

Lethal and sublethal effects of commercial and nano-encapsulated
deltamethrin and matrine against *Habrobracon hebetor* (Hymenoptera:
Braconidae)

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ABSTRACT

Controlling insect pests through nano-based formulation of chemicals is one of the newly applied methods in IPM programs; however, the probable side impacts of nano-pesticides on non-target organisms need to be evaluated. In this study, deltamethrin and matrine were encapsulated with polyethylene glycol (PEG) and chitosan (Cs), respectively, and their toxicity were investigated against *Habrobracon 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 LC₅₀ values were 254.48, 334.90, 760.31 and 1021 mg L⁻¹ in PEG-encapsulated deltamethrin, commercial deltamethrin, Cs-encapsulated matrine, and commercial matrine, respectively. Exposing to the LC₃₀ of the commercial and nano-encapsulated deltamethrin significantly prolonged the total pre-adult period. The adults of *H. hebetor* in PEG-encapsulated deltamethrin treatment had the lowest longevity compared to other treatments and control. Furthermore, the sublethal exposure to the PEG-based nanoformulation of deltamethrin and commercial deltamethrin resulted in a significant reduction of the intrinsic rate of natural increase (r_m) (0.159 and 0.168 day⁻¹, respectively). Same trend was observed for the gross reproductive rate (GRR), net reproductive rate (R_0), and finite rate of increase (λ) of the parasitoid. 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, Insecticides, Parasitoid, Polyethylene glycol, Nano-encapsulation, Toxicity.

1. 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

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31 (Heibatian *et al.*, 2018; Wu *et al.*, 2019). Integrating pesticides with biocontrol agents usually
32 requires critical information about the impact and selectivity of the pesticides on natural enemies
33 including predators and parasitoids (Manjunath, 2022). The parasitoid *Habrabracon hebetor* Say
34 is one of the important species of Braconidae, used for controlling lepidopterous pests (Ghimire
35 and Phillips, 2010). Chemical control is widely used throughout the world for reducing pest
36 populations to prevent crop losses; however, the large-scale utilization of pesticides against
37 agricultural pests has turned out to cause serious problems for either the health of humans or the
38 environment, especially by contamination of air, soil, and underground water (Gill and Grag, 2014;
39 Ochoa and Maestroni, 2018).

40 In recent years, a growing interest has been found in developing nano-based formulations of
41 pesticides to decrease the hazardous impacts of the conventional pesticides (Shao *et al.*, 2022).
42 Nano-pesticides provide not only the successful and long-term control of pests, but reduce the
43 essential dosage of pesticides, frequency of pesticide use, and environmental risks of them
44 (Memarizade *et al.*, 2014; Agathokleous *et al.*, 2020). Polyethylene glycol (PEG) is a synthetic and
45 biocompatible polymer synthesized by ring-opening polymerization of ethylene oxide. For
46 facilitating sustained release of active ingredients of the water-insoluble pesticides, a semisynthetic
47 polymer of hydroxypropyl methyl-cellulose (HPMC) is also used. It forms a strong viscous gel
48 around the particles in contact with aqueous media (Karavas *et al.*, 2006). Chitosan (Cs) is a
49 naturally occurring polysaccharide obtained by deacetylation of chitin from different sources such
50 as fungi, crustaceans, and insects under alkaline conditions (Younes and Rinaudo, 2015). The Cs
51 can readily form spherical nano-capsules by adding a polyanionic salt of tripolyphosphate (TPP)
52 (Dutta *et al.*, 2004; Mason *et al.*, 2006; Ahmadi *et al.*, 2018b).

53 Despite the reported enhanced bioactivity of nano-pesticides against mites or insect pests
54 (Gonzalez *et al.*, 2015; Ahmadi *et al.*, 2018a,b; Ahmadi *et al.*, 2020; Ebadollahi *et al.*, 2022), the
55 impacts of nano-based formulation of pesticides should be evaluated toward natural enemies to
56 guarantee their safety use (Preetha *et al.*, 2018; Yan *et al.*, 2022). Because the nanoformulation of
57 insecticides may exhibit higher toxicity to natural enemies as reported by Sun *et al.* (2020) for
58 nano-formulated abamectin on *Adalia bipunctata* L. larvae or show no adverse impacts on them as
59 reported by Wu *et al.* (2024) for nano-pesticides based on a cationic star polymer (SPc) against
60 *Picromerus lewisi* Scott. So, the current research was aimed to evaluate the probable toxicity of
61 nanoformulations of deltamethrin and matrine insecticides along with their commercial analogues

62 against different growth stages of *H. hebetor*, because according to our unpublished data, both
63 insecticides showed partially less toxicity to *H. hebetor* compared to different insecticides that had
64 been used. Furthermore, the sublethal concentrations of commercial and nanoformulations of both
65 insecticides on the development, reproduction, and life table parameters of *H. hebetor* were
66 assessed.

67 2. Materials and methods

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

70

71 2.1. Insects' rearing

72 The colony of *H. hebetor* was obtained from a mass-rearing insectarium belonging to the
73 Agriculture Organization in Khoda Afarin County, East Azerbaijan Province, Iran. Adults of *H.*
74 *hebetor* were placed in pairs (5 pairs) inside 9 cm in diameter Petri dishes. Inside each Petri dish,
75 20 last instar larvae of *Ephestia kuehniella* were placed as hosts for parasitizing. A narrow strip of
76 paper covered with a thin layer of honey was used as a food source for adult parasitoids. After 24
77 h, the adults were removed from the Petri dishes and the parasitized larvae were kept in a growth
78 chamber at $26 \pm 1^\circ\text{C}$, $60 \pm 5\%$ RH, and 16L: 8D photoperiod until the emergence of the adult
79 parasitoids.

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

85

86 2.2. Materials

87 The commercial formulation of deltamethrin (Decis[®] 2.5% EC, Ariashimi Com., Iran) and
88 matrine (Rui Agro[®] 0.6% SL, Hangzhou Ruigiang Com., China) were used in the current study.
89 Polyethylene glycol-400 (PEG-400) (density 1.128 g/cm³, MW 380–420 g/mol), hydroxypropyl
90 methylcellulose (HPMC) (MW 150 000, HPMC-K100M), and Chitosan (Cs) with a viscosity-
91 average molecular weight of $(5.2 \pm 0.4) \times 10^5$ and a degree of deacetylation larger than 90% were

92 purchased from Sigma-Aldrich (St Louis, MO). All of the other chemicals used in this research
93 were also purchased from Sigma-Aldrich.

94

95 2.3. Nanoparticles

96 PEG-400, acetone, HPMC, distilled water and surfactant were used to prepare
97 nanodeltamethrin. Initially, 0.5% a.i. (w/v) of deltamethrin was added to 12 mL PEG-400, and 2
98 mL acetone (organic phase). Then, 0.2 g of HPMC was dissolved in 20 mL of distilled water and
99 2 mL surfactant (aqueous phase). After that, organic phase were slowly dropped into the aqueous
100 phase and stirred for 30 min at 4000 rpm. The obtained coarse emulsion was diluted with distilled
101 water (30 cc) and then, converted into a nano-emulsion through subjecting to ultrasonic
102 emulsification using a 20 kHz Sonicator (BANDELIN Sonopuls) for 10 min. For the preparation
103 of matrine nanoparticles (with water-soluble substances) (Kowah *et al.* 2023), chitosan, acetic acid
104 solution, and TPP were used. First, chitosan (0.1 g) was dissolved in acetic acid solution (50 mL)
105 (1 % v/v in water) by stirring at room temperature for about 30 min at 4000 rpm. Then, the quantity
106 of 0.5% a.i. (w/v) of matrine was added and allowed to dissolve completely. The TPP solution was
107 separately made by dissolving TPP (0.08 g) in distilled water (5 mL) and later, it was gradually
108 dropped into to the previous solution. The solution was then stirred for almost 60 min at 500 rpm
109 to gain a homogeneous solution (Ahmadi *et al.*, 2018a, b).

110 The size and morphology of PEG-deltamethrin and Cs-matine nanoparticles were assessed by
111 scanning electron microscopy (SEM) (VEGAI, XMU, Czech Republic) at the Central Laboratory,
112 University of Tabriz, Tabriz, Iran. The mean particle size was analyzed by dynamic light scattering
113 (DLS) via a Zetasizer photon correlation spectroscopy (PCS) instrument (Malvern Instruments
114 Limited, UK) at the Central Laboratory, University of Tabriz, Tabriz, Iran. The DLS were
115 replicated three times (Ahmadi *et al.*, 2020; Taktak *et al.*, 2021). Dried samples were imaged by
116 SEM (Ahmadi *et al.*, 2018a). Nanoparticles (5 mL) of PEG-encapsulated deltamethrin and Cs-
117 encapsulated matrine were simply separated from the liquid phase by centrifugation for 20 min at
118 8000 rpm. The supernatants were assessed for deltamethrin or matrine by UV spectroscopy. The
119 solubility of the PEG-deltamethrin and Cs-matine nanoparticles was compared to those of
120 deltamethrin and matrine using UV absorbance (UV-Vis Spectroscopy, Unico, UV-2802, USA) at
121 $\lambda_{\max} = 290$ nm. First, 1 mg of the active ingredient of the examined encapsulated formulations was
122 dissolved in distilled water (1000 μ l) and stirred for 30 min at normal temperature. Then, the

123 absorption amount of deltamethrin or matrine in the supernatants at 200 μ l, 25°C was determined
124 at 0, 0.5, 24, 48, 72, and 96 h.

125

126 2.4. Lethal effects of the chemicals on *H. hebetor*

127 The lethal effects of the commercial and nano-formulated insecticides on the adults of *H.*
128 *hebetor* were evaluated by contact method. By using a micropipette, 3 mL of each concentration
129 (12.5, 9.94, 7.905, 6.287, and 5 mg a.i./L for commercial deltamethrin, 9.5, 7.652, 6.15, 4.965, and
130 4 mg a.i./L for PEG-encapsulated deltamethrin, 12, 8.485, 6, 4.242, and 3 mg a.i./L for
131 commercial matrine, and 9, 6.467, 4.647, 3.339, and 2.4 mg a.i./L for Cs-encapsulated matrine)
132 was poured into the McCartney glass bottles (28 mL) and swirled well to ensure a complete coating,
133 with excess liquid removed. In the control, distilled water plus Tween-80[®] (Merck, Darmstadt,
134 Germany) was used. The bottles were let dry completely for 2 h in the laboratory. Then, 20 newly
135 emerged adults (< 24 h-old) were anesthetized by CO₂ and placed in each bottle and then, the
136 aluminum caps of bottles screw onto the bottles. The wasps were supplied with honey as a food
137 source on narrow strips of paper (5 × 10 mm). All the bottles were kept in the growth chamber at
138 26 ± 1°C, 60 ± 5% RH, and 16:8 h (L:D). The mortality of *H. hebetor* adults in each bottle was
139 recorded 24 h after the initial exposure to the different concentrations of insecticides. Each
140 insecticide's bioassay test was replicated three times. The recommended field concentrations
141 (<https://www.ppo.ir/fa-IR/ppo/5186/>) of deltamethrin, nano-deltamethrin, matrine and nano-
142 matrine were 500, 500, 1000, and 1000 mg liter⁻¹ based on the formulated substance, respectively

143

144 2.5. Sublethal effects study

145 For the evaluation of the sublethal effects of the tested insecticides, 20 pairs of adults of *H.*
146 *hebetor* were placed in Petri dishes (9 cm diameter) with holes (5 cm diameter) in the lids covered
147 by the fine-mesh net for ventilation to parasitize 100 last instar larvae of *E. kuehniella*. Honey was
148 offered to the wasps on narrow strips of paper (5 × 10 mm). After 24 h, the adults were removed
149 and 40 parasitized larvae were kept in a growth chamber at 26 ± 1°C, 60 ± 5% RH, and 16L: 8D
150 photoperiod. Four days later, when one-day old larvae of *H. hebetor* appeared, they were sprayed
151 using a Potter spray tower (Burcard Scientific[®]) with 5 mL of LC₃₀ values of the commercial
152 formulation (6.36 and 4.02 mg a.i./L of deltamethrin and matrine, respectively) and nano-based
153 formulation of insecticides (5.00 and 3.21 mg a.i./L of PEG-encapsulated deltamethrin and Cs-

154 encapsulated matrine, respectively). The larvae in control were treated with distilled water plus
155 Tween-80[®]. The treated larvae were transferred to 9 cm diameter Petri dishes and kept in a growth
156 chamber until the emergence of the adult wasps. For each treatment, 55 pairs of males and females
157 of *H. hebetor* (24 h old) were randomly selected and transferred to Petri dishes (9 cm diameter).
158 Each pair of wasps in each Petri dish was provided with three *E. kuehniella* larvae for oviposition
159 and fed with honey on a thin strip of paper. The host larvae in new Petri dishes were offered to the
160 wasps every 24 h to determine their daily reproduction. The survival, oviposition period, longevity,
161 and fecundity of the parasitoid were daily monitored and recorded until the death of the last
162 individual.

163 2.6. Data analysis

164 The encapsulation efficiency was evaluated according to the following formula (Ahmadi *et al.*,
165 2022):

$$166 \text{EE\%} = \frac{\text{amount of total insecticide} - \text{amount of free insecticide}}{\text{amount of total insecticide}} \times 100$$

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

173 3. Results

174 3.1. Characterization of PEG-deltamethrin and Cs-matine nanoparticles

176 The shape and mean size of the nanoparticles of PEG-based nanoformulation of deltamethrin
177 and Cs-based nano-formulation of matrine were investigated by SEM and DLS techniques,
178 respectively. SEM analysis revealed that the nanoparticles of PEG-deltamethrin were spherical,
179 with a mean size of 100 ± 10 nm (Fig. 1(a)). The same morphology (spherical shape) was detected
180 in nanoparticles of Cs-matine. However, the average diameter of them was not distinguishable
181 due to the aggregation during the drying process (Fig. 1(b)). Based on DLS analysis, the average
182 hydrodynamic diameter of 65 and 70.5 nm with a polydispersity index (PDI) of 195.0 and 16.40
183 was obtained for PEG-deltamethrin and Cs-matine nanoparticles, respectively (Fig. 2 a and b). In

184 comparison to the DLS result, the diameter of PEG-deltamethrin nanoparticles from the SEM result
185 was obtained larger than 70.5 nm (about 100 ± 10 nm). This phenomenon can be attributed to the
186 coating of produced deltamethrin nanoparticles by the PEG ingredient during the drying process.

187

188 3.2. Encapsulation efficiency

189 The encapsulation efficiency (EE%) was measured by UV-Vis spectroscopy using a standard
190 graph for PEG-encapsulated deltamethrin ($y = 0.1597x - 0.0266$, $R^2 = 0.9895$) and Cs-encapsulated
191 matrine ($y = 0.0815x - 0.0086$, $R^2 = 0.9886$) at 290 nm (Fig. 3). The concentrations of deltamethrin
192 and matrine in the supernatant were obtained via the standard curve. Once the insecticide loading
193 efficiencies in nanoparticles were determined, deltamethrin and matrine were found in $89.13 +$
194 0.50% and $91.87 + 0.63\%$ of the nanoparticles. This result suggests that the nanoparticles of PEG
195 and Cs are promising vehicles for encapsulation of the tested insecticides.

196

197 3.3. Water dispersion of the tested chemicals

198 It was revealed that PEG-deltamethrin and Cs-matrine nanoparticles in the absence of organic
199 solvents dissolved more efficiently in water than their commercial forms. After about an hour, the
200 concentration of commercial formulations of deltamethrin and matrine dissolved in water were
201 26.95 and 26.41 mg a.i./mL, respectively (Fig. 4). Furthermore, the solubility of PEG-deltamethrin
202 and Cs-matrine nanoparticles were 47.50 and 47.35 mg a.i./mL (Fig. 4). The results indicated an
203 increase in the rate and extent of both deltamethrin and matrine dissolution for the nano-suspension
204 as compared to the commercial formulations (Fig. 4).

205

206 3.4. Lethal effects of the tested chemicals on *H. hebetor*

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

215

216 **3.5. Sublethal effects study results**

217 A sublethal effect study showed that the incubation and larval period of *H. hebetor* exposed to
218 the LC₃₀ (lethal concentration causing 30% mortality) of the commercial and nanoformulations of
219 deltamethrin and matrine significantly affected by different treatments. The preimaginal period
220 values in commercial and nanoformulations of deltamethrin were higher than those observed in
221 other treatments ($P < 0.05$) (Table 3). The longest pupal period of the parasitoid was observed in
222 PEG-deltamethrin ($P < 0.05$) (Table 3). The total pre-adult period of *H. hebetor* in nano-
223 encapsulated deltamethrin and its commercial formulation was significantly longer than those
224 obtained in nano-encapsulated matrine, commercial matrine, and control ($P < 0.05$) (Table 3). No
225 significant difference was found between the treatments and control in regards to the percentage of
226 pre-adult survival of *H. hebetor* ($P > 0.05$) (Table 3).

227 The adult pre-oviposition period (APOP) of *H. hebetor* was significantly affected when treated
228 with LC₃₀ of the commercial and nanoformulations of either insecticide ($P < 0.05$). The highest
229 APOP was obtained in PEG-based nanoformulation of deltamethrin (Table 4). The total pre-
230 oviposition period (TPOP) was significantly highest in nano-encapsulated deltamethrin and
231 commercial deltamethrin ($P < 0.05$) (Table 4). The oviposition period of *H. hebetor* significantly
232 differed among treatments ($P < 0.05$) and it was shortest in PEG-encapsulated deltamethrin (Table
233 4). Males and females exposed to LC₃₀ of nano-encapsulated deltamethrin had significantly shorter
234 longevity ($P < 0.05$) (Table 4). The fecundity of *H. hebetor* was significantly decreased in the
235 treatments (from 66.48–165.24 eggs) compared to the control (200.84 eggs) ($P < 0.05$). The least
236 fecundity was recorded in PEG-encapsulated deltamethrin and commercial deltamethrin (Table 4).

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

244 The results of the present study showed that the exposure to LC₃₀ of either nano-encapsulated
245 deltamethrin or commercial deltamethrin significantly decreased the gross reproductive rate
246 (GRR), net reproductive rate (R_0), intrinsic rate of natural increase (r_m), and finite rate of increase

247 (λ) of *H. hebetor* ($P < 0.05$) (Table 5). Furthermore, treating *H. hebetor* with the LC₃₀ of nano-
248 encapsulated deltamethrin, commercial deltamethrin, and nano-encapsulated matrine significantly
249 lengthened the mean generation time (T) compared to commercial matrine and control ($P < 0.05$)
250 (Table 5).

251

252 4. Discussion

253 In the present study, the morphology of particles obtained for nanoformulations of the tested
254 insecticides is consistent with the results of Ahmadi *et al.* (2018a) who reported the spherical-like
255 shapes of nanoparticles for *Satureja hortensis* essential oil-loaded Cs/tripolyphosphate
256 nanoparticles and inconsistent with the findings of Ebadollahi *et al.* (2022) that revealed the
257 elliptical shapes of nanoparticles for sodium alginate- and PEG-acetamiprid. According to the
258 obtained results, the mean hydrodynamic diameter of PEG-deltamethrin nanoparticles was about
259 the same size as the Cs-matine nanoparticles. The sizes of the nanoparticles in the present study
260 were somehow in consistent with that reported by Ebadollahi *et al.* (2022) regarding the
261 encapsulation of acetamiprid in PEG (101.2 nm) and were very smaller than the clofentezine-
262 loaded nanoparticles (300 nm) reported by Ahmadi *et al.* (2020). The smaller size of nanoparticles
263 based on DLS in our study compared to the latter study may be resulted from the low aggregation
264 of the nanoparticles in the solution. According to the results of the present study, nanoformulations
265 of the tested insecticides showed improved solubility in water compared to the commercial
266 formulations. Similarly, Pan *et al.* (2015) and Ahmadi *et al.* Worrall *et al.* (2018) stated that normal
267 formulations of insecticides with low water-solubility usually need organic solvents to aid in
268 solubilizing the insecticide, which increases the cost and toxicity of the insecticide; but nano-based
269 formulations of insecticides eliminate the need for organic solvents and can be used to increase the
270 solubility, which leads to reducing their toxicity.

271 Results of the bioassay study showed that nano-encapsulation of deltamethrin with PEG and
272 matrine with Cs decreased the LC₅₀ of the commercial formulations of the insecticides from 334.90
273 to 254.48 mg L⁻¹ and from 1021 to 760.31 mg L⁻¹, respectively. These results revealed that the
274 nano-formulation of the tested insecticides increased their toxicity against *H. hebetor*. Increased
275 performance of nano-based formulations of insecticides against insect pests and their natural
276 enemies has been reported in several studies. For example, Shifa *et al.* (2019) demonstrated that
277 the nanoformulation of deltamethrin caused two times more mortality on *Trialeurodes*

278 *vaporariorum* Westwood than its commercial formulation. The PEG and Cs are generally
279 considered almost non-toxic polymers that are extensively used in the fields of agriculture and
280 medicine (Naskar *et al.*, 2019; Ebadollahi *et al.*, 2022); however, insecticides loaded in
281 aforementioned nano-carriers are usually more effective toward either insect pests or natural
282 enemies than their typical commercial formulations. In the present study, the commercial matrine
283 showed less toxicity in terms of LC₅₀ toward *H. hebetor* than the commercial deltamethrin. The
284 same results were also observed in their nano-based formulations. The variation may be related to
285 the difference in their chemical compositions, mode of action, nano-carriers, encapsulation
286 methods and features of particles. Similar to the findings of the current study, the low toxicity of
287 matrine on natural enemies have been documented in the literature. For instance, the commercial
288 formulation of matrine exhibited less toxicity in terms of LC₅₀ toward adults of *Orius laevigatus*
289 (Fieber) (Kordestani *et al.*, 2022b) and *Amblyseius swirskii* Athias-Henriot (Kordestani *et al.*,
290 2022a). Matrine is a botanical insecticide with a broad spectrum of insecticidal activity, which acts
291 by affecting the insects' acetylcholine receptors (Liu *et al.*, 2007; Qu *et al.*, 2022; Zhou *et al.*,
292 2022). Mahdavi *et al.* (2013) and Heibatian *et al.* (2018) also showed that the commercial
293 formulation of deltamethrin was toxic to *H. hebetor* adults and carabid beetles (Col., Carabidae),
294 respectively. In a study by Garzón *et al.* (2015), deltamethrin was more toxic to *Chrysoperla carnea*
295 Stephens and *Adalia bipunctata* Linnaeus. Deltamethrin is a broad-spectrum insecticide, which
296 disrupts the voltage-gated sodium channels in the nervous system, resulting in neurotoxicity in
297 insects (Pradhan and Mailapalli, 2020).

298 In toxicological studies, life history parameters and other measures of population growth rate
299 provide more detailed information about the impacts of pesticides on targeted and non-targeted
300 organisms than that of lethal dose/concentration 50 (LD₅₀, LC₅₀) (Parsaeyan *et al.*, 2020; Gope *et al.*,
301 2022). According to the results, the exposure of *H. hebetor* larvae to LC₃₀ of either PEG-
302 encapsulated deltamethrin or commercial deltamethrin significantly prolonged the duration of the
303 immature stages and decreased the parasitoid's fecundity. Furthermore, exposure of the parasitoid
304 to the recommended doses of nano-encapsulated deltamethrin shortened its longevity and
305 oviposition period. Similar to our results, nano-encapsulation of acetamiprid using coating
306 materials of sodium alginate and PEG enhanced the sublethal efficiency of the insecticide against
307 the elm leaf beetle (Ebadollahi *et al.*, 2022). Rafiee Dastjerdi *et al.* (2012) showed that *H. hebetor*
308 females exposed to the field-recommended dose of deltamethrin had the shortest longevity and

309 produced fewer eggs (98.08 eggs) than those in control (430.60 eggs). The longevity and fecundity
310 of *H. hebetor* were also affected by the LC_{25} of commercial formulation of fenprothrin
311 insecticides as reported by Faal-mohammadali *et al.* (2014). In contrast, Sarmadi *et al.* (2010)
312 found that the commercial formulation of deltamethrin reduced the fecundity of *H. hebetor*, but it
313 did not affect its longevity. This is probably due to the differences in the population of the parasitoid
314 or the used concentrations of the insecticide.

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

332 For better establishing the eco-friendly control measures in IPM programs, the efficacy of
333 nanopesticides should be evaluated against target and non-target organisms in natural conditions.
334 Al-Azzazy *et al.* (2019) examined the efficiency of silver nanoparticles on phytophagous (*Aculops*
335 *lycopersici* Masee and *Tetranychus urticae* Koch) and predatory (*Euseius scutalis* Athias-Henriot
336 and *Neosiulus cucumeris* Oudemans) mites of tomato plants in greenhouse condition and indicated
337 that the mortality percentages of the mites were increased as the concentrations of nanoparticles
338 raised up. Same result was reported by Abd-Ella *et al.* (2020) for the population of oleander scales,
339 *Aspidiotus nerii* Bouché in field condition. These studies suggest that the nano-formulated

340 insecticides may show no selectivity for either pests or natural enemies. Although the present study
341 was conducted in laboratory, but the obtained results showed that the studied nanopesticides had
342 the potential to negatively affect the *H. hebetor* as the non-target organism. Natural condition
343 investigation could provide more information in this regard.

344 345 5. Conclusion

346 In this study, the lethal and sublethal toxicity of nano and commercial formulations of
347 deltamethrin and matrine were evaluated on *H. hebetor*. The findings showed that the nano and
348 commercial formulations of deltamethrin displayed higher toxicities and caused more sublethal
349 effects on *H. hebetor* compared to nano and commercial forms of matrine. Controlled-release
350 formulations of nano-pesticides may have an important role in reducing their harmful effects on
351 non-target organisms; however, it has been suggested that the application of lower doses of
352 nanoformulations (Shifa *et al.*, 2019) and releasing the natural enemies some days (72 h) after
353 spraying with nano-pesticides can efficiently minimize their negative effects on natural enemies
354 (Ricupero *et al.*, 2022). Therefore, the findings of the current study revealed that commercial
355 matrine and Cs-based nano-formulation of matrine due to their low lethal and sublethal risks to *H.*
356 *hebetor* could be appropriate candidates in integrating chemical control and biological control;
357 however, careful considerations need to be taken regarding the use of commercial and nano-
358 formulation of deltamethrin. For a better understanding of other environmental impacts of the
359 tested nano-insecticides, additional investigations are still required. Furthermore, supplementary
360 inquiries are recommended for future studies to check the potential of loading other conventional
361 insecticides in PEG and Cs and their toxicity on other natural enemies.

362 363 Acknowledgments

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365 technical assistance.

366 367 References

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 501 underlying mechanisms. *Pest Manag. Sci*, 78(8): 3424-3432.

504 **Table 1.** Toxicity of commercial and nano-encapsulated deltamethrin and matrine based on
 505 PEG and Cs (mg/l) against the adults of *Habrabracon hebetor*.

Treatments	χ^2	Slope \pm SE	Lethal concentrations (mg ai/l)		
			LC ₃₀ (95% FL)	LC ₅₀ (95% FL)	LC ₉₀ (95% FL)
Commercial deltamethrin	48.44	4.40 \pm 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 \pm 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 \pm 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 \pm 0.45	3.21 (2.68 – 3.65)	4.56 (4.05 – 5.09)	10.71 (8.83 – 14.52)

506 Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute, 2002).
 507 *PEG: Polyethylene glycol, Cs: chitosan

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

Insecticides	Recommended field concentration (mg liter ⁻¹)	Mortality rate	IOBC classification*
Commercial deltamethrin	500	76.66 \pm 3.33 b	slightly harmful
PEG-deltamethrin	500	100 \pm 0.0 a	Harmful
Commercial matrine	1000	42.67 \pm 2.86 d	slightly harmful
Cs-matrine	1000	65.33 \pm 3.09 c	slightly harmful
Control	Distilled water	1.33 \pm 0.87 e	-

510 * IOBC (International Organization for Biological Control) classification: 1) harmless (mortality<30%),
 511 2) slightly harmful (>30 and <79%), 3) moderately harmful (>80 and <99%), and 4) harmful (>99%)
 512 (Hassan, 1994; Biondi *et al.* 2012).

514

515 **Table 3.** The developmental times and survival (mean \pm SE) of *Habrabracon hebetor* exposed to
 516 LC₃₀ of commercial and nano-encapsulated deltamethrin and matrine based on PEG* and Cs*.

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

517 Means followed by different letters in each column are significantly different ($P < 0.05$, paired bootstrap test).

518 *PEG: Polyethylene glycol, Cs: chitosan.

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525 **Table 4.** The oviposition period, longevity, and fecundity (mean \pm SE) of *Habrabracon hebetor*
 526 exposed to LC₃₀ of commercial and nano-encapsulated deltamethrin and matrine based on PEG*
 527 and Cs*.

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

528 Means followed by different letters in each column are significantly different ($P < 0.05$, paired bootstrap test). *PEG:
 529 Polyethylene glycol, Cs: chitosan. **APOP: adult pre-oviposition period, TPOP: total pre-oviposition period.

530

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

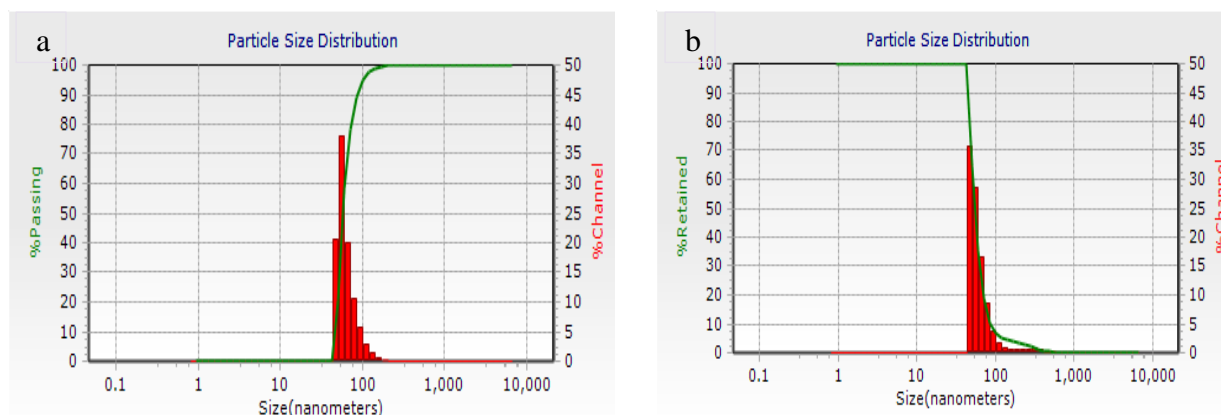
Treatments	GRR^{**} (female/female)	R_0 (female/female)	r_m (day ⁻¹)	λ (day ⁻¹)	T (day)
Commercial deltamethrin	65.32 \pm 8.065 b	37.13 \pm 6.206 b	0.168 \pm 0.008 c	1.183 \pm 0.010 c	21.49 \pm 0.424 a
PEG-deltametrin	69.03 \pm 8.644 b	30.21 \pm 5.394 b	0.159 \pm 0.008 c	1.173 \pm 0.009 c	21.36 \pm 0.278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

533 Means followed by different letters in each column are significantly different ($P < 0.05$, paired bootstrap test).

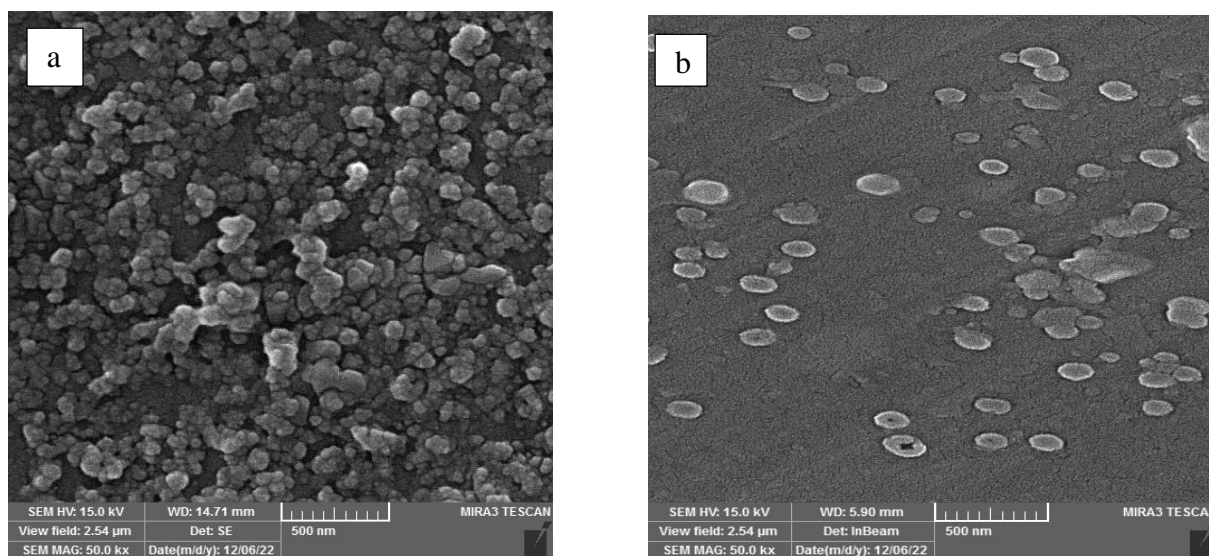
534 *PEG: Polyethylene glycol, Cs: chitosan.

535 ** GRR : gross reproductive rate, R_0 : net reproductive rate, r_m : intrinsic rate of increase, λ : finite rate of increase, T :
 536 mean generation time.

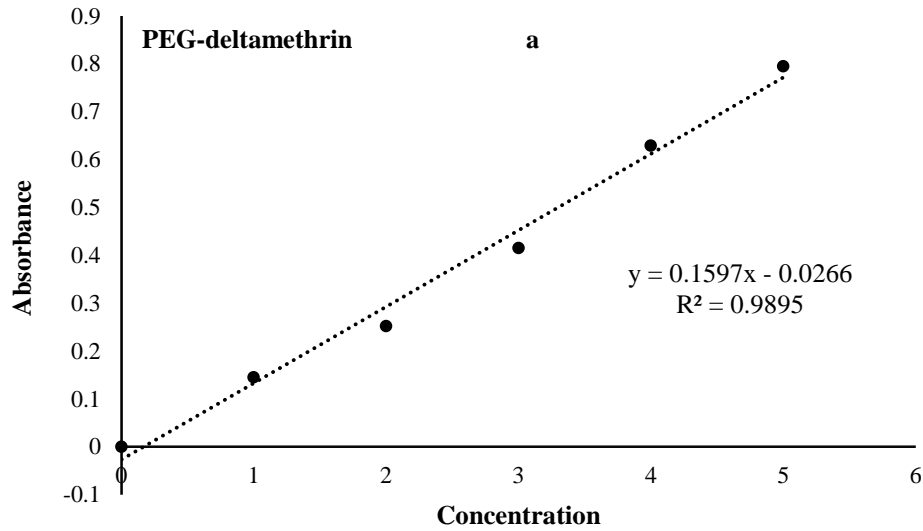
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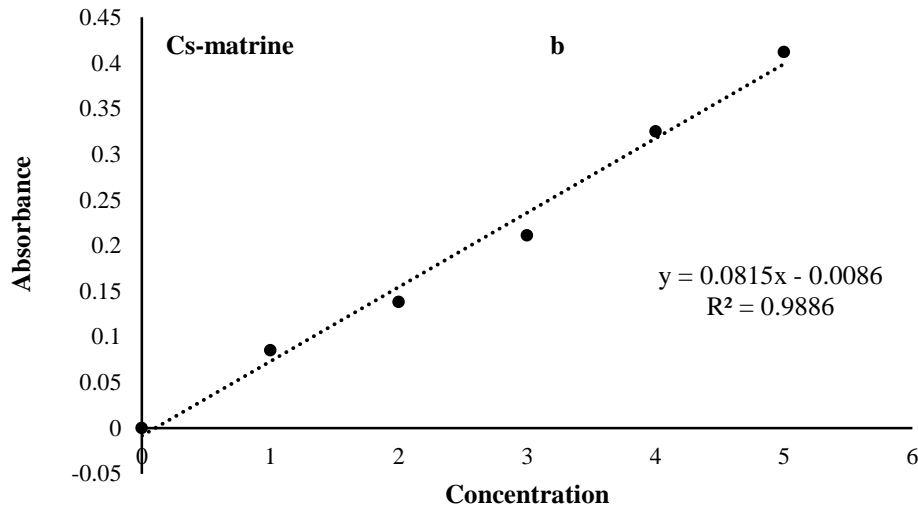
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 539 **Fig. 1.** Dynamic light scattering (DLS) measurement of particle size distribution of PEG
 540 (polyethylene glycol)-deltamethrin (a) and Cs (chitosan)-matrine (b) nanoparticles.
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 543 **Fig. 2.** Scanning electron microscopy (SEM) micrographs of PEG (polyethylene glycol)-
 544 deltamethrin (a) and Cs (chitosan)-matrine (b) nanoparticles.
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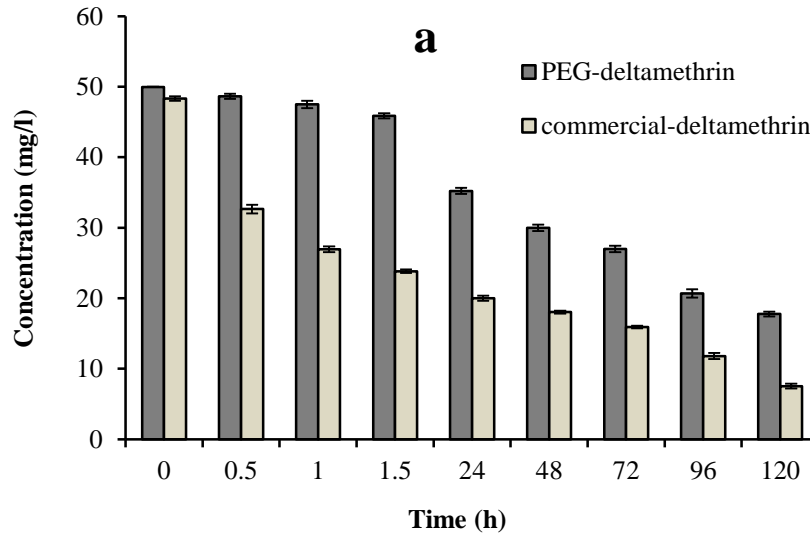
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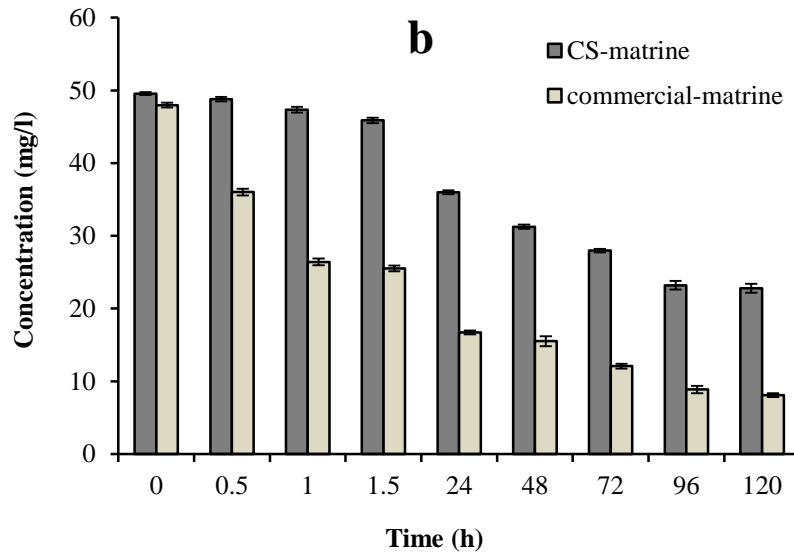
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548 **Fig. 3.** The encapsulation efficiency (EE%) calculated by UV-Vis spectroscopy using a standard
549 graph for nano-encapsulated deltamethrin (a) and matrine (b) based on PEG (polyethylene glycol)
550 and Cs (chitosan), respectively.
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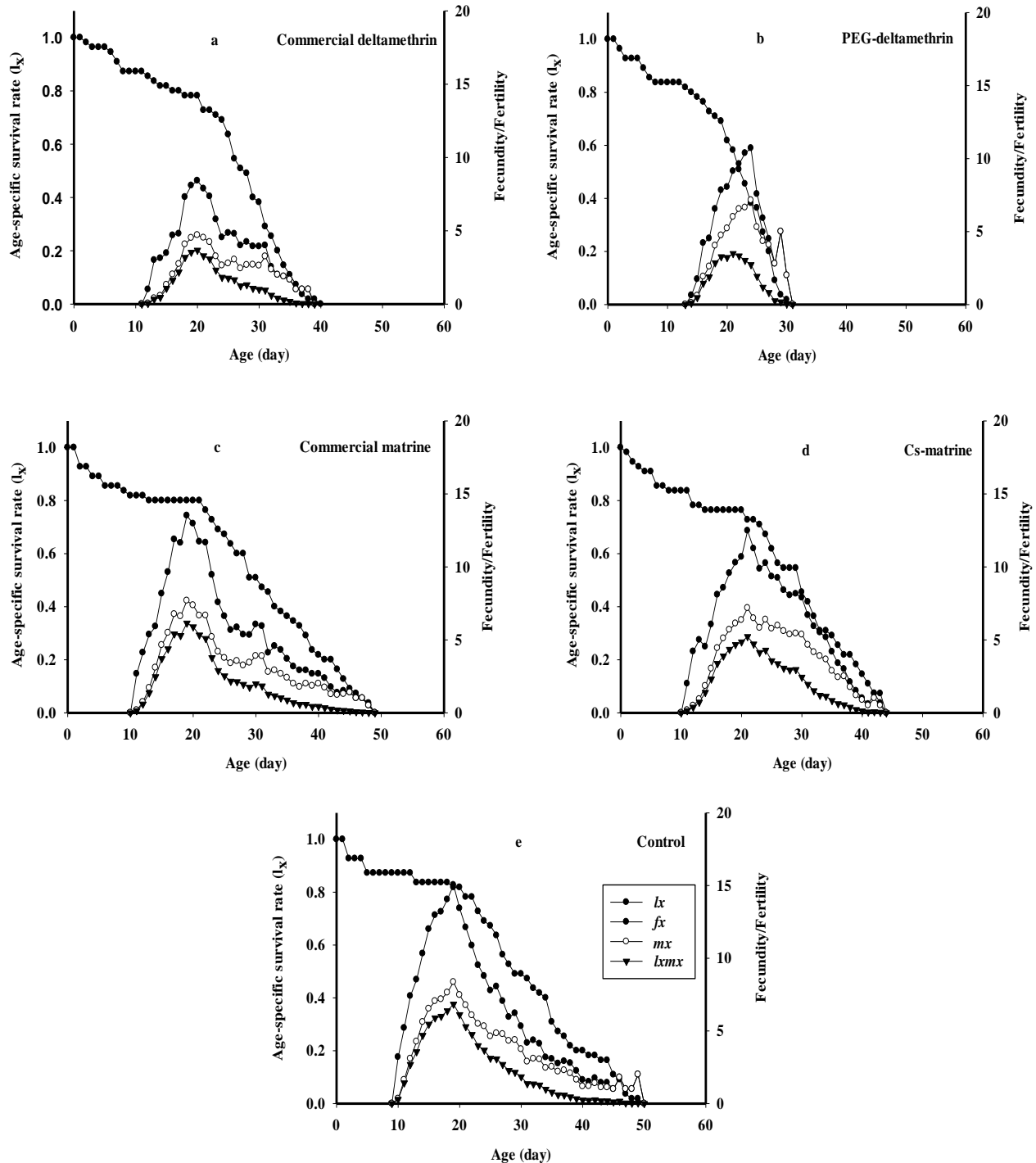


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555 **Fig. 4.** Differences in water solubility of a) PEG (polyethylene glycol)-encapsulated deltamethrin
 556 and commercial deltamethrin and b) Cs (chitosan)-encapsulated matrine and commercial matrine.
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561 **Fig. 5.** Age-specific survival rate (l_x), age-stage specific fecundity (f_x), age-specific fecundity (m_x)
 562 and age-specific fertility ($l_x m_x$) of *Habrabracon hebetor* exposed to LC₃₀ of commercial and nano-
 563 encapsulated deltamethrin (a and b) and matrine (c and d) based on PEG (polyethylene glycol) and
 564 Cs (chitosan), respectively along with control (e).
 565

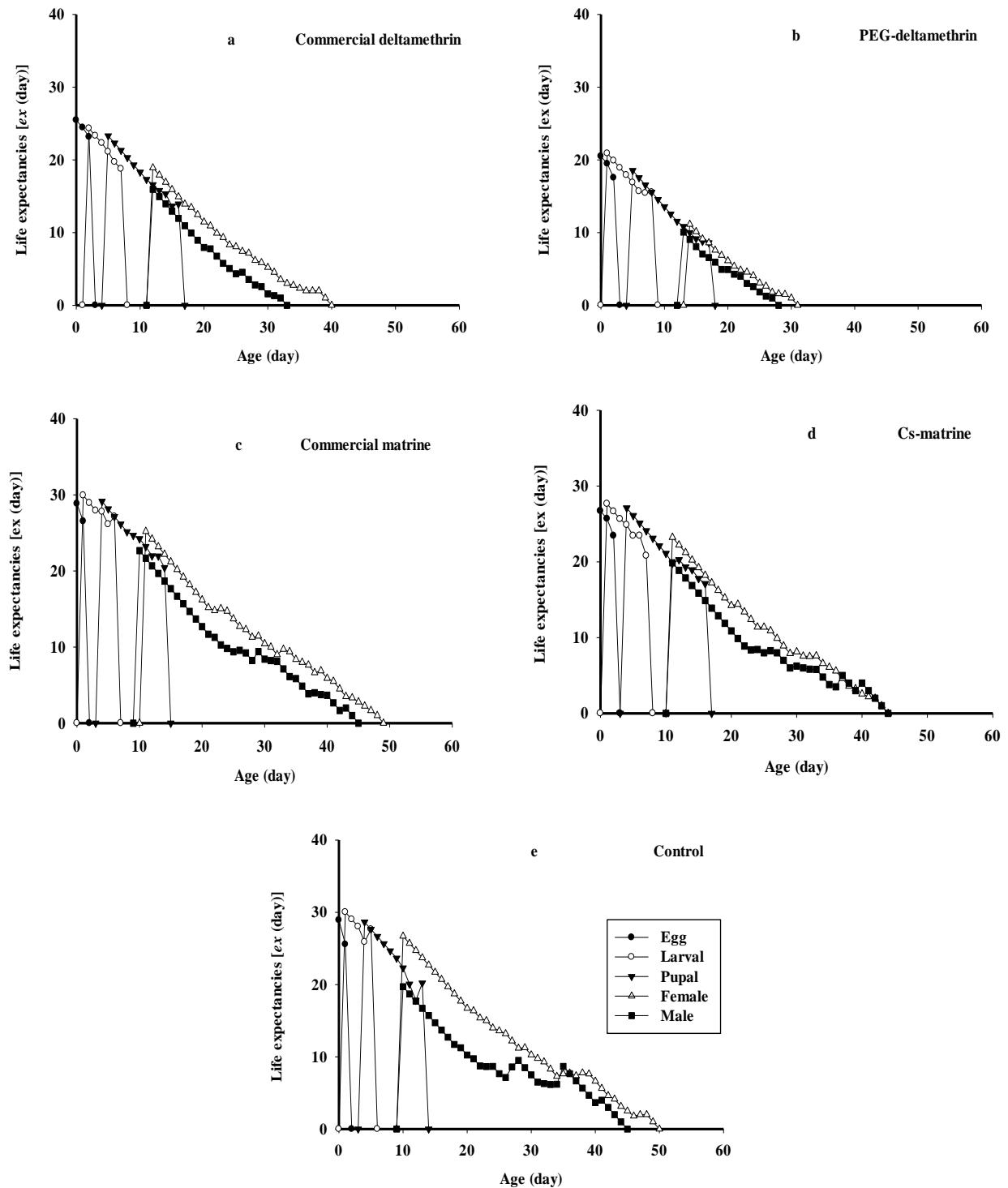


Fig. 6. Life expectancy [e_x (day)] of *Habrabracon hebetor* exposed to LC_{30} of commercial and nano-encapsulated deltamethrin (a and b) and matrine (c and d) based on PEG (polyethylene glycol) and Cs (chitosan), respectively along with control (d).

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

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 *Habrobracon 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 65 و 70.5 نانومتر بود. مقادیر LC50 به ترتیب 254.48، 334.90، 760.31 و 1021 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. hebetor* در 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 0.159 و 0.168 در روز) شد. روند مشابهی برای نرخ تولید مثل ناخالص (GRR)، نرخ تولید مثل خالص (R0)، و نرخ محدود افزایش (λ) از پارسیتوید مشاهده شد. یافته‌های ما نشان می‌دهد که اثرات جانبی منفی فرمولاسیون تجاری و مبتنی بر نانو دلتامترین بر *H. hebetor* باید در برنامه‌های IPM در نظر گرفته شود.