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Comparative selectivity of nano and commercial formulations of pirimicarb on a target pest, *Brevicoryne brassicae*, and its predator *Chrysoperla carnea*

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Abstract

Nanotechnology is a new field in the pesticide industry. Nanopesticides represent an emerging technological tool that offers a range of benefits including increased efficacy, durability, and reduction in the amounts of used active ingredients. However, due to the lack of studies on the toxicity and the sublethal effects on pests and natural enemies, the extent of action and fate of these nanopesticdes is still not fully understood limitting thus their wide use. In this study, we encapsulated the pirimicarb insecticide using nanostructured lipid carriers (NLC) and investigated the toxicity and sublethal effects (LC_{25}) of the resulting nanocapsules against the cabbage aphid, *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae) and its natural enemy the green lacewings *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). Nanoencapsulation of pirimicarb enhanced 12.6-fold its toxicity to cabbage aphids compared to its commercial formulation. Furthermore, analysis of the age-stage, two-sex life table showed that negative effects on the *B. brassicae* aphid population growth were observed on F0 and F1 generations when aphids of parental (F0) generation were exposed to subelethal dose (LC_{25}) of both formulations of pirimicarb. However, negative effects from sublethal exposure to the commercial and nanoformulated pirimicarb resulted in significant reduction on the *net reproductive rate*, *intrinsic rate of natural increase*, and *finite rate of increase* of the green lacewings *C. carnea*. Our findings indicate that the approaches and assumptions used to assess the risks of conventional insecticides may not apply for nanopesticides. Further research is still needed to better understand the environmental impact of these compounds.

Keywords Integrated pest management · Nanopesticides · Nanostructured lipid carriers · Sublethal effect · Life table

Introduction

In modern agriculture, the overreliance on chemical pesticides resulted on a growing build-up of resistance in targeted arthropods (e.g. see Liang et al. 2012; Guedes et al. 2019; Gul et al. 2019), as well as severe consequences for human health and environment (Weisenburger 1993; Desneux et al. 2007) shifting the focus of researches to novel and potentially eco-friendly control tools. In this context, nanotechnology has been suggested as promising source of new materials with enhanced properties for insect pest control (Benelli 2018; Ferreira et al. 2019; Lade and Gogle 2019; Ragaei and Sabry 2014; Athanassiou et al. 2018).

The use of the new concept pesticides based on nanotechnology, commonly referred to as nanopecticides, can increase efficiency and improve quality of pesticides applications and hence reduce their adverse effects on the

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environment (Guan et al. 2010; Guan et al. 2008; Kahru et al. 2008; Campolo et al. 2017; Athanassiou et al. 2018). Nanoformulations of pesticides have attracted much attention due to their greater effect even at very low doses (Camara et al. 2019; Campos et al. 2015; Sciortino et al. 2021). Indeed, nanoparticles (NPs) present altered physiochemical properties with increased surface to volume ratio and high mobility across physiological barriers making them excellent delivery systems (Lade and Gogle 2019; Sasson et al. 2007). Furthermore, nanomaterials had no apparent toxic effects on a number of plants in preliminary research making promising the use of these materials in agriculture (De La Torre-Roche et al. 2013). Pesticides nanoformulation is expected to have significant impacts on the fate of active ingredient improving the performance of conventional insecticides and increasing the control efficiency (Lade and Gogle 2019).

The cabbage aphid, *Brevicoryne brassicae*, is an oligophagous and important pest of Brassicaceae causing sever feeding damages and transmitting various phytopathogenic viruses (Hao et al. 2017; Pereira et al. 2019; Hullé et al. 2020). Application of insecticides is the major tool for managing cabbage aphids with numerous sprayings needed during the plant cycle (Fening et al. 2013). Several classes of insecticides are usually used to control aphids on vegetable, cereal and orchard crops (Lu et al. 2012; Mohammed et al. 2018; Shah et al. 2020). Biological control based on natural enemies is also used to reduce the aphid populations' density (Holland et al. 2012; Desneux et al. 2006a; 2019; Hullé et al. 2020).

However, because the activity of natural enemies cannot prevent virus transmission, earlier and faster aphid control methods (i.e., neurotoxic insecticides) are generally preferred (Ricupero et al. 2020). Pirimicarb is a carbamate aphicide that inhibits acetylcholinesterase activity in the insect nervous system (Hassall 1990). This insecticide is fast acting and presents selectivity toward natural enemies, such as the green lacewings *Chrysoperla carnea*, a genelist predator that can significantly prey upon and is exploited for the biological control of aphids and other soft-bodied arthropods (Darwish and Attia 2017; El-Wakeil et al. 2013; Koczor et al. 2019; Meissle et al. 2014).

Due to the small number of selective available insecticides for aphid control, measures and techniques aiming to reduce the risk of resistance occurrence in aphid populations are urgently needed (Dai et al. 2020). Therefore, nanoformulation of insecticides would help extending the market life of these active compounds. However, only few researches have been focusing on the effects of sublethal doses of such nanoformulations on life table parameters not only of pest but also non-target insects. The present research aimed to better understand and clarify some of the side effects of pirimicarb nanocapsules across generations of aphids. Thus, we prepared nanocapsules of pirimicarb and using a an age-stage and two-sex life table method we investigated the lethal and sublethal effects of nanofurmulated pirimicarb on the life parameters of cabbage aphids, *B. brassicae* and its predator the green lacewing *C. carnea*.

Methods

Insect rearing

The cabbage aphids, *B. brassicae* used in all experiments were obtained from infected cabbage leaves in the greenhouse of Urmia University, Urmia, Iran (37°39'14.88″N, 44°59'7.44″E). The aphids were kept on the cabbage plants *Brassica oleracea* (var. *capitata*) planted in plastic pots (15 cm diameter and 16 cm high) under greenhouse conditions (26 ± 2 °C; 60 ± 10% RH; 16: 8 (L:D)).

The adults of common green lacewing *C. carnea* were obtained from the Iranian Research Institute of Plant Protection of Khorasan (Mashhad, Iran) and were kept under controlled condition of temperature (26 ± 2 °C), relative humidity (60 ± 10%), and photoperiod (16: 8 L:D) in a greenhouse at the University of Tabriz, Iran. Adults of green lacewing were reared on artificial diet in clear plastic cylinders (15 cm in diameter and 25 cm high) with both sides covered with mesh cloth. Larvae were kept individually in Petri dishes and fed with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) which was reared in a insect rearing laboratory at the University of Tabriz.

Insecticides and chemicals

Pirimicarb (50% of active ingredient (ai); Primor) and technical grade of pirimicarb were donated by Ariashimi (Zahedan, Iran). Miglyol 812 (caprylic/capric triglycerides) was obtained from Sasol (Hamburg, Germany). Precirol ATO-5 (Glyceryl distearate) was purchased from Gattefossé (Paris, France); and Poloxamer 407 from Sigma Aldrich (Hamburg, Germany).

Preparation of nanocapsules

Pirimicarb nanocapsules were prepared as described in Maroofpour et al. (2019). Briefly, in a hot water bath, the active ingredient of the insecticide was heated up to 85 °C in the presence of an oily solution containing precirol, miglyol. Then, an aqueous solution containing poloxamer was prepared at the same temperature and added drop by drop into the oil phase containing insecticide under continuous homogenization (Silent Crusher M ultrasonic homogenizer, Heidolph). The resulting solution was then cooled to the room temperature. Size, polydispersity index

(PDI) and zeta potential (ZP) of the nanocapsules were evaluated by Dynamic Light Scattering (DLS) (Malvern Zetasizer Nano ZS 3600). The diameter, PDI and ZP of the nanocapsules were 35.38 ± 0.93 nm, 0.44 and +13.7 mV, respectively (Maroofpour et al. 2019).

Concentration-response bioassays

Insecticide toxicity to cabbage aphid was performed in plastic Petri dishes (6 cm diameter) for both formulations (with and without nanoencapsulation) and a control (non-treated) using leaf-dip method (Koziol and Semtner 1984). The cabbage leaf discs (2 cm in diameter) were dipped for 10 s in the insecticide solution and allowed to dry at room temperature (Moores et al. 1996). After drying, 20 similar-aged adult aphids (<48 h) were transferred to the leaves using a soft brush. Preliminary tests were conducted to determine effective concentration ranges for both formulations. Five concentrations ranging from 7.5 to 14 mg ai/l for commercial product and from 0.5 to 1.5 mg ai/l for nano-formulation, were used. As control for the commercial formulation, distilled water containing Tween-80 0.05% (v/v) was used while for the nanoformulation, the control consisted of distilled water only. Treatments were maintained in the above mentioned greenhouse conditions, and mortality was recorded 48 h after exposure. The experiments were repeated three times.

For the green lacewing *C. carnea*, the toxicity of both insecticidal formulations was determined on 2nd instar larvae by contact exposure to pesticide residues in individual glass tubes (3 cm diameter and 9 cm long). A volume of 150 µl of the insecticide solution (nanoformulated or not) was applied to the inner wall of each tube, and then the tubes were immediately rotated until the solution dried. Four replicates with 15 larvae were used for each concentration. Controls treatments were similar to aphid bioassays.

Sublethal effects on biological traits of *Brevicoryne brassicae* parental generation (F0)

In order to compare the sublethal effects on adult aphids, cabbage leaf discs were treated with LC_{25} of each formulations for 10 s following the above described method and left to dry at room temperature (Koziol and Semtner 1984). After drying, a total of 90 adult aphids (<48 h) were placed on treated leaf discs in individual Petri dishes. Distilled water containing Tween-80 0.05% (v/v) was used as the control treatment. The 70 surviving aphids were transferred to new Petri dish without insecticide after 48-h exposure, and daily observed for progeny nymphs and parental aphid survival until the last aphid died. Leaf discs were replaced every two days during the experiment (26 day).

Transgenerational sublethal effects on biological traits of the *Brevicoryne brassicae* F1

The F1 nymphs obtained from the treated parental generation aphids were used to assess the transgenerational sublethal effects of the commercial, and nanorformulated Primicarb. Control group consisted of F1 nymphs of untreated aphids. The F1 nymphs were kept individually on leaf discs in plastic Petri dishes for each of the formulations until reaching reproductive phase. During the reproductive period, the number of F2 newborn nymphs was counted daily and then removed. The survival of F1 aphids were recorded and the F2 nymphs produced per day were counted and removed until the F1 adult aphids were dead.

Sublethal effects on the green lacewing *Chrysoperla carnea*

Because no mortality was recorded in preliminary tests for 2nd instar larvae of *C. carnea,* even when using the highest dose of nanoformulation corresponding to 400 mg ai/l (see result section), this same dose was chosen to carry out the sublethal effects bioassays. For this purpose, a cohort group consisting of 50 eggs (<24 h) of *C. carnea* was randomly selected and transferred to individual larvae glass tubes for each treatment. After reaching second instar, the larvae were exposed to residues of both formulations of primicarb at 400 mg ai/l as described above. After 24 h exposure period, the larvae were transferred to individual glass tubes without insecticides until adult emergence occured. The larvae were fed with eggs of *E. kuehniella* every day. Then, the emerging *C. carnea* adults were transferred to 250cc clear plastic containers with their opening covered with mesh cloth. An artificial diet including water, yeast, and honey were presented to adults every day. All treatments were maintained under controlled condition of temperature (26 ± 2 °C), relative humidity (60 ± 10%), and photoperiod (16: 8 L:D). Control groups were same as in the concentration–response bioassay.

Data analyses

Concentration-mortality data were subjected to probit analysis (SAS 2008), and 95% confidence intervals for toxicity ratio were estimated following (Robertson et al. 2007) and considered significant when not including the value 1.

The life table data for all individuals were analysed using an age-stage and two-sex life table method (Chi et al. 2020). The *age-stage specific survival rate* (s_{xj}, the probability that a newly laid egg will survive to age x and stage j), the *age-stage specific fecundity* (f_{xj}, the mean fecundity of females at age x), the *age-specific survival rates* (l_x, the probability of a newly offspring surviving to age x), the *age-specific*

fecundity (m_x, the mean fecundity of individuals at age x), the *age-specific maternity* ($l_x m_x$), the *age-stage life expectancy* (e_{xj}, the expected time that an individual of age x and stage j will live), age-stage reproductive value (v_{xj}, the expected contribution of an individual of age x and stage j to the future population) and the population parameters of *intrinsic rate of natural increase* (r, number of females added to the population by each females per day), *finite rate of increase* (λ, the population increases every day compared to the previous day), *net reproductive rate* (R_0, the mean number of offspring that an individual can produce during its lifetime), *mean generation time* (T, the period that a population needs to increase to R_0 -fold of its size as the population reaches the stable age-stage distribution) were estimated and calculated using the computer program TWOSEX-MSChart (Chi 2019; Chi et al. 2020). The means and standard errors of the population parameters were calculated by using the bootstrap technique included in the TWOSEX-MSChart program with 100,000 bootstrap replicates (Chi 2019; Chi et al. 2020).

Results

Concentration-response bioassay results

The probit model satisfactorily described the concentration-mortality data (goodness-of-fit tests showing low χ^2-values [<2] and high P-values [>0.05]). Based on the LC_{50} values obtained for the two formulations, the nanoformulated product (0.8 mg ai/l) was 12-fold (TR = 12.6) more toxic than did the commercial one (10.2 mg ai/l) (Table 1).

Sublethal effects on biological traits of the *Brevicoryne brassicae* parental generation (F0)

Population parameters were evaluated for the parental generation under sublethal concentrations of nano and commercial formulations of pirimicarb. According to the results (Table 2), the exposure to sublethal concentrations of both formulations decreased significantly (paired bootstrap test; $P < 0.05$) all the life table parameters compared to the control. Furthermore, the adverse effects of the nano-formulation were numerically, although not statistically, stronger compared to the commercial product for all the aphids' parameters except the female adult longevity.

The *age-specific survival rate* (l_x), *fecundity* (m_x), and *net maternity* ($l_x m_x$) in parental generation of *B. brassicae* are presented in Fig. 1. The exposure of adults from parental generation to both nanoformulated and commercial pirimicarb seemed to delay and shorter period of oviposition and a decline in age-specific fecundities compared to the control, although no statistical differences were highlighted.

Transgenerational sublethal effects on biological traits of the *Brevicoryne brassicae* F1

The effects of pirimicarb exposure on the development of F1 generation of aphids are reported in Table 3. The results showed different patterns in the effects on larvae

Table 1 Toxicity of commercial and nano formulations tested on *Brevicoryne brassicae*

Formulation	LC_{25} (mg ai/l) (95% FI[a])	LC_{50} (mg ai/l) (95% FI)	Slope ± SE	χ^2 (*df*)	*P*-value	TR_{50}(95% FI)[b]
Commercial formulation	7.8 (6.9–8.4)	10.2 (9.6–10.8)	5.64 ± 0.73	0.26 (3)	0.967	12.6 (12.0–13.1)
Nanoformulation	0.5 (0.4–0.6)	0.8 (0.7–0.9)	3.33 ± 0.48	1.62 (3)	0.654	1

χ^2 = Chi-square for lack-of-fit to the probit model, and *P*-value = Probability associated with the chi-square statistic

[a]*FI* Fiducial Intervals

[b]TR_{50} Toxicity ratio given by LC_{50} of less toxic formulation/LC_{50} of most toxic formulation

Table 2 Life table parameters (mean ± SE) of *Brevicoryne brassicae* parental generation treated with subelethal concentration (LC_{25}) different formulations of pirimicarb

Parameter	Control	Nanoformulation	Commercial formulation
r (day^{-1})	0.27 ± 4.574a	0.16 ± 1.623b	0.19 ± 1.875b
R_0 (offspring/individual)	40.44 ± 1.98a	4.68 ± 0.74b	6.35 ± 1.3b
T (day)	13.72 ± 0.19a	9.42 ± 0.21b	10.0 ± 0.28b
λ (day^{-1})	1.31 ± 5.990a	1.18 ± 1.904b	1.20 ± 2.246b
Total fecundity (offspring/female)	52.8 ± 2.55a	6.1 ± 1.05c	21.6 ± 4.38b
Oviposition period (day)	12.6 ± 0.47a	2.4 ± 0.26b	2.9 ± 0.32b
Female adult longevity (day)	15.68 ± 0.7a	5.39 ± 0.39b	2.89 ± 0.3c

Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Means were compared with paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the treatments

development time and female adult longevity between the two formulations and in comparison with the control. In fact, the commercial product increased the development time of first ($F = 2329.96$; $df = 2$; $P < 0.001$) and third larvae ($F = 376.10$; $df = 2$; $P = 0.002$) and decreased the adult longevity time of female ($F = 1233.45$; $df = 2$; $P < 0.001$) meanwhile the nanoformulated product increased the developmental time only of the 2nd larvae ($F = 105.65$; $df = 2$; $P = 0.003$). Furthermore, compared to the control, both formulations decreased the *longevity* ($F = 5903.74$; $df = 2$; $P_{wp,\ nano} < 0.001$) and *total fecundity* ($F = 1520.56$; $df = 2$; $P_{wp} < 0.001$; $P_{nano} = 0.015$) and increased the *pre-adult period* ($F = 616.86$; $df = 2$; $P_{wp} < 0.001$; $P_{nano} = 0.013$) while the commercial pirimicarb increased the *total pre-oviposition period* (TPOP) ($F = 581.88$; $df = 2$; $P < 0.001$) and decreased the *oviposition period* ($F = 1498.14$; $df = 2$; $P < 0.001$).

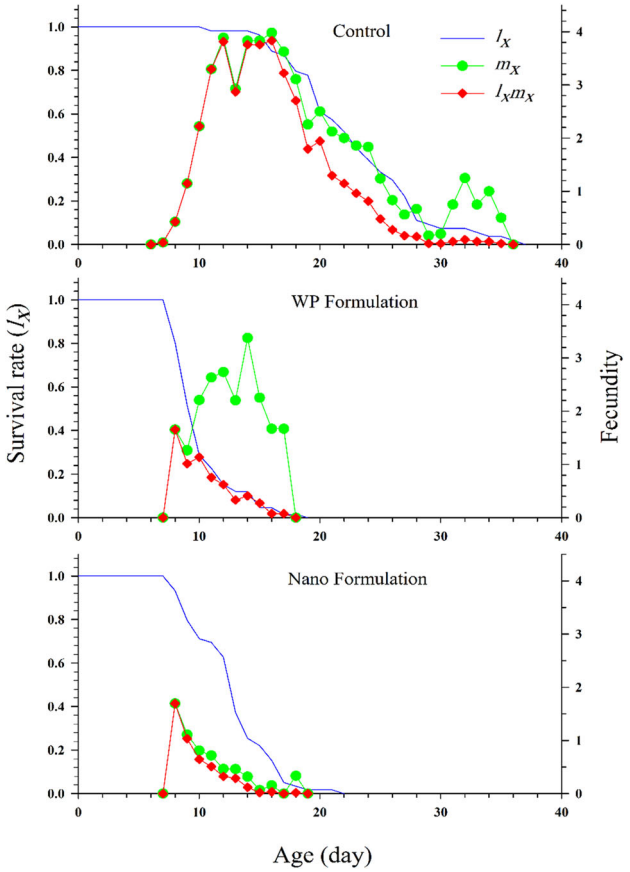


Fig. 1 Age specific survival rate (l_x), fecundity (m_x), and net maternity ($l_x m_x$) of parental generation (F0) in *Brevicoryne brassicae* treated with different formulations of primicarb

Results of the *age-stage survival rate* (s_{xj}) of *B. brassicae* offspring obtained from the sublethaly treated adults showed that the probability of a newborn larva to survive to adult stage tended to decline when parental were exposed to both formulations (i.e., nanoformulationa and commercial pirimicarb) with a more marked decline caused by nano-formulation, although no statistical differences were highlighted (Fig. 2). Furthermore, the *age-specific survival rate* (l_x), *fecundity* (m_x), and *net maternity* ($l_x m_x$) in F1 generation showed a tended to decline beginning of oviposition in both pirimicarb treatments compared with control and in age-specific fecundities caused by the nanoformulation in comparison with commercial formulations (Fig. 2).

Moreover, commercial and nano formulations seemed to reduce the *life expectancy* (e_{xj}) of all stage except second instar larve in commercial (Fig. 3) with the highest decrease registered for the nanoformulated product and the *age-stage-specific reproductive* values (v_{xj}) of the F1 generation of *B. brassicae* females tended to decrease only when parentals were exposed to commercial formulation, although no statistical differences were highlighted (Fig. 3).

Table 3 Life history statistics (mean ± SE) of the F1 generation of *Brevicoryne brassicae* obtained from parental generation (F0) unexposed (control) or sublethaly (LC_{25}) exposed to nanoformulated or commercial product of pirimicarb

Parameters	Stage	Control	Nanoformulation	Commercial formulation
Developmental time (day)	Larvae 1	1.12 ± 0.04a	1.21 ± 0.1a	1.89 ± 0.09b
	Larvae 2	1.88 ± 0.06a	2.42 ± 0.18b	2.27 ± 0.29ab
	Larvae 3	1.89 ± 0.06a	2.44 ± 0.85ab	2.14 ± 0.06b
	Larvae 4	2.29 ± 0.09a	2.25 ± 0.25a	2.36 ± 0.08a
Female adult longevity (day)		15.98 ± 0.76a	13.5 ± 3.63ab	7 ± 0.64b
Preadult (day)		7.19 ± 0.12a	8.75 ± 0.59b	8.70 ± 0.21b
Longevity (day)		21.8 ± 0.90a	5.07 ± 1.05c	13.1 ± 0.75b
APOP (day)		1.04 ± 8.30ab	0.67 ± 0.21b	1.31 ± 0.11a
TPOP (day)		8.24 ± 0.15a	9 ± 0.86 ab	10.06 ± 0.33b
Oviposition period (day)		12.67 ± 0.49a	13.67 ± 2.6a	5.82 ± 0.64b
Total fecundity (offspring/female)		39.98 ± 1.96a	22.5 ± 6.80b	16.76 ± 2.13b

Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Means were compared with paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the treatments

APOP adult preovipositional period, *TPOP* total preovipositional period

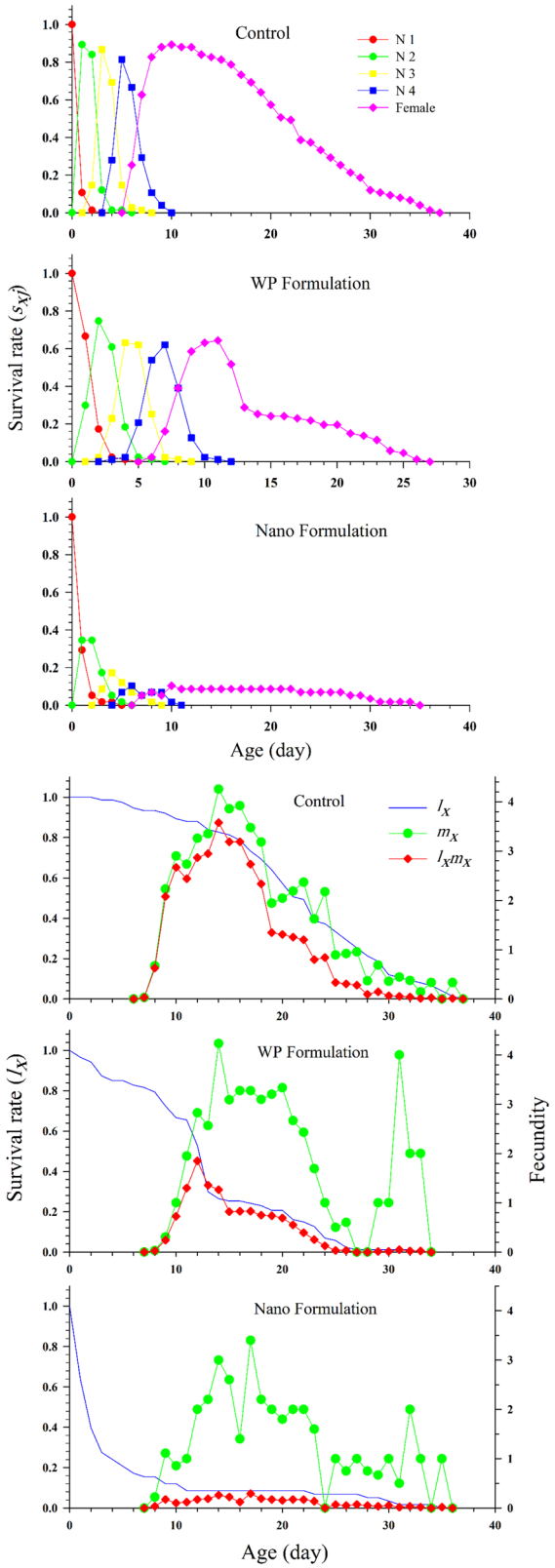


Fig. 2 Age-stage survival rate (s_{xj}) and Age specific survival rate (l_x), fecundity (m_x), and net maternity ($l_x m_x$) of F1 generation in *Brevicoryne brassicae* treated with with different formulations of primicarb

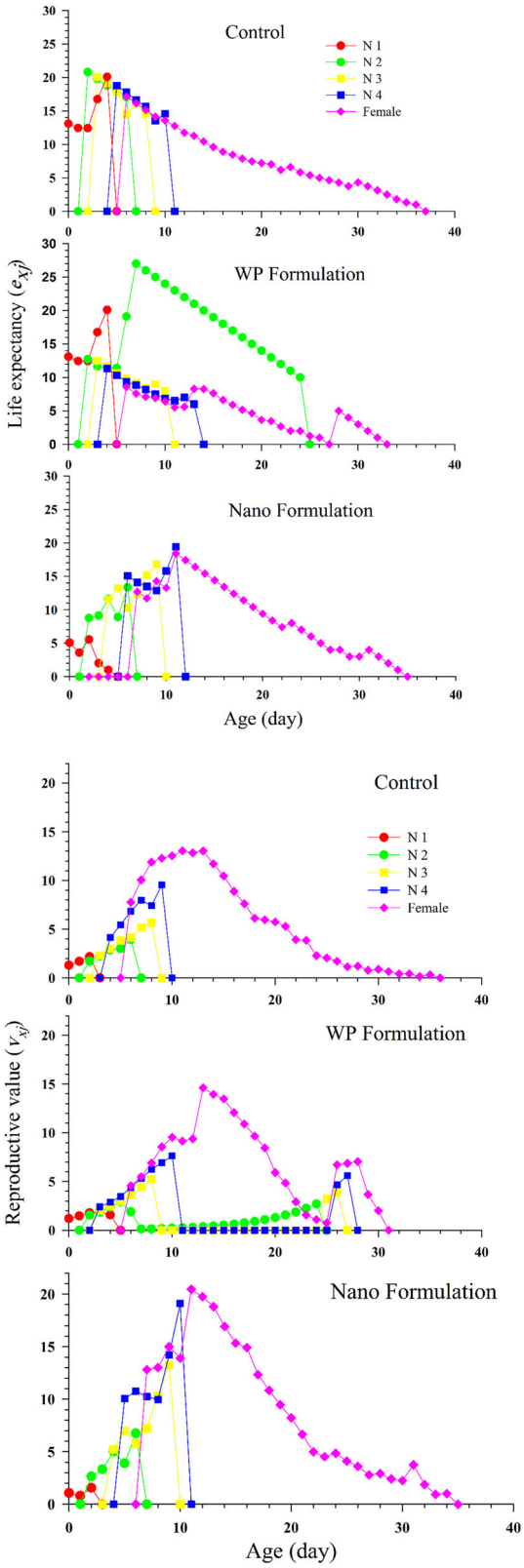


Fig. 3 Age-stage life expectancy (e_{xj}) and Age-stage specific reproductive value (v_{xj}) of F1 generation in *Brevicoryne brassicae* treated with different formulations of primicarb

Table 4 Life table parameters (mean ± SE) of *Brevicoryne brassicae* of the F1 generation of *Brevicoryne brassicae* obtained from parental generation (F0) unexposed (control) or sublethaly (LC_{25}) exposed to nanoformulated or commercial pirimicarb

Parameter	Control	Nanoformulation	Commercial formulation
r (day^{-1})	0.27 ± 0.0053a	0.07 ± 0.038c	0.18 ± 0.009b
R_0 (offspring/individual)	36.79 ± 2.19a	3.10 ± 1.35c	12.90 ± 1.8b
T (day)	13.29 ± 0.17a	16.59 ± 1.66b	13.99 ± 0.29b
λ (day^{-1})	1.31 ± 7.02a	1.07 ± 3.78c	1.20 ± 1.16b

Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Means were compared with paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the treatments

Table 5 Life history statistics (mean ± SE) of *Chrysoperla carnea* treated with different formulation of pirimicarb

Parameters	Stage	Control	Nanoformulation	Commercial formulation
Developmental time (day)	Egg	3.72 ± 0.1a	3.68 ± 0.11a	3.65 ± 0.2a
	Larvae 1	3.24 ± 0.06a	3.28 ± 0.22a	3.23 ± 0.08a
	Larvae 2	3.22 ± 0.06a	3.56 ± 0.09c	3.47 ± 0.07b
	Larvae 3	3.31 ± 0.07a	4.57 ± 0.45b	4.11 ± 0.07b
	Pupa	6.72 ± 0.8a	8.05 ± 0.12c	7.24 ± 0.16b
Female adult longevity (day)		37.04 ± 0.65a	36.58 ± 0.56a	35.28 ± 0.66a
Male adult longevity (day)		22.39 ± 1.2a	21.16 ± 0.96ab	18.7 ± 1.17b
Preadult (day)		20.23 ± 0.17a	22.43 ± 0.18b	21.87 ± 0.26b
Longevity (day)		57.5 ± 0.70a	58.89 ± 0.53a	56.72 ± 0.60a
APOP (day)		4.5 ± 0.19a	4.63 ± 0.11a	4.61 ± 0.12a
TPOP (day)		24.95 ± 0.29a	26.95 ± 0.34c	26.05 ± 0.27b
Oviposition period (day)		27.88 ± 0.72a	27.42 ± 0.65ab	25.89 ± 0.64b
Total fecundity (offspring/adult)		583.08 ± 17.78a	506.93 ± 18.36b	335.5 ± 11.27c

Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Means were compared with paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the treatments

APOP adult preovipositional period, *TPOP* total preovipositional period

Regarding the population parameters, of *B. brassicae* F1 geneartion, significant differences ($P < 0.05$) between the effects of different formulations of pirimicarb and the control were found for the *net reproductive rate* (R_0), *intrinsic rate of natural increase* (r), *finite rate of increase* (λ), and *mean generation time* (T) (Table 4). There was a significant difference between the commercial formulation and nanoformulation except for *mean generation time* (T) ($F = 290.43$; $df = 2$; $P = 0.055$). The nanoformulation significantly reduced the values of *intrinsic rate of natural increase* ($F = 1742.85$; $df = 2$; $P = 0.003$), *finite rate of increase* ($F = 2270.15$; $df = 2$; $P < 0.001$) and *net reproductive rate* ($F = 6139.22$; $df = 2$; $P < 0.001$) (3 and 4 times, respectively) compared to the commercial formulation.

Sublethal effects of exposure to nanoformulation and commercial pirimicarb on the biological traits of the natural enemy *Chrysoperla carnea*

The sublethal exposure of *C. carnea* to nanoformulated and commercial pirimicarb resulted in increased development times for second ($F = 166.86$; $df = 2$; $P_{wp} = 0.008$; $P_{nano} = 0.001$) and third larvae ($F = 223.22$; $df = 2$; $P_{wp} < 0.001$; $P_{nano} = 0.006$), pupae ($F = 1122.43$; $df = 2$; $P_{wp} = 0.004$; $P_{nano} < 0.001$) and male ($F = 39.06$; $P_{wp} = 0.026$) (only for commercial product) and prolonged preadult ($F = 1325.11$; $df = 2$; $P_{wp} < 0.001$; $P_{nano} < 0.001$) and total pre-oviposition (TPOP) periods ($F = 277.06$; $df = 2$; $P_{wp} = 0.005$; $P_{nano} < 0.001$) (Table 5).

The plotted peaks of the *age-stage survival rate* (s_{xj}) for each developmental stage and the probability that a newborn larva survives to adult stage showed similar patterns in prirmicarb treated groups (nanoformulated and commercial) and control (Fig. 4). The beginning time of oviposition in nanoformulation was similar to the untreated control and earlier in the commercial formulation (Fig. 4) the later tended to more decline in *age-specific fecundities* of *C. carnea* than nanoformulations. The *life expectancy* (e_{xj}) in all stages of control was seemed to reduce compared with both formulations (Fig. 5). The *age-stage-specific reproductive* values (v_{xj}) of *C. carnea* females tended to decrease as a result of the commercial formulation only, whereas nano formulation showed almost the same pattern as the

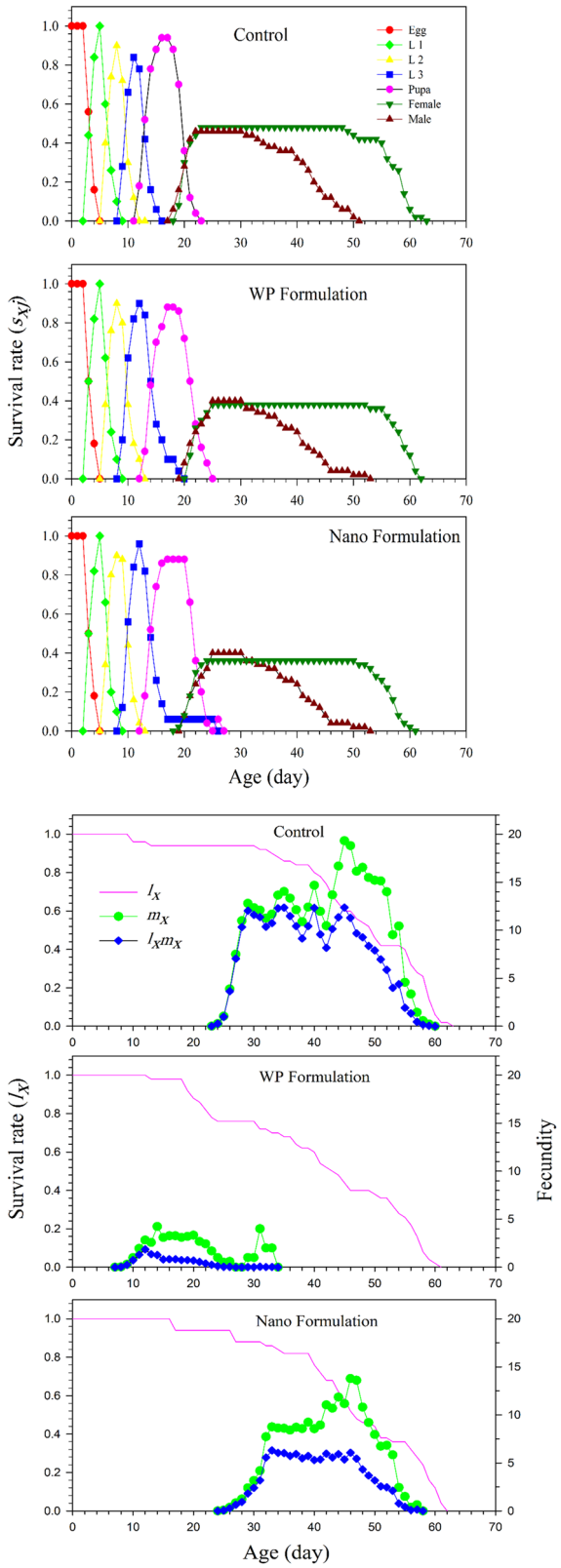


Fig. 4 Age-stage survival rate (s_{xj}) and Age specific survival rate (l_x), fecundity (m_x), and net maternity ($l_x m_x$) of Chrysoperla carnea treated with with different formulations of primicarb

control, although no statistical differences were highlighted (Fig. 5).

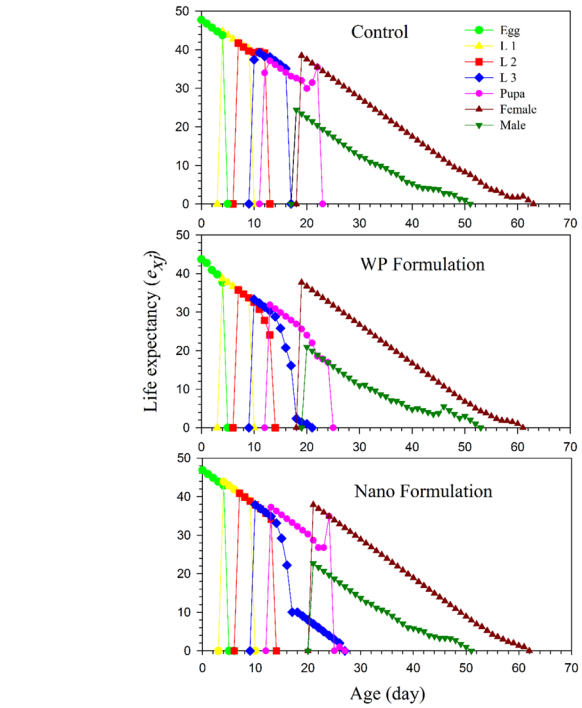
The different pirimicarb formulations contributed to a significant ($P < 0.05$) decrease in the population parameters (e.i., R_0, r, and λ) of *C. carnea* compared to the control (Table 6). No significant difference were found between commercial formulation and nanoformulation, although the adverse effects of commercial formulations on all parameters were numerically higher.

Discussion

Although one of the assets of integrated pest management (IPM) approaches is the reasonable use of pesticides, the variable distribution and gradual degradation of active ingredients under field conditions expose target and non-target arthropod populations to sublethal concentrations of these pesticides (Desneux et al. 2005). Such sublethal exposure to pesticides may induce on arthropods either positive, termed hormesis, or negative effects (Calabrese and Baldwin 2003; Desneux et al. 2007). Therefore, research on the sublethal effects of pesticides on target pest and their natural enemies are relevant for a proper use of these compounds (Desneux et al. 2006b; Xiao et al. 2015; Guedes et al. 2016; Ullah et al. 2019a; 2019b; 2019c; Zhang et al. 2019). The importance of these researches is even critical when technologies like nanoformulations are used to enhance the efficiency of pesticides. Here, results of dose-response bioassay showed that nanoencapsulation increased the toxicity of pirimicarb over its commercial formulation against *B. brassicae*. Furthermore, the sublethal exposure to the nanoformulated active ingredient negatively affected not only the life parameters of both parental and following generations of this pest but also of one of its predator, the green lacewing *C. carnea*.

Nanoencapsulated pirimicarb was 12.6-fold more efficient to kill exposed *B. brassicae* aphids compared to its commercial formulation. Enhanced toxicity after nanoformulation has been reported for pirimicarb (2.9-fold) against *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Maroofpour et al. 2019), beta-cyfluthrin (3- to 4-fold) against *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) (Loha et al. 2012) and for imidacloprid (4.8- to 9.05-fold) against *Glyphodes pyloalis* (Walker) (Lepidoptera: Pyralididae) (Memarizadeh et al. 2014). The improvement in the toxic activity can derive from increased contact with cells of the target organism. Such greater bioavailability is conferred by the small size of the nanoparticles resulting in higher stability, solubility and mobility (Lade and Gogle 2019; Shahzad and Manzoor 2019).

Negative effects on F0 and F1 generations of *B. brassicae* were observed when aphids of parental generation (F0)



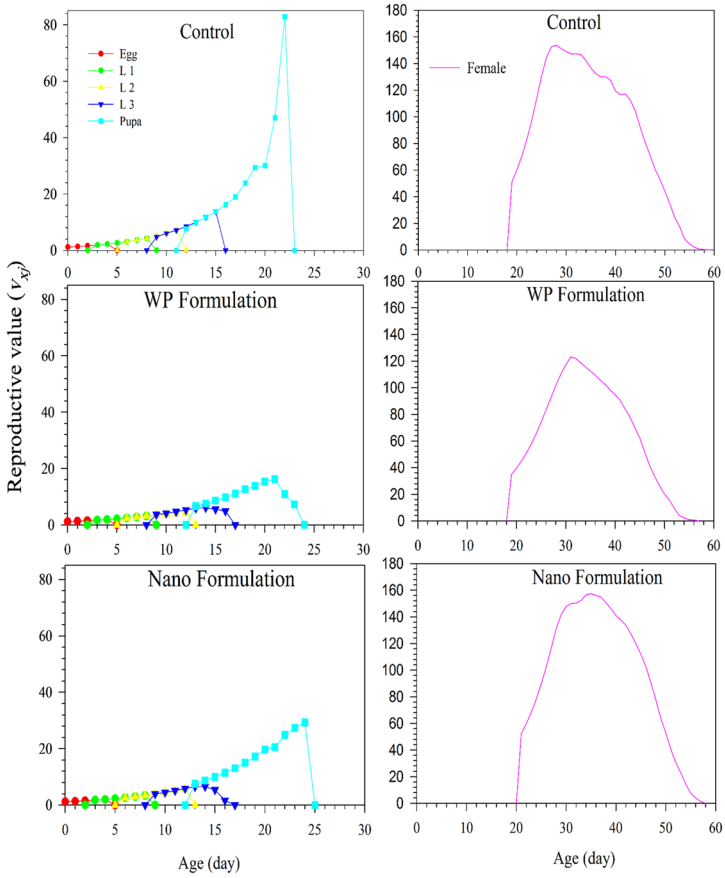


Fig. 5 Age-stage life expectancy (e_{xj}) and Age-stage specific reproductive value (v_{xj}) of *Chrysoperla carnea* treated with different formulations of primicarb

were exposed to subelethal dose (LC_{25}) of both formulations of pirimicarb. The exposure to sublethal concentration resulted in a decrease in the aphid population growth as it seemed to reduce all the parameters (i.e., *net reproductive rate*, *intrinsic rate of natural increase*, *finite rate of increase*, *offspring/female*, *total fecundity*, *oviposition*

Table 6 Life table parameters (mean ± SE) of *Chrysoperla carnea* treated with different formulation of pirimicarb

Parameter	Control	Nanoformulation	Commercial formulation
r (day^{-1})	0.16 ± 0.005a	0.13 ± 0.006b	0.12 ± 0.005b
R_0 (offspring/individual)	279.88 ± 41.92a	192.64 ± 35.46b	120.78 ± 23b
T (day)	35.18 ± 0.39a	38.77 ± 0.34 b	37.91 ± 0.31b
λ (day^{-1})	1.17 ± 0.01a	1.14 ± 0.01 b	1.13 ± 0.01b

Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Means were compared with paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the treatments

period and *female adult longevity*) of the aphid life table in both generations. Similar reductions in reproductive and longevity parameters after sublethal exposure have been reported for pirimicarb also against *Aphis gossypii* (Glover) (Hemiptera: Aphididae) (Amini Jam et al. 2014) and for other carbamates insecticides, such as the aminocarb againts *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (Alford and Holmes 1986), carbofuran against *Hippodamia undecimella* (Schneider) (Coleoptera: Coccinellidae) (Papachristos and Milonas 2008), and carbaryl against *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Kumar and Chapman 1984).

Such negative effects in adults with transgenerational carry-over may be due to disorders caused by pirimicarb in the neurosecretory system that negatively impacted the reproduction process. It is well known that in arthropods reproduction is largely regulated by neurohormones, and neurohormonal deficits due to insecticide poisoning may affect normal reproductive performance (Lee 2000). As these effects have been found independently of the formulation (nanoformulation or commercial one) it is reasonable to think that they are triggered essentially by the action of the active ingredient (pirimicarb). The reduction in the parameters of the aphid life table by pirimicarb indicates that besides its direct toxicity this insecticide can decrease the population growth of this pest through sublethal exposure.

The sublethal negative effects of the treatments (commercial and nanoformulated pirimicarb) on the development of second and third instar larvae of *C. carnea* was more intensive. These negative effects of a believed selective active ingredient need close scrutiny as the larval instars of this insect are the only one presenting active predation.

Furthermore, the adults' longevity of this natural enemy decreased and its *total fecundity* and *preovipositional period* showed a significant decrease in addition to significant reduction on the *net reproductive rate*, *intrinsic rate of natural increase*, and *finite rate of increase* in insects exposed to the two formulations compared to the control. Although short-term sublethal effects of direct exposure to pesticides in natural enemies have been proven, the effect of selective insecticides on predators and parasitoids varies according to natural enemy species, biological stage, and methods of application (Holland et al. 2012; Fogel et al. 2013; Biondi et al. 2015; Yao et al. 2015; Benelli et al. 2019; Morfin et al. 2019; Pereira et al. 2019).

Even though generally of small magnitude, these sublethal effects found here for this generalist predator need further selectivity investigation and highlight the importance of adapted risk assessment when nanoencapsulation technology is used to enhance the efficiency of pesticides. It is clear that, unlike conventional pesticides, nanopesticides are likely to behave differently under field conditions requiring alternative test methods to assess their environmental impact (Kookana et al. 2014; Campolo et al. 2020).

In conclusion, the enhanced uptake, bioavailability, and increased efficacy of nanopesticides represent an attractive technological advancement for their integration in pest management strategies. However, adaptation and improvement of existing methodologies for analysis, characterization, and environmental risk assessment are still needed.

Data availability

Due to its proprietary nature or ethical concerns, supporting data cannot be made openly available.

Author contributions MJH, HH and SI conceived and designed the experiments. NM and MM performed the experiments. NM and KH established and analyzed the bioassay data and life table. NM and MM writing-original draft of manuscript. KH, AB and ND writing-review and editing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Consent to publish The participants have consented to the submission of the case report to the journal.

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