Journal of Zhejiang University-SCIENCE A (Applied Physics & Engineering) 2023 24(5):465-472
www.jzus.zju.edu.cn; www.springer.com/journal/11582
E-mail: jzus_a@zju.edu.cn

Correspondence

https://doi.org/10.1631/jzus.A2200334

Mechanism of the insecticidal effect of lambda-cyhalothrin loaded mesoporous silica nanoparticles with different sizes and surface modifications on *Ostrinia furnacalis* (Guenée) larvae

Yanlong WANG[1], Shuting XIAO[1], Jiang XU[1,2✉], Daohui LIN[1,2]

[1]*Department of Environmental Science, Zhejiang University, Hangzhou 310058, China*
[2]*Zhejiang Provincial Key Laboratory of Organic Pollution Process and Control, Zhejiang University, Hangzhou 310058, China*

1 Introduction

Due to the easy loss and decomposition of traditional chemical pesticides, their repeated application in agriculture results in serious environmental risks to the surrounding environment and organisms. Controlled-release nanopesticides are attracting attention as a promising technology in agriculture due to their unique structure and effects (e.g., nano-size scale, interfacial effects, and effective insecticidal time) (An et al., 2022). Their application could improve insecticidal efficacies, decrease usage amounts, and reduce the potential environmental impacts of chemical pesticides. Various nanocarriers including polymers, clays, metal-organic frameworks, and porous inorganic nanomaterials have been studied for the delivery of pesticides to improve the stability and effective insecticidal time of pesticides (An et al., 2022). Low-priced and non-polluting mesoporous silica nanoparticles (MSNs) are a promising candidate for nanocarriers due to their well-ordered structure, easily tunable particle size, hydrophilic silanol groups, high specific surface area, and easy surface modification. Previous studies have demonstrated that controlled-release nanopesticides with mesoporous silica show good dispersion and photochemical stability, and an effective insecticidal effect (An et al., 2022; Xiao et al., 2022). Clarifying the insecticidal mechanism of controlled-release nanopesticides with mesoporous silica as nanocarriers will advance the understanding of their potential performance, transformation, and biotoxicity in an increasing range of applications.

Pyrethroid insecticides are highly neurotoxic broad-spectrum pesticides with strong selective toxicity and readily decompose into non-toxic compounds in the environment (Soderlund et al., 2002; Ray and Fry, 2006). Their mechanism of action is to delay the closure of sodium ion channels in nerve membranes and break the balance of ions inside and outside the membranes, leading to nerve and behavior disorders in insects (Soderlund et al., 2002; Ray and Fry, 2006). The physicochemical properties of nanocarriers directly affect the properties of controlled released nanopesticides. For example, their size and morphology not only affect the distribution and form of drug molecules in vivo, but also their migration and transformation (Diedrich et al., 2012). The positive and negative charge densities on nanocarriers can enhance or weaken the insecticidal efficacy of prepared nanopesticides by affecting the load and release of drug molecules (Shan et al., 2019; Hamuro et al., 2021). Thus, lambda-cyhalothrin particles (LCNS) loaded on MSNs may result in unpredictable effects on living organisms due to the unique physicochemical properties of the MSNs. The insecticidal mechanism of LCNS-loaded MSNs with different properties (e.g., different sizes, charges, and hydrophobicity) has not been well clarified, but is highly relevant to the design, development, and application of controlled-release nanopesticides in agriculture.

The objective of this study was to investigate the mechanism of the insecticidal effect of LCNS-loaded

✉ Jiang XU, xujiang6@zju.edu.cn
Jiang XU, https://orcid.org/0000-0003-0369-4848

Received July 2, 2022; Revision accepted Nov. 17, 2022; Crosschecked Jan. 15, 2023; Online first Mar. 2, 2023

MSNs with different sizes (M_1 at (130±24) nm, M_2 at (95±19) nm, M_3 at (77±16) nm, and M_4 at (63±12) nm in Fig. 1) and surface modifications (M_4 with amino- or methyl-modifications) on *Ostrinia furnacalis* (Guenée) larvae. The synthesis of bare and functionalized MSNs, pesticide loading on bare and functionalized MSNs, and assessment of the insecticidal effect of LCNS-loaded MSNs on *O. furnacalis* larvae are described in the electronic supplementary materials (ESM). The mortality of *O. furnacalis* larvae in response to LCNS-loaded MSNs ($M_{1\text{-}4}$/L, M_4–NH_2/L, and M_4–CH_3/L) was systematically compared under light and dark conditions. By analyzing the changes in the oxidative stress system and related enzyme activities in *O. furnacalis* larvae, the insecticidal mechanism of LCNS-loaded MSNs in relation to the properties of the MSNs was explored. Our preliminary findings will enhance understanding of the potential of sustained-release nanopesticides for application in agriculture.

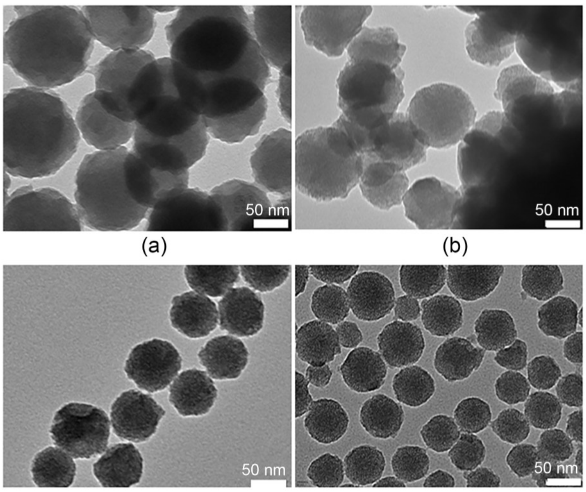

Fig. 1 Transmission electron microscopy images of M_1 (a), M_2 (b), M_3 (c), and M_4 (d) nanoparticles

2 Results and discussion

2.1 Insecticidal assessment of different LCNS-loaded MSNs under light and dark conditions

Toxicity regression equations and half lethal doses (LD_{50}) of LCNS treatment of *O. furnacalis* larvae at 24, 48, and 72 h are shown in Table 1. The LD_{50} of LCNS (dissolved in phosphate buffered saline (PBS) solution containing 1% acetonitrile, pH=7.0) at 24, 48, and 72 h was 341.7, 112.1, and 16.3 mg/kg, respectively. These values were used as the toxic dose reference of drug-loaded nanoparticles. Our previous study suggested that increasing the LCNS dose from 10 to 400 mg/kg increased the mortality rate of free LCNS from 25% to 65%; a low dose of M_4/L (10 mg/kg) resulted in a higher mortality rate (70%) at 24 h (Xiao et al., 2022). Considering the easy photolysis of LCNS (Xiao et al., 2022), insecticidal experiments using 10 or 200 mg/kg LCNS or M_4/L in the diet of the *O. furnacalis* larvae with 12-h starvation were conducted under dark or light conditions to analyze the effect of light on larval mortality.

Table 1 LD_{50} values of LCNS solution at different exposure times

Toxicity time (h)	Toxicity equation	LD_{50} (mg/kg)	*R*-squared, R^2
24	$Y=-1.249+0.493X$	342	0.974
48	$Y=-1.065+0.519X$	112	0.926
72	$Y=-0.806+0.666X$	16	0.926

Compared with 100% mortality under dark conditions, the 24-h mortality of *O. furnacalis* larvae fed 10 or 200 mg/kg LCNS decreased significantly to about 25% under light conditions due to photolysis of LCNS (Fig. 2a). The 24-h mortality caused by 10 or 200 mg/kg M_4/L was more than 70%, and there was no significant difference between the light and dark conditions (Fig. 2a). The improved photochemical stability of LCNS was attributed to the remarkable property of the silica shell to shield entrapped drugs from ultraviolet (UV) light (Hao et al., 2020; Xiao et al., 2022). Another reason for the improved mortality caused by M_4/L could have been that the MSNs as nanocarriers promoted the faster entry of pesticide molecules into insect tissues and cells (Wang et al., 2021). This result indicated that the remarkable light-shielding silica shell (Hao et al., 2020) used for entrapped LCNS could significantly reduce the amount of LCNS used and the potential impact of LCNS on the environment. The size and crystalline structure of nanoparticles significantly affect their biological toxicity (Wang et al., 2021). A negligible effect of MSN size was observed on the mortality of *O. furnacalis* larvae caused by M/L treatments (M_1/L, M_2/L, M_3/L, and M_4/L) under light or dark conditions (Fig. 2b). Compared with the dark condition, lower mortality of *O. furnacalis* larvae caused by M/L was observed

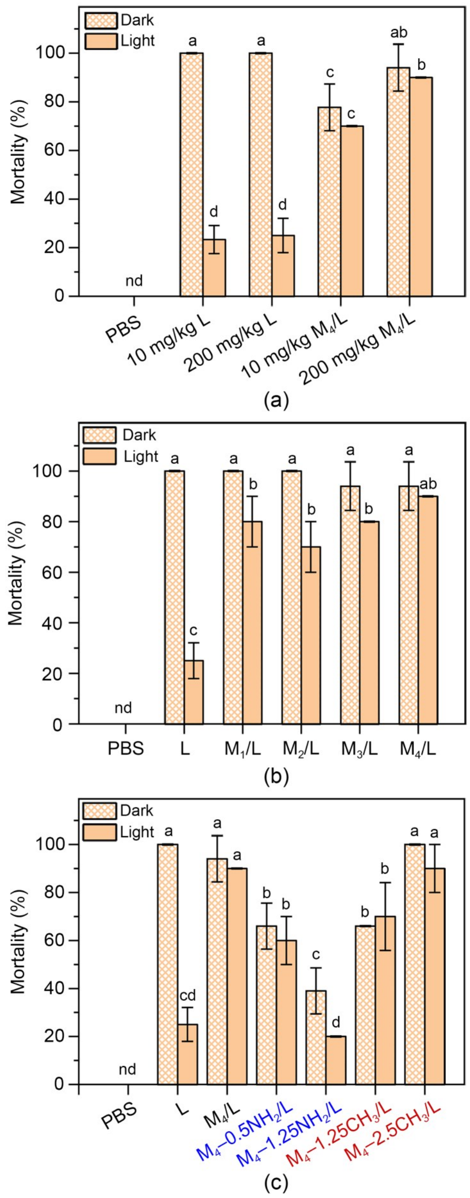

Fig. 2 Mortality of *O. furnacalis* larvae after the 24-h treatments: (a) 10 or 200 mg/kg free LCNS and M_4/L; (b) 200 mg/kg M/L with different particle sizes; (c) 200 mg/kg M_4/L with different surface modifications under dark or light conditions (T=(30±1) °C, relative humidity was 60%, and the light-cycle of the light and dark conditions was 14 h of exposure to light out of 24 h and no light, respectively). Data under the light condition were adopted from our previous study (Xiao et al., 2022) and compared to the dark condition for assessing the alleviated light impacts on the mortality by loading LCNS on modified MSNs. The amino-modified M_4 nanoparticles obtained by using 0.5 or 1.25 mL of 3-Aminopropyl triethoxysilane were marked as M_4–0.5NH_2 and M_4–1.25NH_2, respectively. The methyl-modified nanoparticles obtained by using chlorotrimethylsilane with volumes of 1.25 or 2.5 mL were marked as M_4–1.25CH_3 and M_4–2.5CH_3, respectively. The different letters in panels mean the significant difference (p<0.05) between treatments

under the light condition, with an about 30% decrement (Fig. 2b). However, the light had a negligible effect on the insecticidal effect of the M_4–NH_2/L and M_4–CH_3/L treatments (Fig. 2c). This suggests that loading LCNS onto MSNs with different sizes, charges, and hydrophobicity could be a promising approach to alleviate the negative impact of light (i.e., LCNS degradation) on insecticidal activity. However, the mortality of *O. furnacalis* larvae caused by M_4–NH_2/L or M_4–CH_3/L, but not M_4–2.5CH_3/L, was much lower than that caused by M_4/L, indicating that surface modification of MSN could decrease the biological toxicity of LCNS loaded MSNs (Fig. 2c). The interaction between the positively charged –NH_2 groups and the LCNS molecules is mainly via hydrogen bonding and electrostatic interactions (Maleki et al., 2017; Xiao et al., 2022). These could inhibit the release of LCNS after loading LCNS on the –NH_2 modified M_4 materials. Thus, the 24-h mortality of the M_4–NH_2/L treatment decreased from 66% (caused by M_4–0.5NH_2/L treatment) to 21% (caused by M_4–1.25NH_2/L treatment) with an increasing content of amide groups loaded on the M_4 nanoparticles (Fig. 2c). Small and hydrophobic nanoparticles are easily bound to the cuticle of insects, leading to their dehydration (Benelli, 2018; Wang et al., 2021). The 24-h mortality caused by M_4–2.5CH_3/L was about 90%, which was much higher than that (about 70%) caused by M_4–1.25CH_3/L treatment (Fig. 2c). This was possibly due to the increased loading of LCNS by M_4–2.5CH_3 and the higher hydrophobicity of M_4–2.5CH_3 (Xiao et al., 2022).

2.2 Effects of different LCNS-loaded MSNs on reactive oxygen species accumulation

Usually, adverse conditions (e.g., environmental stimulation, nutrient deficiency, and chemical toxicity) cause oxidative stresses and lead to the accumulation of reactive oxygen species (ROS) (a natural by-product of aerobic metabolism in cells) in organisms (Shen et al., 2015; Benelli, 2018). Due to the instantaneous effects of ROS, changes in ROS levels caused by 10 and 200 mg/kg LCNS treatments were monitored at 6, 12, and 24 h to explore the oxidative stress reaction in *O. furnacalis* larvae. The ROS level in the 10 mg/kg LCNS treatment was almost the same as that in the PBS treatment (blank control), and no significant difference was observed among the LCNS treatments at different times (Fig. 3a). In contrast, the high

LCNS content (200 mg/kg) aggravated the oxidative stress in *O. furnacalis* larvae at 6 h, and then the ROS level declined to a level comparable to that of the PBS treatment at 12 and 24 h (Fig. 3a). This was possibly due to self-regulation by the *O. furnacalis* larvae. The 24-h ROS levels in the larvae increased with increasing concentrations of different M/L treatments, and the various ROS contents were related to MSN sizes. ROS levels in the M/L treatments increased at first and then decreased with decreasing MSN size, and the M_2/L treatment showed the highest concentration of ROS (Fig. 3b). Generally, nanoparticles with smaller particle sizes have a higher affinity for cell surfaces due to their larger specific surface area (Wang et al., 2021). The higher ROS levels in the M_2/L treatment than in the M_1/L treatment were likely due to the easy uptake of small particles by cells. Small-sized nanoparticles could more quickly enter the organisms and cause oxidative stress (Wang et al., 2021). The easy stimulation of smaller nanoparticles triggers oxidative stress in *O. furnacalis* larvae, and the increased contents of antioxidant substances (glutathione, vitamin C, etc.) can decrease the ROS levels (Wang et al., 2021). Thus, lower 24-h ROS levels were observed in the smaller M_3/L (about 77 nm) and M_4/L (about 63 nm) treatments than in the M_2/L treatment.

ROS levels in the 10 mg/kg M_4–NH_2/L treatment were comparable to those in the M_4/L, LCNS, and PBS treatments (Fig. 3c). Amino modification could reduce the hydraulic diameter of nanoparticles, and those with a positive surface charge are more likely to enter cells and produce toxicity (Yu et al., 2012). The high amine-modified LCNS-loaded M_4 treatment (M_4–1.25NH_2/L) resulted in higher ROS levels than the M_4/L and M_4–0.5NH_2/L treatments at 200 mg/kg (Fig. 3c). Compared with hydrophilic nanoparticles, hydrophobic nanoparticles easily penetrate the epidermis of organisms and cause physical contact damage, resulting in rapid toxic effects (Muthukumarasamyvel et al., 2017). Thus, the ROS levels in the M_4–CH_3/L treatments were significantly lower than those in the LCNS, M_4/L, and blank treatments (Fig. 3d). Considering the

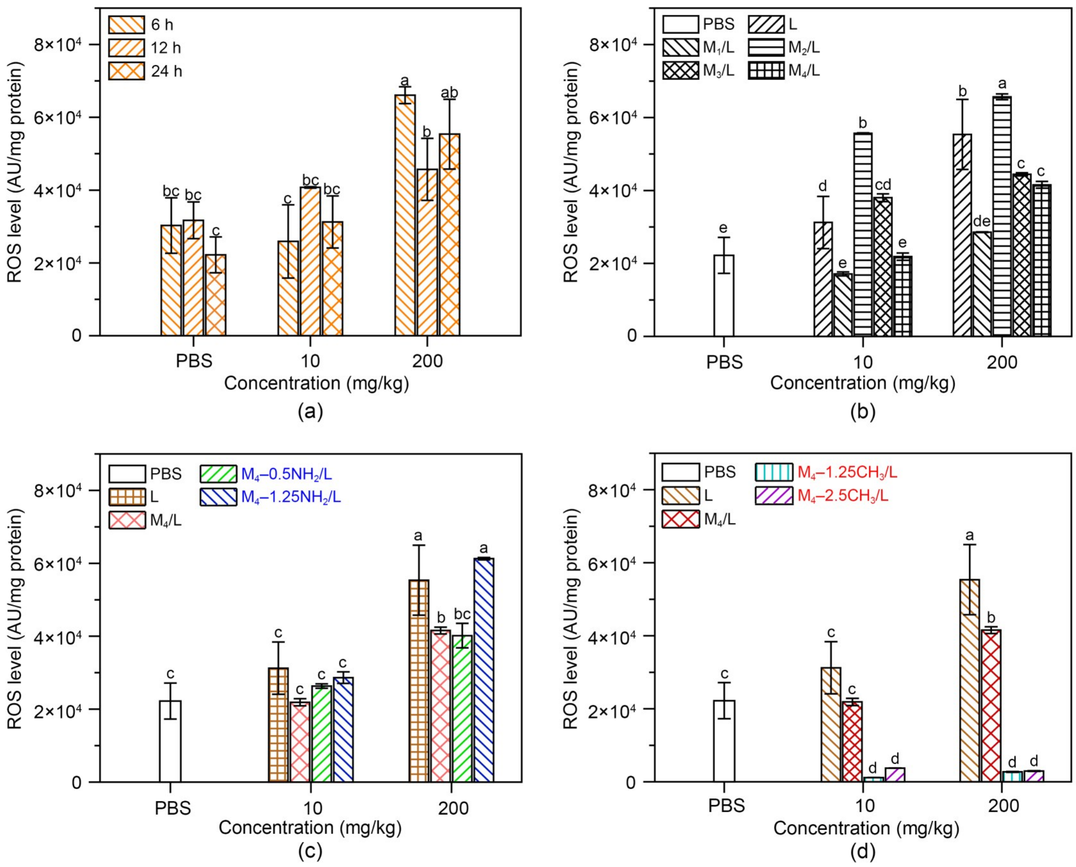

Fig. 3 ROS in the *O. furnacalis* larvae following treatments under the light condition: (a) free LCNS at 6, 12, and 24 h; (b) M/L with different particle sizes; (c) M_4–NH_2/L; (d) M_4–CH_3/L at 24 h

low mortality of *O. furnacalis* larvae caused by the treatments of 200 mg/kg free LCNS under light conditions, the comparable or lower ROS levels in the LCNS-loaded MSN ($M_{1\text{-}4}$/L, M_4–NH_2/L, and M_4–CH_3/L) treatments in comparison with the 200 mg/kg LCNS treatment indicated that oxidative damage in *O. furnacalis* larvae was not the main reason for the high insecticidal activity of LCNS-loaded MSN.

2.3 Effects of different LCNS-loaded MSNs on antioxidase activity

Superoxide dismutase (SOD) can react with $\cdot O_2^-$ to produce a large amount of H_2O_2, and catalase (CAT) can further react with H_2O_2 to produce O_2 and H_2O. Thus, changes of antioxidase (SOD and CAT) activity in organisms could reflect the oxidative damage-related insecticidal efficiency caused by treatment with pesticides and nanoparticles (Xu et al., 2020). When directly exposed to 10 or 200 mg/kg LCNS, the activities of SOD and CAT were comparable to those of PBS treatment at about 60 U/mg protein and 125 U/mg protein respectively (Fig. 4). The lack of a significant difference between the antioxidase activities of LCNS and PBS treatments was likely caused by the low biological toxicity of LCNS with photodegradation (Xiao et al., 2022). The activities of antioxidase (SOD and CAT) significantly decreased with the treatments of 10 or 200 mg/kg LCNS-loaded MSN (M/L, M_4−NH_2/L, and M_4−CH_3/L), compared to the PBS treatment (Fig. 4). The alteration of antioxidase activities by treatments of LCNS-loaded MSNs was size-, dose-, and surface modification-dependent. The SOD activity was the highest in the 10 mg/kg M_2/L treatment (about 40 U/mg protein), but the lowest in the 200 mg/kg M_4−CH_3/L treatment (about 20 U/mg protein). The CAT activity was the highest in the M_4/L treatment at both 10 and 200 mg/kg (about 90 U/mg protein), and the lowest in the 200 mg/kg M_4–1.25NH_2/L treatment (about 30 U/mg protein).

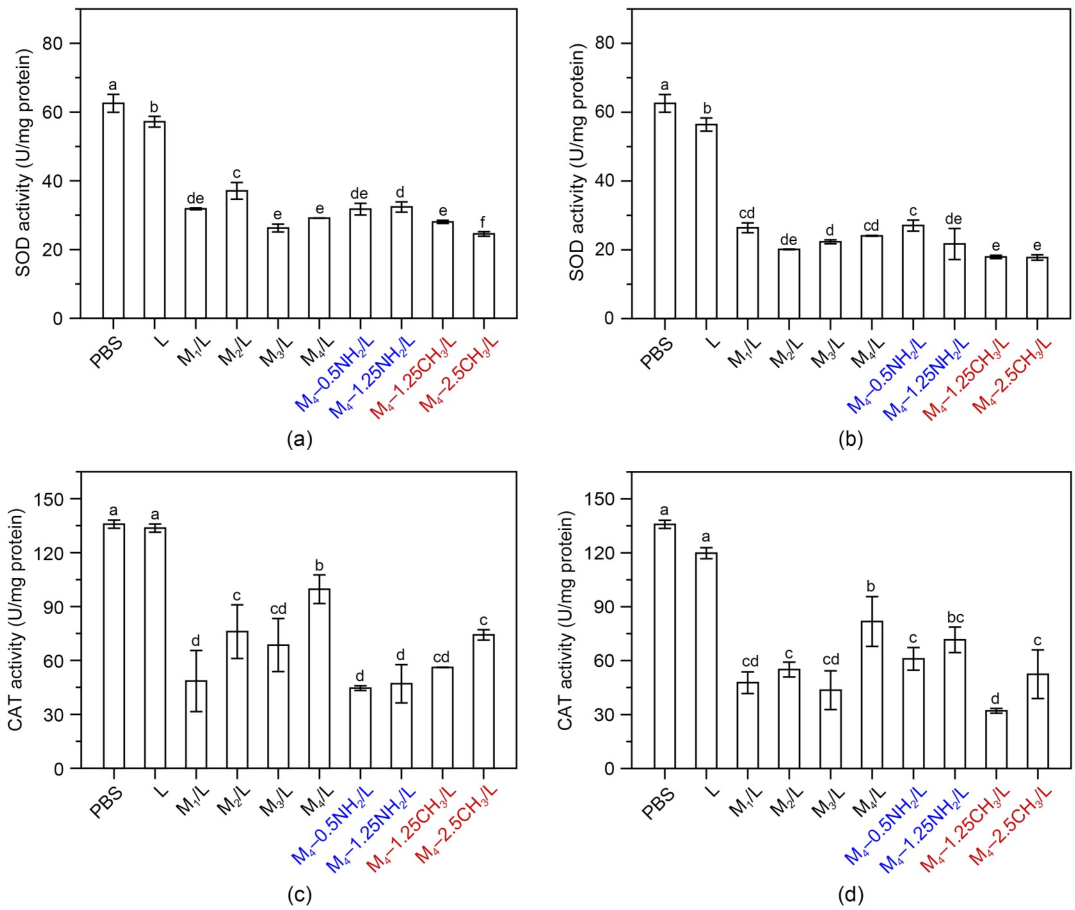

Fig. 4 SOD (a and b) and CAT (c and d) activity in the *O. furnacalis* larvae treated with 10 mg/kg (a and c) or 200 mg/kg (b and d) M/L, M_4–NH_2/L, and M_4–CH_3/L under the light condition

2.4 Effects of different LCNS-loaded MSNs on Na^+/K^+-ATPase activity

Compared to the blank control (PBS treatment), Na^+/K^+-ATPase activity decreased by about 80% and about 90% after short treatments (6 and 12 h) of 10 or 200 mg/kg LCNS, respectively. However, the Na^+/K^+-ATPase activity in the 10 mg/kg LCNS treatment significantly increased to the normal level after 24-h exposure (Fig. 5a), likely due to self-regulation by the *O. furnacalis* larvae (Wang et al., 2021). No significant difference in Na^+/K^+-ATPase activity was observed among the 10 mg/kg LCNS, 10 mg/kg M/L, and PBS treatments. Comparable Na^+/K^+-ATPase activity was observed between the M/L and PBS treatments, while their values were much higher than that following LCNS treatment at 200 mg/kg (Fig. 5b). Compared to the PBS treatment, the M_4–NH_2/L and M_4–CH_3/L treatments also showed no significant inhibition of Na^+/K^+-ATPase activity (Figs. 5c and 5d). The low drug load of the unmodified and modified nanoparticles could be the reason for the lack of significant inhibition of Na^+/K^+-ATPase activity by LCNS-loaded MSN compared with the PBS treatment. Small sized silica can bind to the cuticle of insects and physico-sorption of waxes and lipids may occur, causing major damage, followed by the insect's death (Benelli, 2018). The MSNs with different sizes and surface modifications showed some degree of toxicity to the insects (Fig. S2 of the ESM), with the highest mortality rate (i.e., 33% after 72 h exposure) caused by the most hydrophobic nanoparticles (i.e., M_4–2.5CH_3) (Xiao et al., 2022). The physical contact between the sorbed LCNS-loaded MSNs and the organism would favor the exposure of LCNS to the organism. Our analysis of insecticidal effect, ROS accumulation, changes in Na^+/K^+-ATPase activity, and the toxicity of MSNs with different properties, suggest that the increased exposure of the LCNS on the MSNs was likely the main reason for the biological toxicity of LCNS-loaded MSNs.

3 Conclusions

Understanding the effects of MSN properties on the insecticidal mechanism of action of their

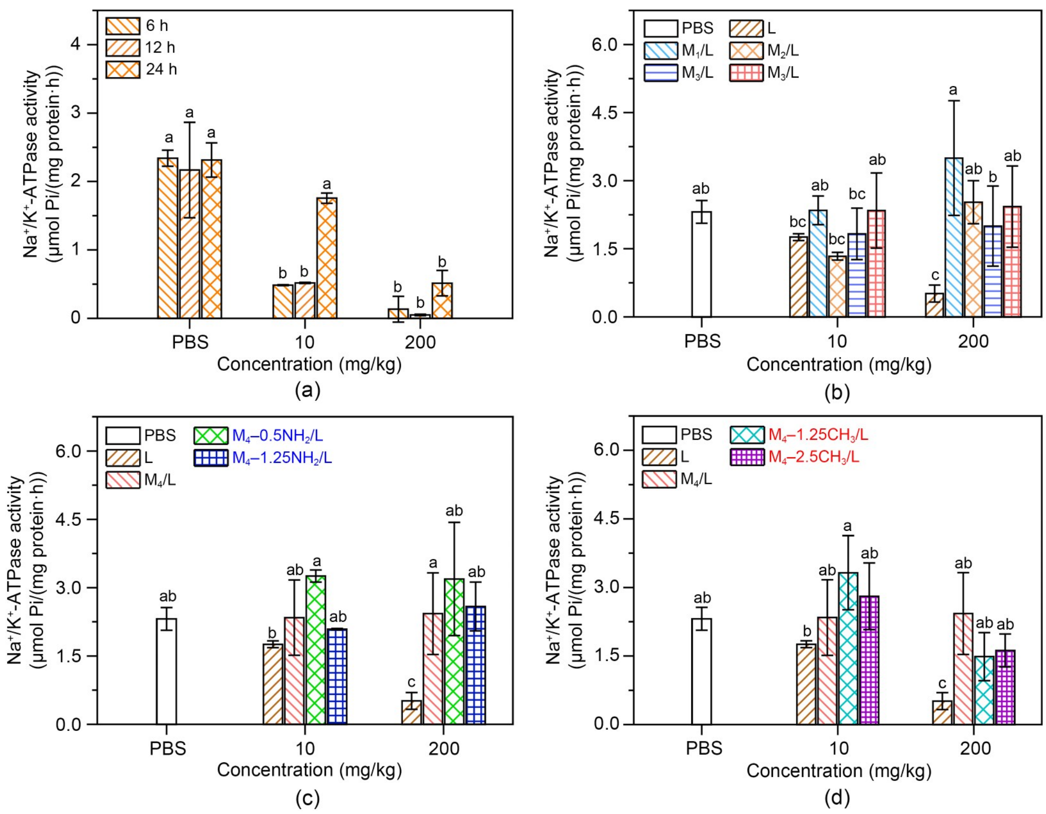

Fig. 5 Na^+/K^+-ATPase activity in the *O. furnacalis* larvae following treatments under the light condition: (a) LCNS at 6, 12, and 24 h; (b) M/L with different particle sizes; (c) M_4–NH_2/L; (d) M_4–CH_3/L at 24 h

drug-loaded versions to pests is of significance for assessing the potential application of MSNs as pesticide nanocarriers in agriculture. The results of this study suggest that light significantly decreases the insecticidal activity of LCNS, while loading LCNS onto bare or modified MSNs could reduce the negative impacts of light. These reduced negative impacts were due mainly to the improved photochemical stability of the loaded LCNS on MSN. The effect of MSN size on the insecticidal effect of LCNS-loaded MSN was negligible, while the surface modifications of $-NH_2$ and $-CH_3$ on MSN decreased the insecticidal effect under both the light and dark conditions. The ROS content in *O. furnacalis* larvae increased following treatment with LCNS-loaded MSNs of medium size (about 95 nm) and a surface modification of $-NH_2$. LCNS-loaded MSNs with different sizes and surface modifications inhibited SOD and CAT activities, but LCNS-loaded MSN treatment had a negligible effect on Na^+/K^+-ATPase activity in the *O. furnacalis* larvae. According to the analyses of insecticidal activity of LCNS-loaded MSNs and the changes in the ROS content and antioxidase and Na^+/K^+-ATPase activities, the high insecticidal activity of LCNS-loaded MSNs was probably caused by the increased exposure of LCNS from the sorbed LCNS-loaded MSNs rather than oxidative damage to *O. furnacalis* larvae. These findings are of significance to understanding the effects of MSN properties on the environmental application and risk assessment of LCNS-loaded MSNs.

Acknowledgments

This work is supported by the Zhejiang Provincial Natural Science Foundation of China (No. LD21B070001).

Author contributions

Yanlong WANG processed the data and wrote the first draft of the manuscript. Shuting XIAO carried out the experiment and processed the data. Daohui LIN helped to organize the manuscript and acquired the funding for this research. Jiang XU designed the research and revised and edited the final version.

Conflict of interest

Yanlong WANG, Shuting XIAO, Jiang XU, and Daohui LIN declare that they have no conflict of interest.

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Electronic supplementary materials

Sections S1 and S2