ of commercial and nano-encapsulated deltamethrin and matrine based on PEG* and Cs*.[a]

| Treatment | APOP (Day) | TPOP (Day) | Oviposition period (Day) | Female longevity (Day) | Male longevity (Day) | Fecundity (Egg) |
|---|---|---|---|---|---|---|
| Commercial deltamethrin | 0.32± 0.11 ab | 15.24± 0.35 a | 15.36 ±1.03 c | 16.00±1.03 c | 13.05±0.95 b | 81.68±6.49 c |
| PEG-deltametrin | 0.44±0.13 a | 15.80±0.26 a | 9.36±0.24 d | 9.88±0.72 d | 8.29±0.77 c | 66.48±6.71 c |
| Commercial matrine | 0.16± 0.07 ab | 13.24± 0.26 b | 22.68 ±1.71 ab | 23.12±1.76 ab | 19.84±1.66 a | 165.24±8.49 b |
| Cs-matrine | 0.24±0.09 ab | 14.12±0.37 b | 19.80±1.34 b | 20.36±1.38 b | 16.94±1.50 a | 157.20±8.45 b |
| Control | 0.08± 0.06 b | 11.56± 0.23 c | 24.68 ±1.59 a | 25.24±1.68 a | 17.57±1.55 a | 200.84±8.79 a |

[a] Means followed by different letters in each column are significantly different (P< 0.05, paired bootstrap test). *PEG: Polyethylene Glycol; *Cs: Chitosan; APOP: Adult Pre-Oviposition Period, TPOP: Total Pre-Oviposition Period.

and extent of both deltamethrin and matrine dissolution for the nano-suspension as compared to the commercial formulations (Figure 4).

### Lethal Effects of the Tested Chemicals on *H. hebetor*

The toxicity results of field-recommended concentrations of the tested insecticides on *H. hebetor* adult females are shown in Table 2. The mortality of adult females was significantly affected by field recommended concentrations of the tested insecticides compared to the control. The highest percentage of mortality was observed in PEG-deltamethrin treatment, followed by deltamethrin, Cs-matrine and matrine treatments, respectively. The result showed that PEG-deltamethrin and deltamthrin had significantly more toxicity on adult females of *H. hebetor* compared to Cs-matrine and matrine insecticides. Therefore, only PEG-deltamethrin was harmful based on International Organization for Biological Control (IOBC) rating.

### Sublethal Effects Results

A sublethal effect study showed that the incubation and larval period of *H. hebetor* exposed to the $LC_{30}$ (lethal concentration causing 30% mortality) of the commercial and nano-formulations of deltamethrin and matrine significantly affected by different treatments. The pre-imaginal period values in commercial and nanoformulations of deltamethrin were higher than those observed in other treatments (P< 0.05) (Table 3). The longest pupal period of the parasitoid was observed in PEG-deltamethrin (P< 0.05) (Table 3). The total pre-adult period of *H. hebetor* in nano-encapsulated deltamethrin and its commercial formulation was significantly longer than those obtained in nano-

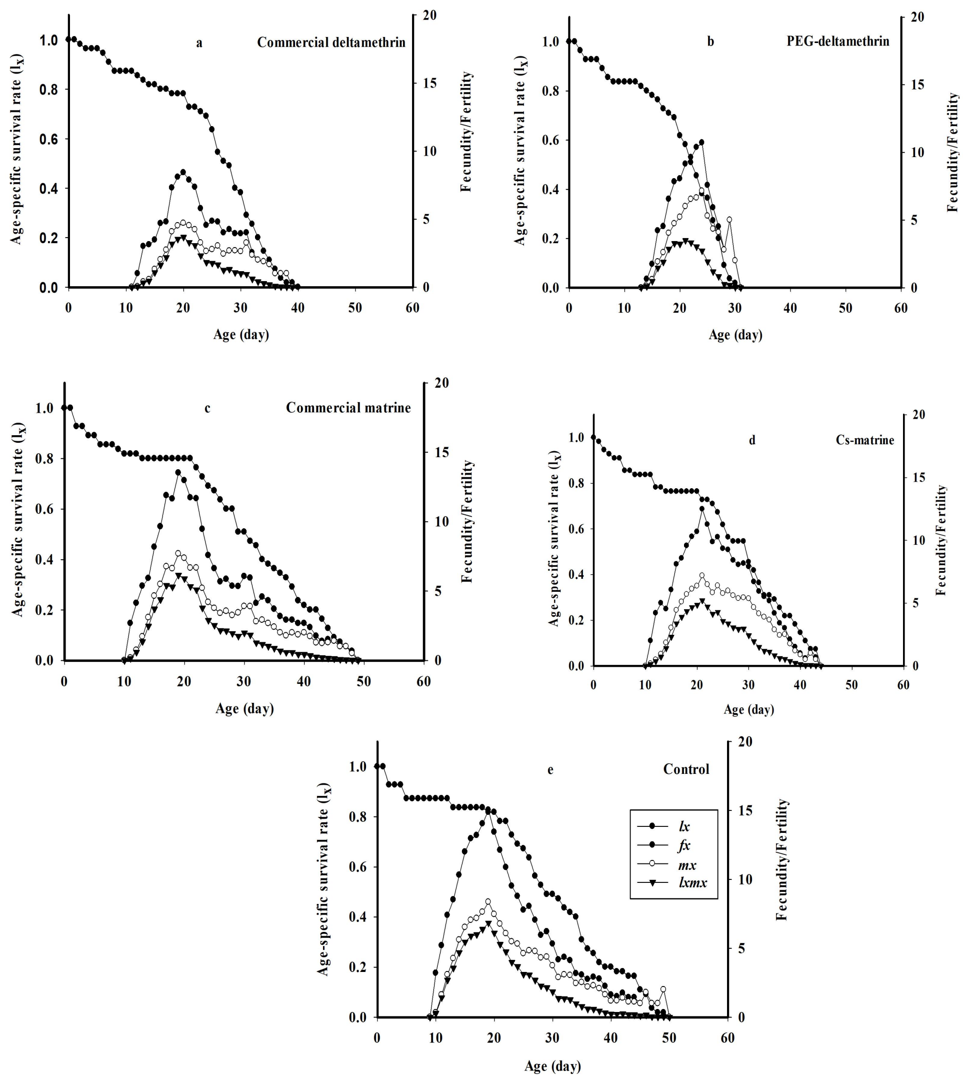



**Figure 5.** Age-specific survival rate ($l_x$), age-stage specific fecundity ($f_x$), age-specific fecundity ($m_x$) and age-specific fertility ($l_xm_x$) of *Habrabracon hebetor* exposed to $LC_{30}$ of commercial and nano-encapsulated deltamethrin (a and b) and matrine (c and d) based on PEG (Polyethylene Glycol) and Cs (Chitosan), respectively along with the control (e).

encapsulated matrine, commercial matrine, and control (P< 0.05) (Table 3). No significant difference was found between the treatments and the control in regards to the percentage of pre-adult survival of *H. hebetor* (P> 0.05) (Table 3).

The Adult Pre-Oviposition Period (APOP) of *H. hebetor* was significantly affected when treated with $LC_{30}$ of the commercial and nanoformulations of either insecticide (P< 0.05). The highest APOP was obtained in PEG-based nanoformulation of deltamethrin (Table 4). The Total Pre-Oviposition Period (TPOP) was significantly highest in nano-encapsulated deltamethrin and commercial deltamethrin (P< 0.05) (Table 4). The oviposition period of *H. hebetor* significantly differed among treatments (P< 0.05) and it was shortest in PEG-encapsulated deltamethrin (Table 4).

Males and females exposed to $LC_{30}$ of nano-encapsulated deltamethrin had significantly shorter longevity (P< 0.05). The fecundity of *H. hebetor* was significantly decreased in the treatments (from 66.48–165.24 eggs) compared to the control (200.84 eggs) (P< 0.05). The least fecundity was recorded in PEG-encapsulated deltamethrin and commercial deltamethrin (Table 4).

The population age-specific survival rate ($l_x$), age-stage specific fecundity ($f_x$), age-specific fecundity of the total population ($m_x$), and the age-specific fertility ($l_xm_x$) of *H. hebetor* in different treatments are given in Figure 5. The $l_x$ of *H. hebetor* decreased in different treatments as the parasitoid became older. The peak of both $f_x$ and $m_x$ happened at 19-24$^{th}$ days in different treatments. For $l_xm_x$, these peaks occurred at 19$^{th}$ -21$^{st}$days. The $E_{xj}$ curves showed that *H. hebetor* tended to live shorter when exposed to commercial deltamethrin and PEG-encapsulated deltamethrin (Figure 6).

The results of the present study showed that the exposure to $LC_{30}$ of either nano-encapsulated deltamethrin or commercial deltamethrin significantly decreased the Gross Reproductive Rate (*GRR*), net Reproductive rate ($R_0$), intrinsic rate of natural increase ($r_m$), and finite rate of increase ($\lambda$) of *H. hebetor* (P< 0.05) (Table 5). Furthermore, treating *H. hebetor* with the $LC_{30}$ of nano-encapsulated deltamethrin, commercial deltamethrin, and nano-encapsulated matrine significantly lengthened the mean generation Time ($T$) compared to the commercial matrine and the control (P< 0.05) (Table 5).

**Table 5.** Population growth parameters (mean±SE) of *Habrabracon hebetor* exposed to LC30 of commercial and nano-encapsulated deltamethrin and matrine based on PEG* and Cs*.

| Treatments | *GRR*** (Female/Female) | $R_0$ (Female/Female) | $r_m$ (d$^{-1}$) | $\lambda$ (d$^{-1)}$ | $T$ (Day) |
|---|---|---|---|---|---|
| Commercial deltamethrin | 65.32±8.065 b | 37.13±6.206 b | 0.168±0.008 c | 1.183±0.010 c | 21.49±0.424 a |
| PEG-deltametrin | 69.03±8.644 b | 30.21±5.394 b | 0.159±0.008 c | 1.173±0.009 c | 21.36±0.278 a |
| Commercial matrine | 121.90±15.629 a | 75.11±11.750 a | 0.212±0.008 b | 1.236±0.011 b | 20.36±0.325 b |
| Cs-matrine | 123.44±15.206 a | 71.43±11.178 a | 0.199±0.008 b | 1.220±0.010 b | 21.49±0.448 a |
| Control | 144.38±17.185 a | 91.29±14.08 a | 0.241±0.009 a | 1.273±0.013 a | 18.72±0.296 c |

Means followed by different letters in each column are significantly different (P< 0.05, paired bootstrap test). *PEG: Polyethylene Glycol, *Cs: Chitosan. ***GRR*: Gross Reproductive Rate, $R_0$: Net Reproductive rate, $r_m$: Intrinsic rate of increase, $\lambda$: Finite rate of increase, $T$: Mean generation time.

## DISCUSSION

In the present study, the morphology of particles obtained for nanoformulations of the tested insecticides is consistent with the results of Ahmadi *et al*. (2018a), who reported the spherical-like shapes of nanoparticles for *Satureja hortensis* essential oil-loaded Cs/tripolyphosphate nanoparticles. However, they were inconsistent with the findings of Ebadollahi *et al*. (2022) that revealed the elliptical shapes of nanoparticles for sodium alginate- and PEG-acetamiprid. According to the obtained results, the mean hydrodynamic diameter of PEG-deltamethrin nanoparticles was about the same size as the Cs-matrine nanoparticles. The sizes of the nanoparticles in the present study were somehow in consistent with that reported by Ebadollahi *et al*. (2022) regarding the encapsulation of acetamiprid in PEG (101.2 nm) and were very smaller than the clofentezine-loaded

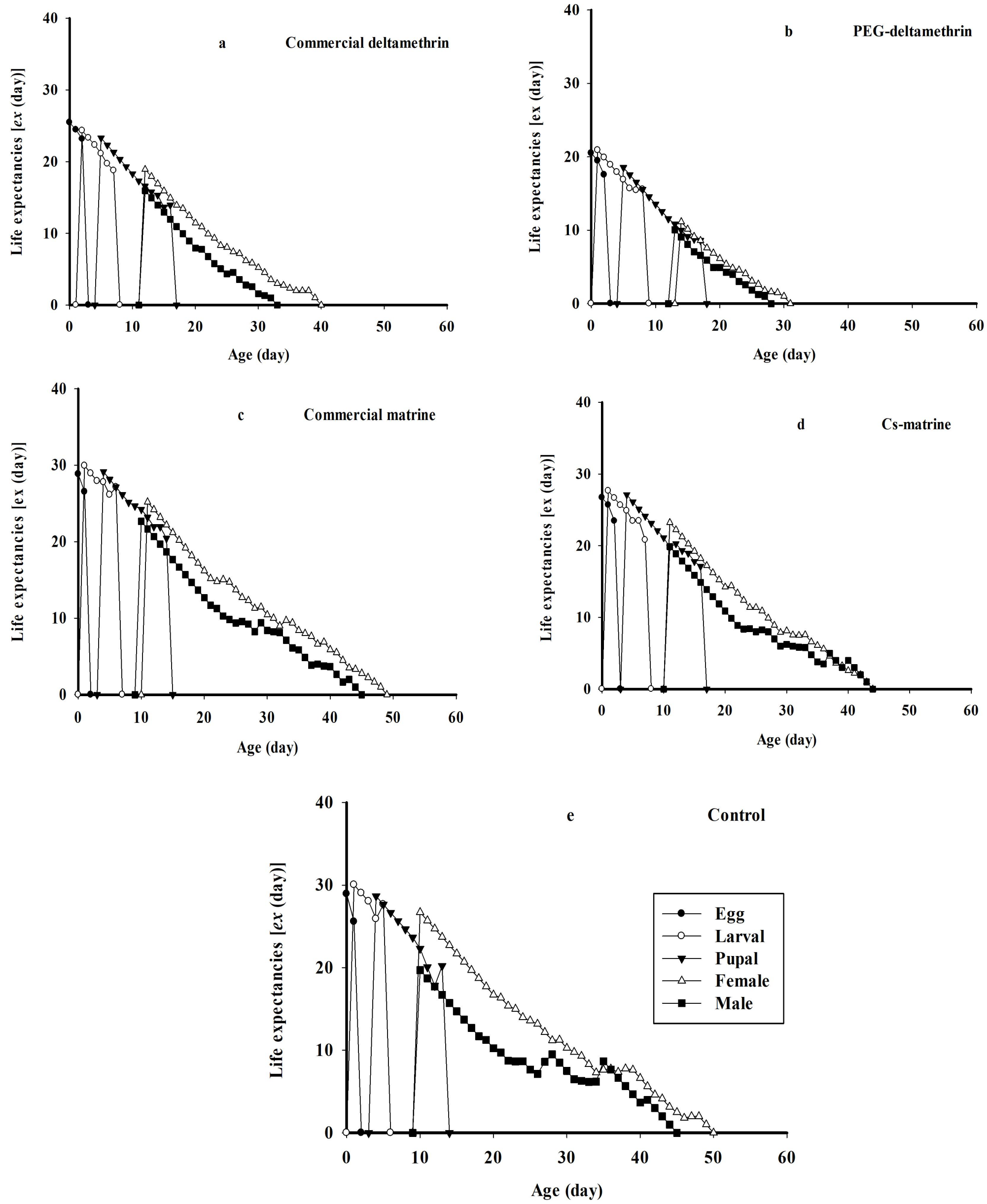



**Figure 6.** Life expectancy [$e_x$ (day)] of *Habrabracon hebetor* exposed to $LC_{30}$ of commercial and nano-encapsulated deltamethrin (a and b) and matrine (c and d) based on PEG (polyethylene glycol) and Cs (chitosan), respectively along with control (d).

nanoparticles (300 nm) reported by Ahmadi *et al*. (2020). The smaller size of nanoparticles based on DLS in our study compared to the latter study may be resulted from the low aggregation of the nanoparticles in the solution. According to the results of the present study, nanoformulations of the tested insecticides showed improved solubility in water compared to the commercial formulations. Similarly, Pan *et al*. (2015) and Ahmadi *et al*. 2021; Worrall *et al*. (2018) stated that normal formulations of insecticides with low water-solubility usually need organic solvents to aid in solubilizing the insecticide, which increases the cost and toxicity of the insecticide; but nano-based formulations of insecticides eliminate the need for organic solvents and can be used to increase the solubility, which leads to reducing their toxicity.

Results of the bioassay study showed that nano-encapsulation of deltamethrin with PEG and matrine with Cs decreased the $LC_{50}$ of the commercial formulations of the insecticides from 334.90 to 254.48 mg $L^{-1}$ and from 1,021 to 760.31 mg $L^{-1}$, respectively. These results revealed that the nano-formulation of the tested insecticides increased their toxicity against *H. hebetor*. Increased performance of nano-based formulations of insecticides against insect pests and their natural enemies has been reported in several studies. For example, Shifa *et al*. (2019) demonstrated that the nanoformulation of deltamethrin caused two times more mortality on *Trialeurodes vaporariorum* Westwood than its commercial formulation. The PEG and Cs are generally considered as almost non-toxic polymers that are extensively used in the fields of agriculture and medicine (Naskar *et al*., 2019; Ebadollahi *et al*., 2022). However, insecticides loaded in the aforementioned nano-carriers are usually more effective in either insect pests or natural enemies than their typical commercial formulations. In the present study, the commercial matrine showed less toxicity in terms of $LC_{50}$ to *H. hebetor* than the commercial deltamethrin. The same results were also observed in their nano-based formulations. The variation may be related to the difference in their chemical compositions, mode of action, nano-carriers, encapsulation methods, and features of particles. Similar to the findings of the current study, the low toxicity of matrine on natural enemies have been documented in the literature. For instance, the commercial formulation of matrine exhibited less toxicity in terms of $LC_{50}$ toward adults of *Orius laevigatus* (Fieber) (Kordestani *et al*., 2022b) and *Amblyseius swirskii* Athias-Henriot (Kordestani *et al*., 2022a). Matrine is a botanical insecticide with a broad spectrum of insecticidal activity, which acts by affecting the insects' acetylcholine receptors (Liu *et al*., 2007; Qu *et al*., 2022; Zhou *et al*., 2022). Mahdavi *et al*. (2013) and Heibatian *et al*. (2018) also showed that the commercial formulation of deltamethrin was toxic to *H. hebetor* adults and carabid beetles (Col., Carabidae), respectively. In a study by Garzón *et al* (2015), deltamethrin was more toxic to *Chrysoperla carnea* Stephens and *Adalia bipunctata* Linnaeus. Deltamethrin is a broad-spectrum insecticide, which disrupts the voltage-gated sodium channels in the nervous system, resulting in neurotoxicity in insects (Pradhan and Mailapalli, 2020).

In toxicological studies, life history parameters and other measures of population growth rate provide more detailed information about the impacts of pesticides on targeted and non-targeted organisms than that of the lethal dose/concentration 50 ($LD_{50}$, $LC_{50}$) (Parsaeyan *et al.*, 2020; Gope *et al*., 2022). According to the results, the exposure of *H. hebetor* larvae to $LC_{30}$ of either PEG-encapsulated deltamethrin or commercial deltamethrin significantly prolonged the duration of the immature stages and decreased the parasitoid's fecundity. Furthermore, exposure of the parasitoid to the recommended doses of nano-encapsulated deltamethrin shortened its longevity and oviposition period. Similar to our results, nano-encapsulation of acetamiprid using coating materials of sodium alginate and PEG enhanced the sublethal efficiency of the insecticide against the elm leaf beetle (Ebadollahi *et al*., 2022). Rafiee Dastjerdi *et al*. (2012) showed that *H. hebetor* females exposed to the field-recommended dose of deltamethrin had the shortest longevity and produced fewer eggs (98.08 eggs) than those in the control (430.60 eggs). The longevity and fecundity of *H. hebetor* were also affected by the $LC_{25}$ of commercial formulation of fenpropathrin insecticides as reported by Faal-mohammadali *et al*. (2014). In contrast, Sarmadi *et al*. (2010) found that the commercial formulation of deltamethrin reduced the fecundity of *H. hebetor*, but it did not affect its longevity. This is probably due to the differences in the population of the parasitoid or the used concentrations of the insecticide.

In the present study, the sublethal exposure to PEG-based nanoformulation of deltamethrin and commercial deltamethrin resulted in significant reduction of the parasitoid's *GRR*, $R_0$, $r_m$, and $\lambda$ in comparison with the control and other treatments. A significant reduction in population growth parameters of *H. hebetor* has also been detected with the commercial formulation of some other insecticides (Rafiee-Dastjerdi *et al*., 2012; Faal-mohammadali *et al*., 2014). According to Kordestani *et al*. (2022a, b), the $LC_{25}$ of commercial formulation of matrine stimulated reproduction in *A. swirskii* and *O. laevigatus* by significantly increasing their population growth parameters of $R_0$ and $r_m$. The results of the two latter studies are partly comparable with the findings of the present study for Cs-based nanoformulation of matrine and commercial matrine treatments, in which the *GRR* and $R_0$ of *H. hebetor* were not significantly different from the control. These findings imply that the low lethal concentration of some insecticides, especially nano and commercial forms of matrine in our study, can be marginally compatible with the use of natural enemies. In the current research, *H. hebetor* had the highest mean generation Time (*T*) when exposed to the $LC_{30}$ of nano-encapsulated deltamethrin, commercial deltamethrin, and nano-encapsulated matrine. As mentioned earlier, *H. hebetor* in nano-encapsulated deltamethrin and commercial deltamethrin had the lowest intrinsic rates of increase. Therefore, it seems quite probable that producing more generations in a given amount of time will be constrained in the mentioned treatments.

For better establishing the eco-friendly control measures in IPM programs, the efficacy of nanopesticides should be evaluated against target and non-target organisms in natural conditions. Al-Azzazy *et al.* (2019) examined the efficiency of silver nanoparticles on phytophagous (*Aculops lycopersici* Massee and *Tetranychus urticae* Koch) and predatory (*Euseius scutalis* Athias-Henriot and *Neosiulus cucumeris* Oudemans) mites of tomato plants in greenhouse condition and indicated that the mortality percentages of the mites were increased as the concentrations of nanoparticles increased. The same result was reported by Abd-Ella *et al.* (2020) for the population of oleander scales, *Aspidiotus nerii* Bouché, in field condition. These studies suggest that the nano-formulated insecticides may show no selectivity for either pests or natural enemies. Although the present study was conducted in laboratory, but the obtained results showed that the studied nano-pesticides had the potential to negatively affect the *H. hebetor* as the non-target organism. Natural condition investigation could provide more information in this regard.

## CONCLUSIONS

In this study, the lethal and sublethal toxicity of nano and commercial formulations of deltamethrin and matrine were evaluated on *H. hebetor*. The findings showed that the nano and commercial formulations of deltamethrin displayed higher toxicities and caused more sublethal effects on *H. hebetor* compared to the nano and commercial forms of matrine. Controlled-release formulations of nano-pesticides may have an important role in reducing their harmful effects on non-target organisms. However, it has been suggested that the application of lower doses of nanoformulations (Shifa *et al*., 2019) and releasing the natural enemies some days (72 hours) after spraying with nano-pesticides can efficiently minimize their negative effects on natural enemies (Ricupero *et al*., 2022). Therefore, the findings of the current study revealed that commercial matrine and

Cs-based nano-formulation of matrine could be appropriate candidates in integrating chemical and biological control, due to their low lethal and sublethal risks to *H. hebetor*. However, careful considerations need to be taken regarding the use of commercial and nano- formulation of deltamethrin. For a better understanding of other environmental impacts of the tested nano-insecticides, additional investigations are still required. Furthermore, supplementary information are recommended for future studies to check the potential of loading other conventional insecticides in PEG and Cs and their toxicity effect on other natural enemies.

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# اثرات کشنده و غیرکشنده دلتامترین و ماترین تجاری و نانوکپسوله شده علیه
**(Hymenoptera: Braconidae)** ***Habrobracon hebetor***

**اکرم احمدی، موسی صابر، و غلامرضا مهدوی نیا**

## چکیده

کنترل آفات حشرات از طریق فرمولاسیون مواد شیمیایی مبتنی بر نانو یکی از روش‌های جدید بکار رفته در برنامه‌های مدیریت تلفیقی آفات (IPM) است، با این حال، اثرات جانبی احتمالی نانو آفت‌کش‌ها بر ارگانیسم‌های غیر هدف باید ارزیابی شود. در این مطالعه دلتامترین و ماترین به ترتیب با پلی اتیلن گلیکول (PEG)و کیتوزان (Cs) کپسوله شدند و سمیت آنها بر علیه *Habrabracon hebetor* Say با استفاده از روش تماسی بررسی شد. با توجه به میکروسکوپ الکترونی روبشی(SEM) ، نانوذرات کروی برای هر دو فرمولاسیون مشاهده شد. میانگین قطر نانوذرات هیدرودینامیکی برای دلتامترین و ماترین ٦٥ و ٧٠.٥ نانومتر بود. مقادیر LC50 به ترتیب ٢٥٤.٤٨، ٣٣٤.٩٠، ٧٦٠.٣١ و ١٠٢١ mg L−1 در دلتامترین کپسوله شده با PEG، دلتامترین تجاری، ماترین کپسوله شده با Cs و ماترین تجاری بود. قرار گرفتن در معرض LC30 دلتامترین تجاری و نانو کپسوله شده به طور قابل توجهی کل دوره قبل از بزرگسالی را طولانی کرد. بزرگسالان H. hebetorدر تیمار دلتامترین کپسوله شده با PEG کمترین طول عمر را در مقایسه با سایر تیمارها و شاهد داشتند. علاوه بر این، مواجهه کشنده با نانوفرمولاسیون مبتنی بر PEG دلتامترین و دلتامترین تجاری منجر به کاهش قابل توجهی از نرخ ذاتی افزایش طبیعی (rm) (به ترتیب ٠.١٥٩ و ٠.١٦٨ در روز) شد. روند مشابهی

برای نرخ تولید مثل ناخالص(GRR) ، نرخ تولید مثل خالص(R0) ، و نرخ محدود افزایش ($\lambda$) از پارسیتوید مشاهده شد. یافته های ما نشان می دهد که اثرات جانبی منفی فرمولاسیون تجاری و مبتنی بر نانو دلتامترین بر H. hebetorباید در برنامه های IPM در نظر گرفته شود.