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Quaternary ammonium cationic surfactants increase bioactivity of indoxacarb on pests and toxicological risk to *Daphnia magna*

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ABSTRACT

Agricultural researchers have always been pursuing synergistic technique for pest control. To evaluate the combined effects of quaternary ammonium compounds (QACs) and indoxacarb, their independent and joint toxicities to two insects, *Spodoptera exigua* and *Agrotis ipsilon*, and the aquatic organism, *Daphnia magna*, were determined. Results showed that all of five tested QACs increased the toxicity of indoxacarb to *S. exigua* and *A. ipsilon*. Both of benzyldimethyltetradecylammonium chloride (TDBAC) and benzododecinium chloride (DDBAC) exhibited significantly increased toxicities to *S. exigua* with synergic ratios of 11.59 and 6.55, while that to *A. ipsilon* were 2.60 and 3.45, respectively. When exposed to binary mixtures of QACs and indoxacarb, there was synergism on *D. magna* when using additive index and concentration addition methods, but only TDBAC, STAC and ODDAC showed synergistic effect in the equivalent curve method. The results indicate that the surfactants can be used as the synergists of indoxacarb in the control of Lepidoptera pests. However, their environmental risks should not be neglected owing to the high toxicity of all mixtures of indoxacarb and five QACs to *D. magna*.

1. Introduction

Quaternary ammonium compounds (QACs) are molecules with at least one hydrophobic long alkyl chain that is attached to a positively charged nitrogen atom (Nałęcz-Jawecki et al., 2003). They are regarded as high consumption chemicals in the list of Organization for Economic Co-operation and Development (OECD) (Tezel, 2009) and are widely used to make emulsifiers, fabric softeners, corrosion inhibitors and surfactants in the textile industry (Di Nica et al., 2017). Moreover, they are used as personal care products and disinfectants in healthcare (García et al., 2001; Patrauchan and Oriel, 2003; Sütterlin et al., 2008). Research and application of QACs in agriculture also increased in recent years owing to their significant effect on sterilization and disinfection. Several QACs exhibited high antifungal activity on *Fusarium oxysporum* (Nel et al., 2007), *Sclerotinia sclerotiorum* (Yu et al., 2008), *Botrytis cinerea* (Chen et al., 2007) or other plant pathogenic fungi. However, whether QACs can be used as insecticides or as synergists of insecticide is rarely reported (Liu et al., 2011).

QACs are mainly generated by the textile industry and the health industry, and then become ubiquitous contaminants in sewage and wastewater. As reported by Tezel (2009), approximately 25% of the QACs consumed annually are directly discharged into the environment without appropriate disposal. Tremendous threats brought by QACs should not be neglected because their worldwide annual consumption was reported to be as large as 500,000 t in 2004 (Zhu et al., 2010). In agricultural ecosystem, these QACs can leach into the soil, drift into water and thus threaten the ecosystem (Tezel, 2009; Utsunomiya et al., 1989). Biodegradation peculiarities of QACs determine whether they can cause long-term influence on the environment. However, all of the QACs with different molecular structures are hard to biodegrade because large carbon chain will enhance the stability of QACs (Chen et al., 2003). In addition, for most QACs, their presence may decrease the biodegradation efficiency of linear alkylbenzene sulfonates (Kümmerer et al., 1997). Thus, the biocidal activity of QACs is a serious potential threat to environmental organisms and also aquatic ecosystems. Yu et al. (2012) and Wang et al. (2006) have reported, the growth and vitality of various algae, such as *Chlorella vulgaris*, *Scenedesmus obliquus*, *Alexandrium tamarense* and *Heterosigma akashiwo*, could be strongly inhibited by QACs. Meanwhile, QACs are toxic not only to bacteria but also to protozoa, crustaceans and other non-target organisms (García et al., 2001; Hrenovic et al., 2008; Nałęcz-Jawecki et al., 2003). *Daphnia magna* is a very sensitive organism upon chemicals and thus is

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regarded as a recommended model organism in the environmental toxicity assessments of chemicals in OECD guidelines (OECD, 2004). Lithner et al. (2009) have used *D. magna* as a model organism to screen plastic consumer products with low toxicity. Syberg et al. (2008) and Barata et al. (2006) have reported the mixture toxicity of toxicants to *D. magna*. But the toxicities of QACs to *D. magna* were barely reported.

Numerous methods have been proposed for testing joint effects and interactions of chemical mixtures, such as concentration addition (CA), independent action (IA), accelerated failure time mode (AFT) and so on (Qiu et al., 2017; Zhu et al., 2016; Cedergreen, 2014; Altenburger et al., 2012; Belden et al., 2007; Deneer, 2000). Among them, additive index (AI) method (Marking, 1977) and equivalent curve method (Boillot and Perrodin, 2008; Calamari and Alabaster, 1980; Altenburger et al., 1990), are always recommended in the joint toxicity test. AI is mathematically calculated using the equation $S = (A_m/A_i) + (B_m/B_i)$, where A_i and B_i are the independent toxicities of component A and B, respectively; A_m and B_m are the toxicities of A and B in the mixture that give the same effect of A_i and B_i; and S is the joint toxicity of the mixture (LC_{50} or EC_{50} values are usually used for these calculations). Generally, when $S \leq 1$, the equation AI = (1/S)–1.0 was used for calculation; otherwise, AI = (−1) S + 1.0. The final judgment of the joint toxicity was the value of AI; when AI > 0, AI = 0 and AI < 0, the combined effect was defined as synergism, simple addition and antagonism, respectively. In equivalent curve method, independent toxicities of two ingredients A and B in the mixtures are first tested, and the EC_{50} values and 95% confident intervals are determined. Subsequently, EC_{50} values and confident intervals of A and B are marked on the horizontal and vertical coordinate axes respectively, as shown in Fig. 1. Then, the data points of EC_{50} values, lower limit and upper limit of 95% confident intervals are connected. The labelled red dots represent where the joint toxicities of these two chemicals in combination fall. In general, if the red point site between the two lines of confident intervals, it represents additive effect; if it site under the lower line, then it means synergistic effect; similarly, it indicates antagonistic effect when the red point site above the upper line. In the current study, we intended to compare the two methods in assessing the joint toxicity of indoxacarb and QACs.

Indoxacarb, an oxadiazine pesticide, works as a sodium channel blocker and thus results in paralysis and death of targeted pests. It has been reported to have favorable efficacy in controlling a number of Lepidoptera as well as certain Homoptera and Coleoptera insects and exhibits low environmental risk and mammalian toxicity (Wing et al., 2000). Indoxacarb is proved relatively stable at 20 °C and pH 7, with the aqueous hydrolysis DT_{50} = 22 days according to International Union of Pure and Applied Chemistry. Furthermore, the half-life of indoxacarb is 7.6 days in the soil at an initial residue of 0.202 mg/kg (Zhou and Li, 2008). As it is applied in agricultural production

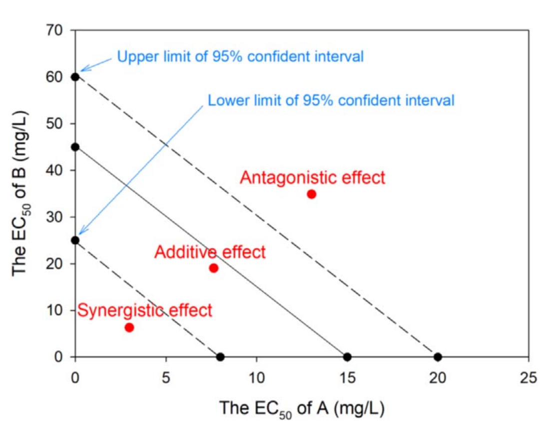

Fig. 1. The joint toxic effects predicted by the equivalent curve method for binary mixture of A and B. The lower limit and upper limit of 95% confident intervals of A and B are marked on the horizontal and vertical coordinate axes respectively, then the data points are connected.

(Brantley and Holmes, 2017), part of the active ingredient would be washed out and drift into the water, and then persist for a long period in the soil and ground water (Fenoll et al., 2014). The objective of the present study was to evaluate the combined effects of indoxacarb and each of five QACs on two target insects following the immersion test for insecticide activity (Busvine, 1980). Besides, the joint toxicity of QACs and indoxacarb to *Daphnia magna* was also tested to evaluate their environmental risks. Simultaneously considering the synergism to the pesticide and the joint toxicity to the non-target organisms is the foundation for assessing the prospect of QACs that are applied as synergists of pesticides.

2. Materials and methods

2.1. Test organisms

A *Spodoptera exigua* (Hübner) population was collected from Tai'an, Shandong Province, China (Site: 36.18°N, 117.13°E) during October 2012, and an *Agrotis ipsilon* (Rottemberg) population was obtained from laboratory culture at the Key Laboratory of Pesticide Toxicology and Application Techniques in Shandong Agricultural University, Tai'an, Shandong Province, China. The moths of two insects were kept in cages with meshed sides to maintain ventilation at toxicity test laboratory. The adults were fed on a solution containing sucrose (100 g/L) and a vitamin C (ascorbic acid) solution (20 ml/L) in a soaked cotton wool ball. All of the larvae were fed a diet that was recommended by Mu et al. (2002). The 3rd instar larvae were used in the test.

The *D. magna* was the pure strain introduced from the Research Center for Pesticide Environmental Toxicology (Beijing, China). Glass beakers (2-L capacity) were kept with ground water from Tai'an, Shandong Province, China (Site: 36°15′17″N, 117°06′15″E) with pH of 6–9, hardness of 140–250 mg/L and the dissolved oxygen concentration is more than 4.0 mg/L even at the end of the test (valid according to OECD Guideline 202). The *D. magna* were fed daily with the algae *Scenedesmus obliquus* at a concentration of 3×10^5 cells/ml. The tested *D. magna* were younger than 24 h old.

2.2. Test chemicals

Five QACs were tested in the present study. Benzyldimethyltetradecylammonium chloride (TDBAC), benzododecinium chloride (DDBAC) and octyl-decyl dimethyl ammonium chloride (ODDAC) (diluted with distilled water to yield 45% stock solutions (450,000 mg/L) for the sake of convenience) were purchased from Shandong Taihe Water Treatment Co., Ltd (Shandong, China). N-Hexadecyltrimethylammonium chloride (CTAC) and stearyltrimethylammonium chloride (STAC), supplied by Jintong Letai Chemical Product Co., Ltd(Beijing, China), were made into 70% stock solutions (700,000 mg/L) using distilled water. Technical material of indoxacarb with a high purity of 98% was supplied by Rainbow Chemical Co., Ltd (Shandong, China), and then it was fabricated to a 15% stock suspension using Tween-80 0.1% aqueous solution (the average diameter of technical particles was approximately 1 µm). CAS numbers and chemical structural formulas of five QACs and indoxacarb were listed in Table S1 (Supporting Information).

2.3. Acute independent toxicity on Daphnia magna

The acute toxicity test was carried out in accordance with OECD guidelines (OECD, 2004) to determine the mobility of *D. magna*. The toxicants were diluted with groundwater (pH, hardness and other quality parameters can be found in 2.1 section) in toxicity test. After the pre-test, five or more concentrations and a control treatment with only ground water were designed in the indoxacarb and QACs independent toxicity test. The diluents with indoxacarb concentrations of 0.005, 0.05, 0.1, 0.5 and 1 mg/L were used for the official tests of *D. magna*. All