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Toxic effects of cadmium on the growth and predation capacity of the predator Orius sauteri

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Abstract

The heavy metal cadmium (Cd) leads to significant bottom-up effects on food chains of plants, herbivores, and predators in agroecosystems. Through the transfer and accumulation of Cd. In addition to the indirect effects of this cascading effects, predators *Orius sauteri* fed with artificial diets containing Cd also have a direct effect. We found that *O. sauteri* fed with sucrose solutions containing Cd at high concentrations of 125 mg/L and 625 mg/L significantly decreased the survival of the 3rd and 5th instar, female, and male adults (except 125 mg/L) of *O. sauteri*. However, the survival of *O. sauteri* nymphs was significantly increased when they fed with low concentrations of 1 mg/L, 5 mg/L and 25 mg/L of sucrose solutions. Cd stress has differing effects on the LC₅₀ of each age of *O. sauteri*, the lowest LC₅₀ for male adults (153 mg/L), followed by 3rd instar nymphs (192 mg/L), 5th instar nymphs (289 mg/L) and female adults (383 mg/L). It is interesting that 1 mg/L, 5 mg/L, and 25 mg/L of sucrose solutions containing Cd significantly improved the predation capacity of the nymphs of *O. sauteri* to the *Bemisia tabaci* pupa. The predation ability of the male adults of *O. sauteri* on *B. tabaci* pupa were significantly improved, but significantly decreased in the female adults when they fed with 125 mg/L and 625 mg/L sucrose solutions containing Cd. This difference needs to be studied in depth in the future, controlling Cd contamination and protecting natural enemies to enhance the biological control of pests.

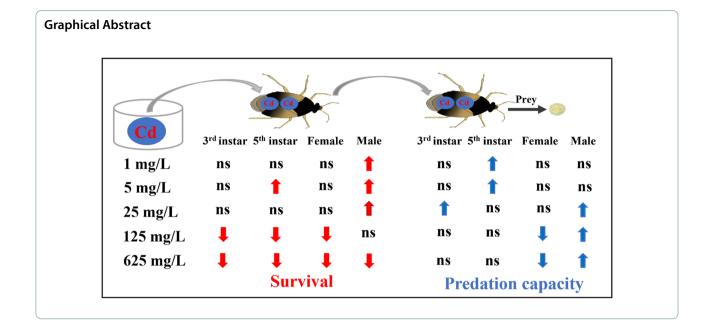
Keywords Heavy metal, Cadmium, Survival, Predation capacity, Biological control

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Introduction

In agroecosystems, agricultural production is continuously threatened by both biotic factors, such as pathogen infections and herbivorous insect-driven damage, and abiotic stressors including drought, high and cold temperatures, nutrient deficiencies, salt toxicity, and toxic metal contamination in the soil (Zhu 2016; Li et al. 2023). Toxic heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) accumulate in crop plants, jeopardizing food safety and human health (Zhao et al. 2022). Heavy metal pollution, particularly that of Cd, is critically serious in Chinese agroecosystems (Huang et al. 2015; Zhao et al. 2015; Guan et al. 2018). The overall exceedance rate of soil environmental standards in China has reached 16.1% and Cd has the highest exceedance rate of 7%, making it the primary pollutant in arable land (Ren et al. 2022a; Shi et al. 2023). This heavy metal enters ecosystems via various anthropogenic activities, including mineral resource extraction, metal processing and smelting, industrial emissions, wastewater irrigation and the application of pesticides and fertilizers in agriculture (Li et al. 2022a, b).

Even at low concentrations, Cd can have significant impacts on ecosystems due to its high solubility, rapid absorption by plants and transfer through the food chain, exerting toxic effects on all organisms (plants, animals, and humans) (Winter et al. 2012; Wei et al. 2020; Sun et al. 2021; Liu et al. 2023). Cd can directly affected plant growth and development, causing nutrient deficiencies, inhibiting chlorophyll synthesis, reducing photosynthesis, suppressing plant growth and ultimately causing plant death (Hermans et al. 2011). Cd also has an impact on herbivorous insects, serving as crucial mediators for the transmission and accumulation of Cd through the food chain (Tibbett et al. 2021; Liu et al. 2023; Wang et al. 2024). Herbivorous insects accumulate Cd in their bodies after consuming plants or food containing this metal, leading to negative impacts on their growth and reproduction (Kazemi-Dinan et al. 2014; Vlahović et al. 2017; Huang et al. 2023; Yan et al. 2023). This includes weight loss, reduced survival rate (Wei et al. 2020), decreased egg production and lower hatching rate (Chen et al. 2022) and prolonged developmental duration (Winter et al. 2012). Laboratory experiments showed that artificial diets with Cd significantly prolonged the developmental period of 4th to 6th instar larvae of Lymantria dispar and reduced their body weight (Vlahović et al. 2009; Mircic et al. 2013; Vlahović et al. 2014).

Through accumulation in herbivorous arthropods, Cd can produce bottom-up effects impacting the fitness of organisms in third trophic level (Gardiner and Harwood 2017; Akhtar et al. 2021; Malematja et al. 2023; Wang et al. 2024). This was found in particular in the aphid predator *Coccinella transversalis* (Naikoo et al. 2021a). Similarly, Cd was transmitted and accumulated through the food chain from soil to kidney bean to Western flower thrips (*Frankliniella occidentalis*) to the predator *Orius sauteri* (Liu et al. 2023), and in the *Vicia faba* L.—*Megoura crassicauda*—*Harmonia axyridis* food chain (Wang et al. 2022). However, reports documenting direct toxic effects of Cd on insect natural enemies at the third trophic level remain rare.

Predatory bugs belonging to the Orius genus (Hemiptera: Anthocoridae) are major natural enemies of agricultural pests (Desneux et al. 2006; Desneux and O'Neil 2008; Ren et al. 2022b; Zuma et al. 2023) and Orius sauteri (Poppius) is widely used as an efficient biological control agent in China (Zhao et al. 2017), notably for field application (Di et al. 2022; Zhu et al. 2024). It can be released in a variety of crops in both greenhouse and field settings, demonstrating its efficacy in the control of small insect pests such as thrips, whitefly, mites and aphid (Wang et al. 2014, 2018; Zhu et al. 2024). The sweet potato whitefly, Bemisia tabaci (Gennadius) is a serious, destructive, global pest of vegetable, field and ornamental crops, severely reducing crop yields and quality, and causing significant economic losses (Horowitz et al. 2020). Wang et al. (2013) reported that O. sauteri had an excellent control efficiency against whitefly and could prey on all stages of B. tabaci. Given this role in biological control, it is important to examine the direct toxic effect of Cd on O. sauteri and comprehensively document the effects of Cd exposure on its predation capacity on B. tabaci (Guo et al. 2023). Comprehensive documentation of such factors will help to better understand the direct toxicity of heavy metal stress on pests' natural enemies and the efficiency of biological control. Such information could ultimately lead to better optimization for the protection and utilization of natural enemies under field conditions.

We therefore tested the effect of various concentrations of Cd in artificial diet on *O. sauteri* life history traits, including survival of adults and nymphal stages. We also determined Cd LC_{50} in *O. sauteri*, and assess its predatory ability towards *B. tabaci* pupa under Cd stress.

Materials and methods

Insects rearing

Orius sauteri (Poppius) (Hemiptera: Anthocoridae) were initially collected from maize fields of the Beijing Academy of Agriculture and Forestry Sciences (BAAFS) (Haidian District, Beijing, China). A colony was subsequently established and reared using hyacinth bean *Lablab purpureus* (L.) (Fabales: Fabaceae) pods as oviposition substrate with *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) eggs as food source (Di et al. 2022). To obtain *O. sauteri* cohorts of the same age, a new substrate was replaced every two days. Adults and nymphs of the same age (2–3 days) were used for the experiments.

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) was collected from tomato crops at the Beijing Noah Agricultural Science and Technology Co., LTD. (Pinggu District, Beijing, China). The colony was reared on cotton plants in insect-rearing cages ($45 \times 45 \times 45$ cm) made from aluminum frames and nylon yarn net (pore size: 125 µm). Individuals of *B. tabaci* were then transferred to each cotton plant and transferred to a climate-controlled

incubator (MH-351, Sangyo, Japan) set to 26 ± 1 °C, $60 \pm 5\%$ RH, 16:8 L:D photoperiod, 5000 Lux fluorescent light. New four-leaf stage cotton plants were placed in the cages for 5 days to allow oviposition and obtain whitefly adults of the same age. Whitefly adults were then removed and eggs were allowed to develop on cotton plant leaves. After about15 days, pupa of *B. tabaci* were collected for use in experiments described below.

Cd treatment

Acute toxicity of different concentrations of Cd (0, 1, 5, 25, 125, and 625 mg/L) on *O. sauteri* was tested using 30% sucrose aqueous solution, following Liu et al. (2023). Cadmium chloride (CdCl₂, Shanghai Aladdin Bio-Chem Technology Co., LTD, China, 99%) was dissolved in 30% (sucrose/water, weight/volume=30%) sucrose aqueous solution containing 625 mg/L of Cd, which was subjected to a series of dilutions to obtain a gradient of Cd concentrations at 125 mg/L, 25 mg/L, 5 mg/L and 1 mg/L, respectively. The pure 30% sucrose solution was used as a control (0 mg/L of Cd).

The effect of feeding with Cd on the survival of Orius sauteri

For each Cd concentration, 0.5 g of cotton wool with 5 mL of the sucrose solutions was placed in a Petri dish. Twenty 3rd instar nymphs, 5th instar nymphs, female, and male adults (2–3 days after emergence) of *O. sauteri* at the same age were collected and transferred in the above rearing boxes. Each treatment (Cd concentration and *O. sauteri* life stage) was repeated eight times, making it a total of 160 *O. sauteri* tested for each Cd concentration and *O. sauteri* life stage. The number of *O. sauteri* surviving was counted and recorded every day and they were supplemented with 1 mL of new solution until all adults and nymphs had died.

Effect of direct feeding with Cd on predation by Orius sauteri

Orius sauteri (3rd instar nymphs, 5th instar nymphs, female adults, and male adults) were fed with Cd-sucrose solutions as above for 3 days, before being starved for 6 h. More than 100 *O. sauteri* were initially treated in each Cd treatment, and 9 individuals were randomly selected among the surviving ones after three days to test their predation ability. A tomato leaf containing 20 *B. tabaci* pupae (4th instar nymphs) was then added in each Petri dish and stabbed into wet cotton wool to maintain leaf moisture. The number of pupa of *B. tabaci* preyed upon by *O. sauteri* was recorded after 24 h.

Statistical analyses

The Gehan-Breslow-Wilcoxon test of GraphPad Prism 9.0.0 was used to analyze the survival of *O. sauteri* exposed to Cd sucrose solution at differing concentrations. The LC_{50} values of *O. sauteri* at each age were calculated using the toxicity dose response Log-dose probit analysis in SPSS 25.0 (IBM, NY, USA). The predation of *O. sauteri* on *B. tabaci* following exposure to sucrose solutions containing Cd was analyzed using Student's *t*-tests with SPSS. All graphs were created by GraphPad Prism 9.0.0 (GraphPad Software, CA, USA).

Results

Effect of direct Cd feeding on the survival of Orius sauteri

There was no significant difference in the survival rate of 3rd instar nymphs of *O. sauteri* when fed with sucrose solutions containing low doses of Cd at 1 mg/L (χ^2 =0.281, *P*=0.596), 5 mg/L (χ^2 =0.166, *P*=0.684), and 25 mg/L (χ^2 =0.889, *P*=0.346) compared to the control

group (no Cd exposure). However, the treatment of 3rd instar nymphs of *O. sauteri* with 125 mg/L (χ^2 =12.54, *P*=0.004) and 625 mg/L (χ^2 =133.7, *P*<0.001) of Cd in sucrose solutions significantly reduced survival compared to the control (Fig. 1a).

There was no significant difference in the survival rate of 5th instar nymphs of *O. sauteri* when fed with sucrose solutions containing low doses of Cd at 1 mg/L (χ^2 =2.899, *P*=0.089) and 25 mg/L (χ^2 =2.065, *P*=0.151), but survival was significantly higher in the group exposed to 5 mg/L (χ^2 =6.893, *P*=0.009) Cd sucrose solutions. However, 5th instar nymphs of *O. sauteri* fed with sucrose solutions containing high doses of Cd at 125 mg/L (χ^2 =8.136, *P*=0.004) and 625 mg/L (χ^2 =74.10, *P*<0.001) experienced significantly reduced survival compared to the control group (Fig. 1b).

There was no significant difference in the survival rate of female adults *O. sauteri* when fed with sucrose solutions containing low doses of Cd at 1 mg/L (χ^2 =2.038,

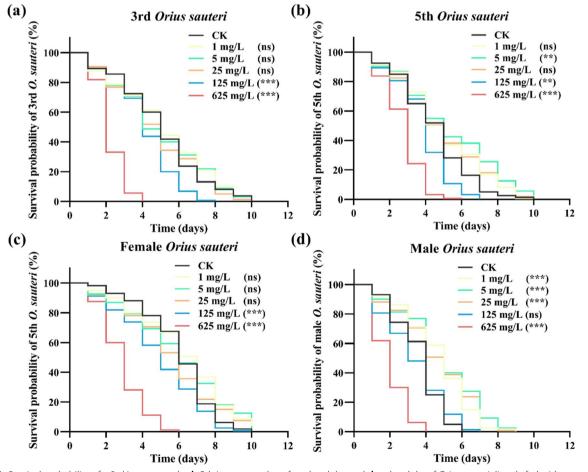


Fig. 1 Survival probability of **a** 3rd instar nymphs, **b** 5th instar nymphs, **c** female adults, and **d** male adults of *Orius sauteri* directly fed with sucrose solutions containing different concentrations of Cd. Mean probabilities for Gehan–Breslow–Wilcoxon statistical tests comparing each Cd concentrations with the control (** P < 0.01; *** P < 0.001; ns, not significant). CK refers to the control group (no Cd). N = 160 individuals for each concentration

P=0.153), 5 mg/L (χ^2 =0.068, *P*=0.795) and 25 mg/L (χ^2 =2.739, *P*=0.098). However, female adults *O. sauteri* fed with 125 mg/L (χ^2 =18.21, *P*<0.001) and 625 mg/L (χ^2 =170.3, *P*<0.001) of Cd sucrose solutions experienced significantly reduced the survival compared to the control (Fig. 1c).

The survival rate of male adults fed with sucrose solutions containing low doses of Cd at 1 mg/L (χ^2 =32.70, P<0.001), 5 mg/L (χ^2 =36.81, P<0.001) and 25 mg/L (χ^2 =22.48, P<0.001) was significantly higher than that of the control group. There was no significant difference in the survival rate of male adults of *O. sauteri* fed with 125 mg/L (χ^2 =1.820, P=0.177) Cd sucrose solution but survival was significantly reduced at 625 mg/L compared to the control (χ^2 =110.1, P<0.001) (Fig. 1d).

Median lethal concentration and confidence intervals of direct Cd feeding

When nymphs and adults were fed with Cd-containing sucrose solution for 3 days, the LC_{50} varied between treatments with the lowest level in male adults followed by 3rd instar nymphs, 5th instar nymphs and finally female adults (Table 1). This indicated that the toxicity of heavy metal Cd to male adults of *O. sauteri* was greatest after 3 days but had the smallest effect on female adults.

The impact of direct Cd feeding on the predation of *Orius sauteri*

Third instar nymphs of *O. sauteri* fed with 25 mg/L of Cd sucrose solution significantly increased their predation ability on *B. tabaci* pupa (t=-3.295, P=0.005) compared to the control. However, there was no significant difference in the predation of *O. sauteri* 3rd instar nymphs on *B. tabaci* after feeding on sucrose solutions containing Cd at 1 mg/L (t=0.561, P=0.583), 5 mg/L (t=0.429, P=0.673), 125 mg/L (t=-1.538, P=0.144) and 625 mg/L (t=-0.600, P=0.557) compared with the control group (Fig. 2a).

The ability of *O. sauteri* 5th instar nymphs to prey on pupae of *B. tabaci* was significantly higher than that of the control group after feeding on sucrose solutions containing Cd at concentrations of 1 mg/L (t=-2.350, P=0.032) and 5 mg/L (t=-2.434, P=0.027). However,

there was no significant difference in their predation of *B. tabaci* pupae after feeding on 25 mg/L (t=-0.403, P=0.697), 125 mg/L (t=-1.008, P=0.328) and 625 mg/L (t=-0.279, P=0.784) Cd sucrose solutions compared to the control group (Fig. 2b).

Female adults of *O. sauteri* fed with 125 mg/L (t=3.212, P=0.005) and 625 mg/L (t=3.780, P=0.002) of Cd sucrose solutions significantly reduced their predation on *B. tabaci* pupae. In contrast, there was no significant difference in their predation ability after feeding on sucrose solutions containing Cd at concentrations of 1 mg/L (t=1.512, P=0.150), 5 mg/L (t=0.603, P=0.555) and 25 mg/L (t=1.414, P=0.176) compared to the control group (Fig. 2c).

Male adults of *O. sauteri* feeding on 25 mg/L (t=-2.262, P=0.038) and 625 mg/L (t=-3.212, P=0.005) of Cd sucrose solutions significantly increased their predation on *B. tabaci* pupae. However, there was no significant difference in their predation of *B. tabaci* pupae when fed with sucrose solutions containing Cd at low doses of 1 mg/L (t=-0.839, P=0.414), 5 mg/L (t=-1.342, P=0.198), 125 mg/L (t=-2.364, P=0.031) compared to the control group (Fig. 2d).

Discussion

Cd can be transferred and accumulated in the food chain of plants, herbivores and natural enemies in agroecosystems, thereby causing significant impacts through bottom-up effects (Tibbett et al. 2021; Han et al. 2022; Wang et al. 2024). However, few reports document the effects of direct feeding on food containing Cd by natural enemies. In this research, we focused our attention on the effects of direct feeding on a heavy metal (Cd) on the survival, predation ability and LC_{50} of *O. sauteri*. The results presented in the current study demonstrated that Cd is potential detrimental to natural enemies in agricultural ecosystems. The survival and predation ability of O. sauteri were significantly affected by feeding on sucrose solution containing Cd. While relatively low concentrations of Cd sometimes increased survival and predation on B. tabaci, high concentrations of Cd systematically led to increased mortality and reduced predation in O. sauteri.

Table 1 Mean lethal concentrations (LC₅₀) for Orius sauteri exposed to sucrose solution containing Cd

Stage of Orius sauteri	Slope±SE	LC ₅₀ (mg/L) 95% confidence interval	X ²	df	<i>p</i> -value
3rd instar nymph	0.519±0.049	191.950 (133.200–276.192)	9.427	4	0.051
5th instar nymph	0.365 ± 0.048	288.606 (240.501-349.420)	5.761	4	0.218
Female adult	0.454 ± 0.050	383.086 (335.062-443.459)	5.606	4	0.231
Male adult	0.632 ± 0.051	153.329 (86.629–249.691)	13.445	4	0.009

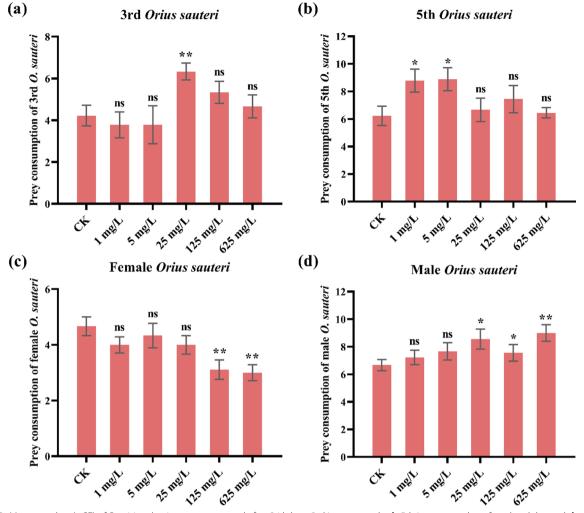


Fig. 2 Mean number (±SE) of *Bernisia tabaci* pupae consumed after 24 h by a 3rd instar nymphs, b 5th instar nymphs, c female adults, and d male adults of *Orius sauteri* after feeding sucrose solution containing Cd. Significant differences based on *t*-tests (* *P* < 0.05; ** *P* < 0.01; ns, not significant)

The impact of Cd stress on natural enemies usually occurs through food chain accumulation whereby it produces indirect toxic effects on the third trophic level (Han et al. 2022; Liu et al. 2023; Wang 2023). Comparatively less studies investigated direct effects of Cd on pests' natural enemies. Here, we found that the survival of the 3rd and 5th instar nymphs, female, and male adults of O. sauteri were significantly decreased when they were fed with sucrose solutions containing high concentration Cd. Similarly, Kafel et al. (2014) showed that survival of Spodoptera exigua exposed to Cd and Zn was significantly reduced. Stolpe et al. (2017) reported that when Zn was added to an artificial diet, the body weight of Myzus persicae increased but the opposite occurred when Cd was added to the diet, leading to a decreased survival rate when both were used together. In addition, prior research has indicated that the heavy metal Cd reduced the survival of European honeybees (Hesketh et al. 2016).

Overall, we found a direct toxicity of Cd resulting in a reduced survival rate of the predator *O. sauteri*. However, toxicity effects were did not increase linearly with Cd concentration (Huang et al. 2023). Past a certain threshold and with increased concentrations of Cd, the survival rate of *O. sauteri* gradually decreased: a concentration of 625 mg/L and 125 mg/L significantly inhibited survival, leading to premature death. Conversely at low concentrations (1 mg/L, 5 mg/L and 25 mg/L), the survival of *O. sauteri* was promoted. These results suggests a possible hormetic effect (Nascarella et al. 2003; Wang et al. 2024).

Effects of direct Cd exposure on *O. sauteri* predation were varied. With low Cd concentrations of 1 mg/L and 5 mg/L, predation of 5th instar nymphs on *B. tabaci* significantly increased, while a Cd concentration of 25 mg/L increased predation in 3rd instar nymphs and male adults. The fifth instar is a key sensitive stage just before adult emergence in O. sauteri, where exposure to low Cd concentrations may stimulate predation, and likewise in early adulthood. Conversely, younger third instar nymphs are at a stage of rapid growth, and may therefore be less sensitive to Cd exposure through active metabolism. Only exposure to higher Cd concentrations may lead to a similar stimulation of predation in these less sensitive stage to exposure to low Cd concentrations, compared to the more sensitive stages of 5th instar nymphs (just before adult emergence) and early adulthood. In both 3rd and 5th instars however, higher concentrations of Cd exposure did not impact predation. This may be because such higher concentrations are toxic and do not stimulate metabolism.

The predation capacity of female adults was significantly reduced when exposed Cd solution concentrations of 125 mg/L and 625 mg/L. This is in total contrast with male adults whereby feeding Cd at 25 mg/L, 125 mg/L and 625 mg/L significantly increased their predation capacity. Similar to predation ability of nymph and female adult of O. sauteri, feeding aphids with low doses of Cd had no effect on the predation rate of 4th instar ladybirds, but exposure to high doses of Cd resulted in a significantly reduced predation rate (Naikoo et al. 2021b). The same results were found for Pb and Ni (Naikoo et al. 2019, 2021a). The abnormal increase in the predation ability of male adults of O. sauteri (relative to the other tested life stages) may be due to their higher tolerance or investment in feeding activity to increase detoxification, especially since Cd had the greatest toxicity on male adults, as shown by their lowest LC_{50} compared to other life stages. This could be further investigated in future work. Perhaps in the future we could consider using the stimulatory effect of this external stress to enhance the predation capacity of natural enemies.

Heavy metal stress had differing effects on the LC_{50} of each life stage of *O. sauteri*. The results of this experiment showed this clearly with the lowest LC_{50} for male adults (153 mg/L), followed by 3rd instar nymphs (192 mg/L), 5th instar nymphs (289 mg/L) and female adults (383 mg/L). This provides conclusive information showing that the heavy metal Cd has the greatest toxicity to male adults of *O. sauteri* and the lowest to female adults. While examining the toxicity of pesticides is common, including in *O. sauteri*, determining the toxicity determination of heavy metals is much less common. In one study in honeybees however, the LC_{50} of Cd was 78 mg/L for worker bees fed with sucrose solution containing Cd, and 0.275 mg/L for larvae (Di et al. 2016). Compared to this previous study, the LC_{50} of *O. sauteri* is several hundred times higher than honeybee larvae, while in adults is several dozen times higher. Factors affecting LC_{50} through pesticide exposure also include sublethal effects in addition to direct effects on mortality (Desneux et al. 2007). For instance, the toxicity of imidacloprid on *O. sauteri* adults was high, with an LC_{50} of 0.30 mg/L, but acute toxicity was the lowest with an LC_{50} of 44.5 mg/L (Lin et al. 2020). Comparing these results with our own findings reveal significant disparities on an order of magnitude, indicating that the toxic effects induced by Cd stress on *O. sauteri* are considerably lower compared to those caused by insecticides. They remain nonetheless important to quantify, notably because potential positive effects could provide routes for improved biological control efficiency.

Conclusion

The present study was conducted under laboratory conditions to investigate the direct toxic effects of Cd feeding on the predator O. sauteri. High concentrations of Cd were highly inhibitory while medium and low concentrations had some positive consequences, although this depended on the life stage considered. Moreover, exposure to low Cd concentration improved the predation ability of the nymphs of O. sauteri to B. tabaci pupa, whereas high concentrations of Cd were highly detrimental to the predation ability of female adults while simultaneously increasing the predation ability of male adults. This research helps to further understand the impact of Cd pollution on agricultural ecosystems and biological control services. This is of great significance for protecting natural enemies of crop pests and for controlling harmful organisms.

Author contributions

Ning Di, Lan Jing, Nicolas Desneux and Su Wang designed the assay; Jing Kou and Zheng-Yang Zhu conducted the experiments; Jing Kou, Zheng-Yang Zhu, Yu Zhang, Jie Wang, and Lan Jing analyzed the data; Jing Kou, Zheng-Yang Zhu, Ning Di, Coline Jaworski and Su Wang wrote the manuscript. All authors read and approved the manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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