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Original Research Articles

The acute toxicity of cypermethrin, emamectin benzoate and imidacloprid on red swamp crayfish (*Procambarus clarkia*)

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Pesticide contamination is commonly found as a mixture of different pesticides rather than individual compounds. However, the regulatory risk evaluation is mostly based on the effects of individual pesticides. In the present study, we aimed to investigate the individual and combined toxicities of cypermethrin (CYP) with emamectin benzoate (EMB) and imidacloprid (IMI) to crayfish using acute indices and various sub-lethal endpoints. Semi-static bioassay procedures were followed in the experiment. The 24, 48, and 72 h LC_{50} values (with 95% confidence limits) of CYP for crayfish were calculated as 0.141, 0.137, and 0.135 ug/ml, respectively, which were higher than those of IMI (75.813, 72.345, 70.568 μg/ml) and EMB (34.581, 27.930, 22.298 ug/ml). Pesticide mixtures of CYP and EMB displayed a synergistic response to crayfish; the LC50 was 0.053, 0.050, and 0.048 ug/ml, which was lower than when only CYP was present. Pesticide mixtures of CYP and EMB were found to be highly toxic to crayfish. At the physiological level, both individuals and mixtures of pesticides caused severe injury to the internal organs of crayfish. Taken together, the synergistic effects indicated that it was highly important to include joint toxicity studies when assessing the risk of pesticides.

INTRODUCTION

Rice field integrated farming, which combines aquaculture and rice cultivation, effectively stabilizes food security and maintains sustainable agricultural development.[1] Integrated farming ecosystems are critical in stabilizing food synergy, improving soil fertility, and decreasing the incidence of insect pests and pesticide use.[2] As a typical integrated agricultural-aquaculture system, rice field farming activities include rice tilapia, rice carp, rice crayfish, and rice-turtle.[1,3,4] However, the risk assessment indicated that the exposure to toxic levels of pesticides for aquaculture is significant, at least shortly after pesticide application.[5] Less research has been done in rice field habitats, particularly in rice-crayfish culture systems without artificial diets for crayfish.

Cypermethrin (CYP), the α-cyano-3-phenoxybenzyl ester of 2,2dimethyl- 3-(2,2-dichlorovinyl)2-2-dimethyl cyclopropane carboxylate, has been widely used to control of pests as a public health agent, often enters wastewater effluent because of its use in agriculture. CYP residues have been detected in water and sediment samples from streams and rivers draining major agricultural districts.[6] According to previous reports, the concentration of CYP in surface water was <1 μg/L in most cases, but it can be as high as 2.8 μg/L.[7] Chemical exposure may cause oxidative stress and reactive oxygen species (ROS) generation in fish.[8,9] Under normal physiological conditions, ROS is detoxified by antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).[9,10] According to previous studies, CYP is more toxic to crustaceans[11] than to fish species.[12] Further, its toxicological effects were found to be species-specific.[13]

Emamectin benzoate (EMB) is a neurotoxic insecticide of the avermectin group. The careless use of EMB could cause environmental pollution and serious harm to other, non-target organisms. Research has shown that juvenile lobsters exposed to the highest dosages of EMB with longer exposure increased mortality, with LC_{50} values decreasing with increasing test material concentration.[14] In addition, other reports have shown tubule degeneration in the kidney, mild glycogen vacuolation in the liver, loss of absorptive vacuoles, inflammation and disintegration of the epithelial layer in the intestine of Nile tilapia fed the 1X EB-diet.[15] Although toxicity to some natural enemies and non-target arthropods was reported, this insecticide was

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considered less harmful to beneficial arthropods than broad-spectrum compounds. The insecticide primarily acts through ingestion, causing paralysis in the insect by activating the glutamate-gated chloride channels in the synapses allosterically. Because EMB is widely used as an insecticide for controlling rice leaf roller on paddy in China and applied to agricultural products just before harvest, the development of a simple, accurate detection method for this insecticide in paddy is highly desirable.

Imidacloprid [IMI; 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2imidazolidinimine] is the most widely used neonicotinoid insecticide, and its use continues to increase.[16] The residues of IMI may enter the food chain, making it important to investigate the potential adverse effects of imidacloprid exposure. This neurotoxic insecticide of the neonicotinoid group is an acetylcholine receptor agonist with a broad spectrum and highly systemic activity, acting by ingestion and direct contact and causing neuronal hyperexcitation at the level of synapses.[17] IMI exhibited high toxicity to Mysid shrimp and aquatic insect midges, black flies, and mosquito larvae.[18-20] Given IMI's widespread environmental persistence and high solubility in water, there is a significant need for research on the aquatic toxicity of mixtures of IMI with other pesticides. In the Wu et al.[21] study, pesticide mixtures of triazophos (TRI) and IMI synergistically affected zebrafish embryos. Activities of carboxylesterase (Car-E) and catalase (CAT) were significantly changed in most of the individual and joint exposures to pesticides compared to the control group.

Aquatic organisms in natural environments are commonly exposed to chemical mixtures rather than individual compounds.[22] The behavior of chemical mixtures may not correspond to that predicted from data for individual compounds.[23] Therefore, the evaluation of mixture toxicity, a more realistic approach to evaluating the toxicity of chemicals in the ecosystem, is crucial for risk assessment.[24,25]

The synergistic effects indicated that it is important to incorporate joint toxicity studies, especially at low concentrations, when assessing the risk of pesticides. In this study, the toxicity of CYP, EMB, IMI, and their mixtures to crawfish was explored by immersion, and the LC_{50} of the five pesticides was calculated. It can provide data to support future toxicological repeated drug administration experiments and provide some data references for pest control in aquatic environments of the aquaculture industry. In addition, determining the toxicity value can also provide some data reference for clinical medicine toxicity and side reaction detection, and the LC_{50} value can also provide a basis for formulating relevant water quality standards in water pollution prevention and control.

METHODS AND MATERIALS

TEST ORGANISMS AND CHEMICAL

All crayfish handling followed the guidelines for the care and use of animals for scientific purposes established by the Institutional Animal Care and Use Committee (IACUC) of Yancheng Institute of Technology, China. Red swamp crayfish were obtained from Jiangsu Jinfeng Agricultural Technology Co., Ltd. A commercial feed (Tongwei (Dafeng) Feed Co., Ltd., Jiangsu, China) was used to feed the crayfish twice daily (7:00 and 19:00), and they were temporarily cultured to acclimate to the experimental conditions for two weeks (average body weight: (4.04 ± 1.13) g). The conditions for temporary culture were temperature ((25 ± 1) °C), pH 7.60–8.40, NH_3 and H_2S less than 0.04 mg/L and 0.03 mg/L, respectively, and dissolved oxygen kept more than 6.0 mg/L throughout the experiment.

A pure cypermethrin (4.5%) was supplied from Zhejiang Well-done Chemical Co., Ltd., China. Purified grade of imidacloprid (10%) was supplied by Yancheng Limin Agrochemical Co., Ltd., Jiangsu, China. A purified grade of emamectin benzoate (5%) was supplied by Henan Yongguan Agricultural Technology Co., Ltd., China. Pesticide mixtures of CYP and IMI were supplied by Jiangsu Gushun Agrochemical Co., Ltd., China. Pesticide mixtures of CYP and EMB (4.2%) were supplied by Qingdao Haina Biotechnology Co., Ltd., Shandong, China. Pesticide mixtures of CYP and IMI, pesticide mixtures of CYP and EMB are emulsifiable oils, and EMB and IMI are both wettable powders. The above reagents were used as mother liquor and diluted according to the experimental concentration.

TOXICITY TESTS

To investigate the five pesticides' toxicity on crayfish, a 28-day exposure experiment in a semi-static manner was carried out under laboratory conditions. The five pesticides were arranged into five concentration gradients (CYP mother liquor was 0.060, 0.105, 0.135, 0.180, 0.270μg/ml; EMB mother liquor was 40, 60, 70, 80, 100μg/ml; IMI mother liquor was 14, 17, 20, 25, 36 μg/ml, pesticide mixtures of CYP and EMB mother liquor 0.01, 0.03, 0.05, 0.08, 0.1μg/ml, the ratio was 37:3, pesticide mixtures of CYP and IMI mother liquor were 0.06, 0.12, 0.18, 0.24, 0.30μg/ml, the ratio was 1:1 (**Table 1**), 30 crayfish were selected for each concentration to set up three groups of parallel experiments. Then 30 crayfish without drug impregnation were selected to set up three groups and cultured in aerated water as the control group, and the toxicity was determined by the liquid immersion method. The soaking process involved immersing the crayfish in pesticide for approximately 10 minutes. After the soaking, the extra pesticide on the body surface of the crayfish was wiped off with absorbent paper, and then the crayfish was put into the aerated water for cultivation, and the crayfish was fed regularly. In the process of culture, the survival status of crayfish was checked at 12, 24 and 36 h after treatment, and the dead crayfish were picked out in time. The dead crayfish was assessed based on the criterion that there was no reaction to the crayfish when it was touched gently. Regression equations, χ^2, LC_{50} values, and 95% confidence intervals were calculated and the mortality of crayfish toxicity of the five pesticides.

The color changes and shapes of each organ and tissue of crayfish were observed, and photographed, compared with the clear water.

Table 1. Individual and mixture toxicities of cypermethrin, emamectin benzoate and imidacloprid to crayfish

medicament	*Exposure time (h)*	*Virulence regression equation*	χ^2	*95% confidence limits (μg/ml)*	LC_{50} *(μg/ ml)*
CYP	24	y=4.165x+3.546	1.748	(0.123, 0.161)	0.141
	48	y=4.392x+3.791	2.126	(0.121, 0.156)	0.137
	72	y=4.478x+3.889	2.274	(0.119, 0.153)	0.135
EMB	24	y=7.186x-13.507	2.399	(70.311, 82.434)	75.813
	48	y=8.166x-15.187	1.413	(67.448, 77.632)	72.345
	72	y=7.648x-14.138	3.319	(65.528, 75.967)	70.568
IMI	24	y=4.444x-6.839	5.546	(25.996, 79.892)	34.581
	48	y=4.712x-6.814	2.545	(24.913, 33.191)	27.930
	72	y=6.460x-8.710	5.878	(17.967, 30.403)	22.298
Mixtures of CYP and EMB (37:3)	24	y=3.017x+3.849	2.153	(0.044, 0.064)	0.053
	48	y=3.193x+4.147	2.678	(0.042, 0.060)	0.050
	72	y=3.21x+4.244	3.265	(0.039, 0.057)	0.048
Mixtures of CYP and IMI (1:1)	24	y=5.334x+3.979	5.662	(0.134, 0.239	0.180
	48	y=4.244x+3.379	2.956	(0.140, 0.181)	0.160
	72	y=4.244x+3.379	2.956	(0.140, 0.181)	0.160

Note: LC means the median lethal concentration.

STATISTICAL ANALYSIS

The experiment data was arranged using Excel 2016, and IBM SPSS Statistics24 performed the analysis.

RESULTS

TOXICITY OF CYP, EMB, AND IMI ON CRAYFISH

As shown in (**Table 1**), the experimental results displayed that the LC_{50} of CYP, EMB, and IMI after exposure for 24h, 48h, and 96h were 0.141, 0.137, 0.135, 75.813, 72.345, 70.568, 34.581, 27.930, 22.298 μg/ml, respectively, the death rate of crayfish in parallel with concentration and the extension of poisoning time. The experimental results show that the LC_{50} of EMB was the highest among the three pesticides, and its toxicity was the lowest. The LC_{50} of EMB was about 700 times higher than that of CYP, so the toxicity of CYP was significantly greater than that of EMB. The toxicity of IMI was between CYP and EMB.

JOINT TOXICITY OF CYP WITH EMB AND IMI ON CRAYFISH

To evaluate the interaction of CYP and EMB against crayfish, we determined the LC_{50} values of pesticide mixtures after 24h, 48h, 96h exposure. From **Table 1**, the experimental results show that the LC_{50} of the mixture of CYP and EMB was 0.053, 0.050, and 0.048 μg/ml for 24, 48 and 72h, respectively. The LC_{50} increased when the exposure time was extended as shown in Figure 1, indicating that the joint toxicity was positively correlated with exposure time. Interestingly, the mortality of crayfish exposed to the mixture of CYP and IMI for 48h and 72h was identical, resulting in LC_{50} of 0.160 μg/mL. In addition, when CYP was mixed with IMI, the toxicity of the pesticide was lower than that of CYP alone, contrary to the results of changes in CYP toxicity after mixing with EMB.

MANIFESTATIONS OF CRAYFISH POISONING

The behavior of crayfish to exposure was observed every 12 h during the toxicity tests. Upon exposure to all pesticides in the initial period, most crayfish showed transient excitement phenomena, including enhanced activity and quick swimming. The changes in behavioral responses initiated half an hour following dosing in all exposure groups. With the prolonged time and concentration, the movements of the crayfish slowed down and they tended to congregate in the corners of the aquarium. Some crayfish attempted to climb the vertical walls, whilst others settled in the middle of the aquaria. Fighting incidents among crayfish increased. Before death, crayfish lost equilibrium and presented themselves in a knock-down position. Then crayfish flipped over and turned upside-down on their backs.

ANATOMY AND OBSERVATION

As shown in **Figure 1**, the gut of crayfish in all experimental groups changed from dark brown to blue, others became empty, and some sections lost elasticity. The size of the gills did not change, but they turned black in color. The color of the hepatopancreas did not change significantly, but gradually changed from solid to liquid with the increase of concentration, and obvious lesions occurred. In contrast to the other groups, the color of the gastric pouch changed from dark brown to white, and obvious lesions occurred in the mixture CYP and EMB group (**Figure 1e K, L**), while

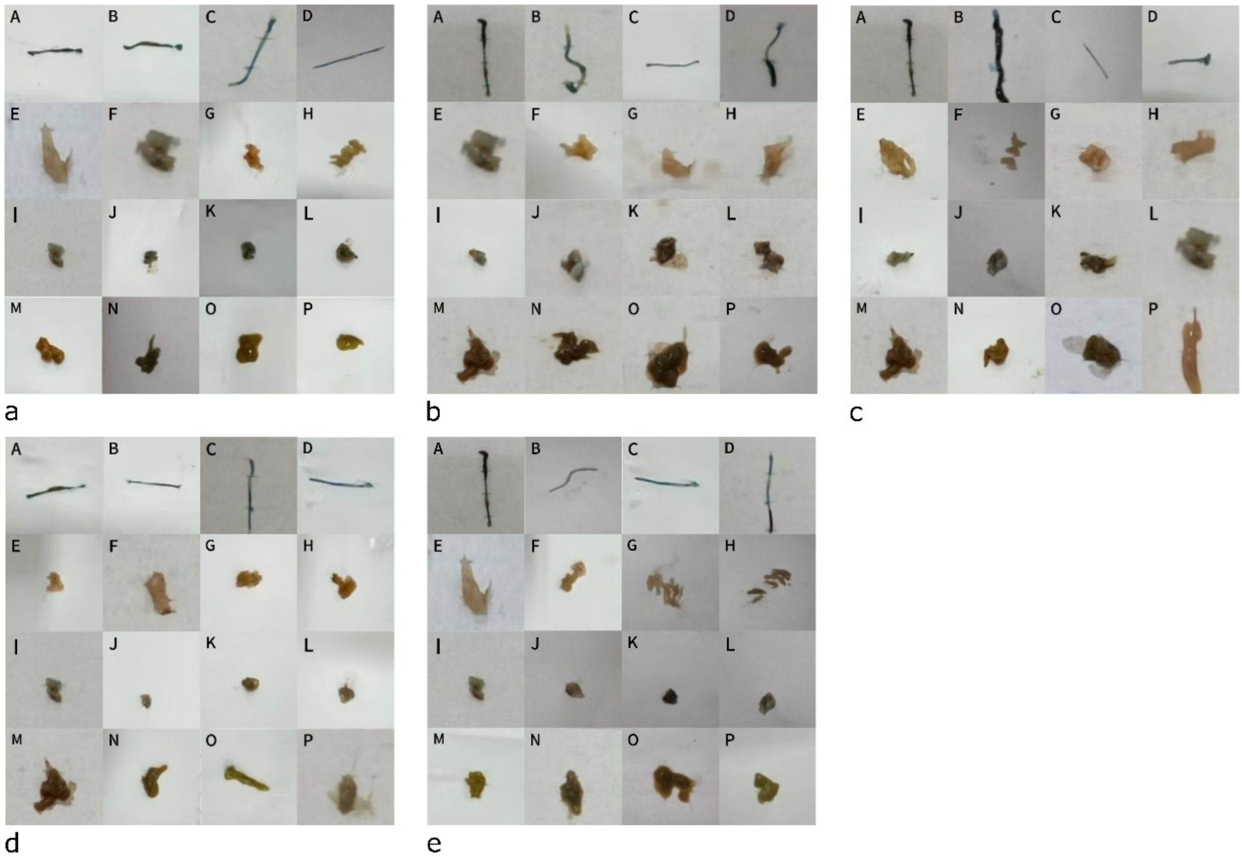

Figure 1. Tissue pathological responses of crayfish

Tissue pathological responses of CYP, EMB, IMI, CYP and EMB, and mixtures of CYP and IMI to crayfish were shown in a, b, c, d, and e. And show the effects of increasing concentration on the lesions of the gut (A-D), gills (E-H), gastric sac (I-L), and hepatopancreas (N-P) of crayfish, respectively.

the color of the gastric pouch changed to dark cyan in the other groups. Nonetheless, no CYP, EMB, and IMI response to heart and muscle tissue was observed in our study.

DISCUSSION

Various types of pesticides are widely used in China for a wide range of purposes. CYP is extensively used to control moths and pests in cotton, fruits, rice, vegetables and horticulture, as well as kill miscellaneous aquatic biota in ponds.[26] However, excessive use of pesticides can lead to the destruction of the ecological environment and death of aquatic organisms, as documented in numerous species of aquatic organisms.[27,28] CYP has been reported for a novel biodegradation pathway with Bacillus sp. strain SG2.[29] Meanwhile, EMB has been experimentally confirmed as a pesticide that is readily biodegradable.[30] In a study on the extensive use of imidacloprid in rice ecosystems, the total soil microbial biomass carbon content was reduced following imidacloprid application, concluding that IMI had transient negative effects on soil microbes.[31] Waseem Hayat et al.[32] pointed out that adding tert-butyl alcohol to nanoscale zero-valent iron (n-ZVI)/sodium persulfate (SPS) system decreased degradation, suggesting OH primarily degraded IMI. Previous studies have shown that CYP, EMB, and IMI can be efficiently degraded, but whether they can be degraded after combining them requires more research in the future. In this study, the 24, 48, and 72h acute LC_{50} values (with 95% confidence limits) of CYP for crayfish were 0.141, 0.137, and 0.135 μg/ml, respectively, the results were lower than CYP on juvenile crayfish in Yuan's experiment (1.305, T0.424, 0.287 μg/ml).[33] The cause of this experiment's LC_{50} being smaller may be attributed to different age-related crayfish immunity, but more work is needed to explain the cause of the phenomenon. Pesticide contamination was often found as a mixture of different pesticides in water bodies than individual compounds.[34] This study demonstrated, for the first time, that the joint action of CYP with EMB and IMI was highly toxic to crayfish. Mixtures of CYP and IMI exerted a strong synergistic effect on the acute toxicity to crayfish; the LC_{50} was 0.180, 0.160, and 0.160 μg/ml, indicating the higher toxicity of mixtures compared with individual pesticides, the synergistic effect of CYP and EMB was more obvious, the LC_{50} was 0.053, 0.050, 0.048 μg/ml. Lakshmi et al.[35] supported the cypermethrin's effectiveness of synergism; the cypermethrin and Croton bonplandianum Baill, the synergistic effect was found to provide better larvicidal activity. In Wu et al.[21] study, pesticide mixtures of triazophos (TRI) and IMI displayed synergistic response on zebrafish embryos. The activities of carboxylesterase (Car-E) and catalase (CAT) were significantly altered in most individual and joint pesticide exposures compared with the control group. Therefore, we should consider the toxic effects of both individual pesticides and their mixtures in the risk management of pesticides.

In an experiment on acute toxicity testing of crayfish,[36] the behavioral responses of crayfish to various pesticides were consistent, ranging from initial transient excitement to eventual slow action until death. This study did not conduct in-depth research on the sectioning of the heart and muscles, so it was impossible to determine whether pesticide exposure could have harmed the multinucleated and branched myocardial cells or if the muscles exhibited myofiber fracture and lysis.[37]

Since the natural aquatic environment encounters pesticide complexity, it is necessary to assess the joint effects of pesticides. Based on the abovementioned results, the mixed pesticide elicits a more significant and pronounced lethal effect on crayfish, providing a more comprehensive insight into joint toxic effects at the physiological level. Furthermore, the results also provided theoretical and scientific significance for the safety application of CYP, EMB, and IMI to farmers and promote the healthy development of the modern eco-agriculture model.

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AUTHOR CONTRIBUTION - CREDIT

Conceptualization: Kaixin Zhang (Lead). Funding acquisition: Kaixin Zhang (Lead). Investigation: Kaixin Zhang (Lead). Validation: Kaixin Zhang (Lead). Writing – original draft: Kaixin Zhang (Equal), Jiyi Chen (Equal). Data curation: Jiyi Chen (Lead). Formal Analysis: Jiyi Chen (Lead). Methodology: Shuxin Zhang (Lead). Project administration: Xinxin Chen (Lead). Visualization: Xinxin Chen (Lead). Software: Ke Sun (Lead). Supervision: Pujie Liu (Lead). Resources: Hongshan Li (Lead). Writing – review & editing: Yanming Sui (Lead).

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

All crayfish handling followed the guidelines for the care and use of animals for scientific purposes established by the Institutional Animal Care and Use Committee (IACUC) of Yancheng Institute of Technology, China.

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REFERENCES

1. Dong S, Gao Y, Gao Y, et al. Evaluation of the trophic structure and energy flow of a rice-crayfish integrated farming ecosystem based on the Ecopath model. *Aquaculture*. 2021;539. doi:10.1016/j.aquaculture.2021.736626

2. Bhattacharyya P, Sinhababu DP, Roy KS, et al. Effect of fish species on methane and nitrous oxide emission in relation to soil C, N pools and enzymatic activities in rainfed shallow lowland rice-fish farming system. *Agriculture, Ecosystems & Environment*. 2013;176:53-62. doi:10.1016/j.agee.2013.05.015

3. Zhang J, Hu L, Ren W, et al. Rice-soft shell turtle coculture effects on yield and its environment. *Agriculture, Ecosystems & Environment*. 2016;224:116-122. doi:10.1016/j.agee.2016.03.045

4. Billah MM, Uddin MK, Samad MYA, et al. Impact of feeding schedule on the growth performances of tilapia, common carp, and rice yield in an integrated rice-fish farming system. *Sustainability*. 2020;12(20):8658. doi:10.3390/su12208658

5. Anyusheva M, Lamers M, La N, Nguyen VV, Streck T. Fate of pesticides in combined paddy rice-fish pond farming systems in northern Vietnam. *J Environ Qual*. 2012;41(2):515-525. doi:10.2134/jeq2011.0066

6. Burr SA, Ray DE. Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. *Toxicological Sciences*. 2004;77(2):341-346. doi:10.1093/toxsci/kfh027

7. Jergentz S, Mugni H, Bonetto C, Schulz R. Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. *Chemosphere*. 2005;61(6):817-826. doi:10.1016/j.chemosphere.2005.04.036

8. Jin Y, Zhang X, Shu L, et al. Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (Danio rerio). *Chemosphere*. 2010;78(7):846-852. doi:10.1016/j.chemosphere.2009.11.044

9. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety*. 2006;64(2):178-189. doi:10.1016/j.ecoenv.2005.03.013

10. Zhang X, Xie P, Li D, Tang R, Lei H, Zhao Y. Time-dependent oxidative stress responses of crucian carp (Carassius auratus) to intraperitoneal injection of extracted microcystins. *Bull Environ Contam Toxicol*. 2009;82(5):574-578. doi:10.1007/s00128-009-9671-2

11. Lidova J, Buric M, Kouba A, Velisek J. Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe. *Veterinární medicína*. 2019;64(3):125-133. doi:10.17221/136/2018-vetmed

12. Soares MP, Jesus F, Almeida AR, et al. Endemic shrimp Macrobrachium pantanalense as a test species to assess potential contamination by pesticides in Pantanal (Brazil). *Chemosphere*. 2017;168:1082-1092. doi:10.1016/j.chemosphere.2016.10.100

13. Eyckmans M, Celis N, Horemans N, Blust R, De Boeck G. Exposure to waterborne copper reveals differences in oxidative stress response in three freshwater fish species. *Aquatic Toxicology*. 2011;103(1-2):112-120. doi:10.1016/j.aquatox.2011.02.010

14. Daoud D, McCarthy A, Dubetz C, Barker DE. The effects of emamectin benzoate or ivermectin spiked sediment on juvenile American lobsters (Homarus americanus). *Ecotoxicology and Environmental Safety*. 2018;163:636-645. doi:10.1016/j.ecoenv.2018.06.075

15. Julinta RB, Abraham TJ, Roy A, et al. Safety of emamectin benzoate administered in feed to Nile tilapia Oreochromis niloticus (L.). *Environmental Toxicology and Pharmacology*. 2020;75:103348. doi:10.1016/j.etap.2020.103348

16. Furlan L, Kreutzweiser D. Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. *Environ Sci Pollut Res*. 2015;22(1):135-147. doi:10.1007/s11356-014-3628-7

17. Cruces L, Peña E de la, De Clercq P. Seasonal phenology of the major insect pests of quinoa (Chenopodium quinoa Willd) and their natural enemies in a traditional zone and two new production zones of Peru. *Agriculture*. 2020;10(12):644. doi:10.3390/agriculture10120644

18. Stoughton SJ, Liber K, Culp J, Cessna A. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates Chironomus tentans and Hyalella azteca under constant- and pulse-exposure conditions. *Arch Environ Contam Toxicol*. 2008;54(4):662-673. doi:10.1007/s00244-007-9073-6

19. Roessink I, Merga LB, Zweers HJ, Van den Brink PJ. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Enviro Toxic and Chemistry*. 2013;32(5):1096-1100. doi:10.1002/etc.2201

20. Smit CE, Posthuma-Doodeman CJAM, van Vlaardingen PLA, de Jong FMW. Ecotoxicity of imidacloprid to aquatic organisms: derivation of water quality standards for peak and long-term exposure. *Human and Ecological Risk Assessment: An International Journal*. 2015;21(6):1608-1630. doi:10.1080/10807039.2014.964071

21. Wu S, Li X, Liu X, et al. Joint toxic effects of triazophos and imidacloprid on zebrafish (Danio rerio). *Environmental Pollution*. 2018;235:470-481. doi:10.1016/j.envpol.2017.12.120

22. Key P, Chung K, Siewicki T, Fulton M. Toxicity of three pesticides individually and in mixture to larval grass shrimp (Palaemonetes pugio). *Ecotoxicology and Environmental Safety*. 2007;68(2):272-277. doi:10.1016/j.ecoenv.2006.11.017

23. Chen X, Li H, Zhang J, Ding Y, You J. Does cadmium affect the toxicokinetics of permethrin in Chironomus dilutus at sublethal level? Evidence of enzymatic activity and gene expression. *Environmental Pollution*. 2016;218:1005-1013. doi:10.1016/j.envpol.2016.08.051

24. Scheil V, Köhler HR. Influence of nickel chloride, chlorpyrifos, and imidacloprid in combination with different temperatures on the embryogenesis of the zebrafish Danio rerio. *Arch Environ Contam Toxicol*. 2009;56(2):238-243. doi:10.1007/s00244-008-9192-8

25. Moghiseh Z, Jaafarzadeh N, Jorfi S, Takdastan A, Kalantar M, Prosser R. Risk of pesticides mixtures in rice to birds and humans in Iran. *Arch Environ Contam Toxicol*. 2023;85(2):147-164. doi:10.1007/s00244-023-01024-y

26. Zhao P, Dong X, Chen X, Guo X, Zhao L. Stereoselective analysis of chiral pyrethroid insecticides tetramethrin and alpha-cypermethrin in fruits, vegetables, and cereals. *J Agric Food Chem*. 2019;67(33):9362-9370. doi:10.1021/acs.jafc.9b01850

27. Buřič M, Kouba A, Máchová J, Mahovská I, Kozák P. Toxicity of the organophosphate pesticide diazinon to crayfish of differing age. *Int J Environ Sci Technol*. 2013;10(3):607-610. doi:10.1007/s13762-013-0185-4

28. El-Sheikh ESA. Comparative toxicity and sublethal effects of emamectin benzoate, lufenuron and spinosad on Spodoptera littoralis Boisd. (Lepidoptera: Noctuidae). *Crop Protection*. 2015;67:228-234. doi:10.1016/j.cropro.2014.10.022

29. Pankaj, Sharma A, Gangola S, Khati P, Kumar G, Srivastava A. Novel pathway of cypermethrin biodegradation in a Bacillus sp. strain SG2 isolated from cypermethrin-contaminated agriculture field. *BioTech*. 2016;6(1):45. doi:10.1007/s13205-016-0372-3

30. Deng L, Chen L, Guan S, et al. Dissipation of emamectin benzoate residues in rice and rice-growing environments. *Molecules*. 2020;25(3):483. doi:10.3390/molecules25030483

31. Mahapatra B, Adak T, Patil NKB, et al. Imidacloprid application changes microbial dynamics and enzymes in rice soil. *Ecotoxicology and Environmental Safety*. 2017;144:123-130. doi:10.1016/j.ecoenv.2017.06.013

32. Hayat W, Zhang Y, Hussain I, et al. Efficient degradation of imidacloprid in water through iron activated sodium persulfate. *Chemical Engineering Journal*. 2019;370:1169-1180. doi:10.1016/j.cej.2019.03.261

33. Yuan J, Guo J, Wang H, Guo A, Lian Q, Gu Z. Acute toxicity of cypermethrin on the juvenile of red claw crayfish Cherax quadricarinatus. *Chemosphere*. 2019;237:124468. doi:10.1016/j.chemosphere.2019.124468

34. Tarla DN, Erickson LE, Hettiarachchi GM, et al. Phytoremediation and bioremediation of pesticide-contaminated soil. *Applied Sciences*. 2020;10(4):1217. doi:10.3390/app10041217

35. Lakshmi KV, Sudhikumar AV, Aneesh EM. Synergistic effect of Croton bonplandianum Baill. with cypermethrin and lambda-cyhalothrin against Aedes aegypti Linn, a Dengue fever vector. *South African Journal of Botany*. 2021;140:103-109. doi:10.1016/j.sajb.2021.03.034

36. Woodman SG, Steinkey D, Dew WA, Burket SR, Brooks BW, Pyle GG. Effects of sertraline on behavioral indices of crayfish Orconectes virilis. *Ecotoxicology and Environmental Safety*. 2016;134:31-37. doi:10.1016/j.ecoenv.2016.08.011

37. Yu J, Xu E, Li W, et al. Acute toxicity of an emerging insecticide pymetrozine to Procambarus clarkii associated with rice-crayfish culture (RCIS). *IJERPH*. 2018;15(5):984. doi:10.3390/ijerph15050984