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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

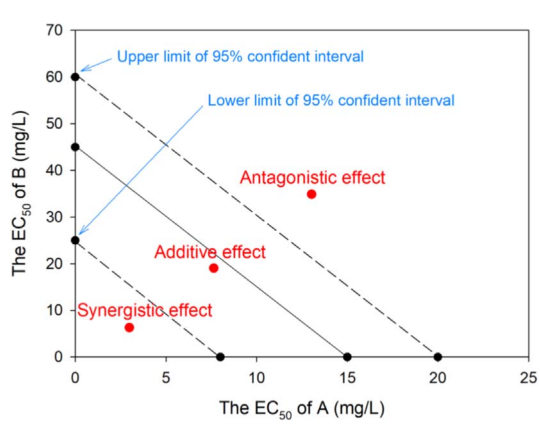

Fig. 1. The joint toxic effects predicted by the equivalent curve method for binary mixture of A and B. The lower limit and upper limit of 95% confident intervals of A and B are marked on the horizontal and vertical coordinate axes respectively, then the data points are connected.

(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 of the concentrations of the five tested QACs used in the official tests were the same, being 0.001, 0.005, 0.01, 0.05 and 0.1 mg/L, respectively. Ten neonatal *D. magna* were added in 50 ml of dilution. Test organisms were not fed during the 48 h-toxicity testing. The test vessels were maintained at 20 ± 1 °C and a photoperiod of 16:8 h (L:D) until the end of experiment. The examined endpoint was immobilisation, which was defined as the inability to swim after 15 s of gentle agitation. The measurements were repeated in triplicate with 30 *D. magna* for each concentration of chemicals. The pH, hardness and dissolved oxygen concentration were measured before and every 24 h after chemical exposure.

2.4. Acute joint toxicity on Daphnia magna

In joint toxicity test, the concentrations of each treatment were designed on the basis of their independent toxicities to *D. magna*. Additive index (Marking, 1977) and the equivalent curve (Boillot and Perrodin, 2008; Calamari and Alabaster, 1980; Altenburger et al., 1990) were recommended in the joint toxicity test of indoxacarb (a sodium channel blocker) and QACs (a permeability-increasing agent) in the current study. Joint toxicities of indoxacarb and QACs were also assessed using concentration addition method as a reference according to a previous protocol (Zhu et al., 2016). When the ratio of experimental EC_{50} value of a mixture to EC_{50} value predicted by concentration addition is greater than 1, then it indicates antagonism; otherwise it means synergism. We designed the joint concentrations based on one toxic unite, which was defined as the ratio 1:1 of EC_{50}s of indoxacarb (A) and QACs (B). Concentrations in joint toxicity testing were determined as $0.125 \times (EC_{50-A} + EC_{50-B})$, $0.25 \times (EC_{50-A} + EC_{50-B})$, $0.50 \times (EC_{50-A} + EC_{50-B})$, $0.75 \times (EC_{50-A} + EC_{50-B})$ and $1.00 \times (EC_{50-A} + EC_{50-B})$ to calculate the toxicity of the mixture.

2.5. Increased toxicities of indoxacarb + QACs on S. exigua and A. ipsilon

Bioassays were conducted on newly emerged third-instar larvae of *S. exigua* and *A. ipsilon* from laboratory cultures using the standard method of the immersion test for insecticide activity (Busvine, 1980). General concentrations rational for official tests were determined based on preliminary range-finding tests. Then diluents with indoxacarb concentrations of 1, 5, 10, 25, 50 and 75 mg/L were used for the official tests of *S. exigua*, whereas that of 10, 20, 40, 60 and 80 mg/L were used for *A. ipsilon* treatments. For the evaluation of indoxacarb + QACs, the concentration of QACs was kept at 450 mg/L, a typical concentration used in agricultural production (Liu et al., 2011), whatever indoxacarb concentrations. Simultaneously, deionized water without any chemicals was regarded as blank control. The larvae were placed in a dip net (5-cm diameter) and then dipped into the tested solutions for 5 s. After the insects were allowed to dry between two pieces of filter paper, they were transferred to 24-cell culture plates (Zhang et al., 2016). Fresh cabbage leaves without any contaminants were used to feed the larvae while the temperature and relative humidity were maintained at 27 ± 1 °C and 65 ± 10%, respectively (with a photoperiod of L:D = 14:10 h). The mortality was assessed after 48 h, and a larva was considered dead if it did not move when prodded with a camel hair brush. To enhance the experimental precision, each experiment was repeated in triplicate and 72 larvae were used in each concentration. The synergic ratios of QACs to indoxacarb were calculated as the LC_{50} values of indoxacarb to those of indoxacarb + QACs (Liu et al., 2011).

2.6. Data analysis

The EC_{50} values were calculated by the probit regression of the *Daphnia* immobilisation rates against the log10 values of chemical concentrations. All of the statistical analyses were performed using the SPSS statistical package (ver.17.0, USA).

Table 1
Limit of detection (LOD) and limit of quantification (LOQ) five quaternary ammonium cationic surfactants and indoxacarb.

Chemicals	Calibration range (μg/L)	Calibration curve (y =)	LOD (μg/L)	LOQ (μg/L)
DDBAC	0.1–100	1320.0x − 73.453	0.2	0.5
TDBAC	0.1–100	1137.2x + 28.227	0.2	0.5
CTAC	0.1–100	1273.9x + 97.779	0.4	1.5
STAC	0.1–100	1341.8x + 30.069	0.4	1.5
ODDAC	0.1–100	1360.1x − 178.94	0.2	0.5
Indoxacarb	0.01–10	2525.5x − 40.105	1.0	3.0

TDBAC is benzyldimethyltetradecylammonium chloride; DDBAC isbenzododecinium chloride; ODDAC is octyl-decyl dimethyl ammonium chloride; CTAC is N-Hexadecyltrimethylammonium chloride; and STAC is stearyltrimethylammonium chloride.

3. Results and analysis

3.1. Validation of the acute test on D. magna

Chemical analyses of exposure medium contamination (i.e. indoxacarb and five QACs) were determined using HPLC method (Table S2, Supporting Information). Reliable analytical methods for the quantification of the tested substances in the test solutions with limit of detections and limit of quantifications were listed in Table 1. Fortunately, all the measurements provided favorable basic conditions for further toxicity tests. To assess contamination accuracy, indoxacarb and QACs analyses were performed for two samples with the lowest and the highest concentrations at the beginning and end of the test according to OECD Guideline 202. The results showed that measured concentrations varied generally less than 10% for indoxacarb and 5% for QACs from the nominal concentrations (Table S3, Supporting Information). As the Guideline describes, if evidence is available to demonstrate that the concentration of the test substance has been satisfactorily maintained within 20% of the nominal or measured initial concentration throughout the test, then the results can be based on nominal or measured initial values. So, all calculations were based on nominal concentrations in the current study in order to simplify calculations. At the end of experiments, controls in each treatment were observed no more than 10% of the *D. magna* be immobilized. The pH values of dilutions were 7.5 ± 0.5 while the hardness was kept at 140–250 mg/L and the dissolved oxygen concentrations were more than 4.0 mg/L. All the mentioned performance criteria were favorable for the acute tests to be valid according to OECD Guideline 202 on "*Daphnia* sp., Acute Immobilisation Test and Reproduction Test".

3.2. Independent toxicity of QACs and indoxacarb on D. magna

As shown in Table 2, the 48-h EC_{50} of indoxacarb on *D. magna* was 2.37×10^{-2} mg/L, which indicates an extremely high toxicity level.

Table 2
Independent toxicity of QACs or indoxacarb on waterflea (*Daphnia magna*).

Chemicals	48 h-EC_{50} and 95% CL (mg/L)	Regression	R^2
DDBAC	6.61×10^{-3} (3.09×10^{-3}–1.21×10^{-2})	$Y = 0.827X + 1.802$	0.922
TDBAC	8.27×10^{-3} (4.19×10^{-3}–1.49×10^{-2})	$Y = 0.854X + 1.780$	0.858
CTAC	1.49×10^{-2} (6.19×10^{-3}–4.05×10^{-2})	$Y = 0.593X + 1.084$	0.818
STAC	1.19×10^{-2} (5.54×10^{-3}–2.57×10^{-2})	$Y = 0.698X + 1.343$	0.745
ODDAC	2.85×10^{-2} (1.44×10^{-2}–7.77×10^{-1})	$Y = 0.718X + 1.109$	0.914
Indoxacarb	2.37×10^{-2} (1.25×10^{-2}–3.87×10^{-2})	$Y = 1.105X + 1.791$	0.941

Table 3
Joint toxicity of QACs and indoxacarb on waterflea (*Daphnia magna*).

Treatments	48 h-EC_{50} and 95% CL (mg/L)		AI	Joint toxicity
	QACs	Indoxacarb		
Indoxacarb + DDBAC	1.94×10^{-3} (1.42×10^{-3}–2.48×10^{-3})	6.97×10^{-3} (5.11×10^{-3}–8.90×10^{-3})	0.702 (0.15–1.3)	synergism
Indoxacarb + TDBAC	1.38×10^{-3} (8.39×10^{-4}–1.85×10^{-3})	3.95×10^{-3} (2.41×10^{-3}–5.30×10^{-3})	1.998 (1.54–2.83)	synergism
Indoxacarb + CTAC	4.98×10^{-3} (3.64×10^{-3}–6.46×10^{-3})	7.92×10^{-3} (5.79×10^{-3}–1.03×10^{-2})	0.496 (−0.05–1.35)	synergism
Indoxacarb + STAC	2.95×10^{-3} (2.02×10^{-3}–3.86×10^{-3})	5.88×10^{-3} (4.01×10^{-3}–7.67×10^{-3})	1.016 (0.46–1.87)	synergism
Indoxacarb + ODDAC	7.27×10^{-3} (4.65×10^{-3}–9.79×10^{-3})	6.04×10^{-3} (3.87×10^{-3}–8.14×10^{-3})	0.961 (0.58–3.49)	synergism

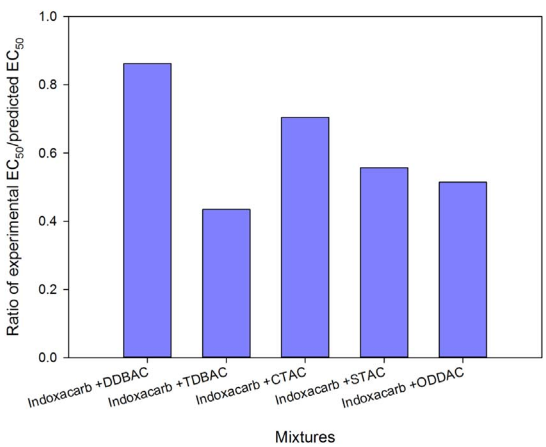

Fig. 2. The ratios of experimental EC_{50}/predicted EC_{50} of the mixtures calculated using concentration addition model.

The 48-h EC_{50} values of the five QACs ranged from 6.61×10^{-3} to 2.85×10^{-2} mg/L. Single long-chain alkyl benzyl ammonium chloride showed the higher toxicities than the other two series of QACs, with the DDBAC and TDBAC exhibited extremely high toxicity level, followed by STAC, CTAC and ODDAC which were high toxicity level.

3.3. Joint toxicity of QACs and indoxacarb on D. magna

Joint toxicities of five of the QACs and indoxacarb on *D. magna* were evaluated by the AI model (Table 3), CA model (Fig. 2) and the equivalent curve model (Fig. 3). AI method results indicated that when QACs and indoxacarb were combined with one toxic unit, all of five mixtures exhibited synergism on *D. magna*. Single long-chain alkyl benzyl ammonium chloride showed the highest toxicity (at EC_{50} level) when combined with indoxacarb, with TDBAC gaining the highest AI value of 1.998, then was STAC, ODDAC, DDBAC and CTAC. Results of CA model showed similar tendency (Fig. 2). The mixture of indoxacarb + TDBAC yielded the lowest ratio of experimental EC_{50}/predicted EC_{50} of the mixture, namely the highest synergism.

The equivalent curves illustrated different joint toxicities (at EC_{50} level) of five QACs with indoxacarb on *D. magna* (Fig. 3). Three out of five QACs, TDBAC (Fig. 3b), STAC (Fig. 3d) and ODDAC (Fig. 3e), displayed synergistic effect combined with indoxacarb on *D. magna* in the equivalent curve method. The other two QACs revealed additive effect on *D. magna*.

3.4. Increased toxicities of QACs + indoxacarb in controlling S. exigua and A. ipsolon

At the end of all tests, no mortality of insects was observed for the control with distilled water only. It was also demonstrated that all the five QACs with the concentration of 450 mg/L had no lethal bioactivity upon *S. exigua* and *A. ipsolon*. As Fig. 4 shows, the magnitudes of the increased toxicities differed among different types of surfactants. All of the five QACs increased the toxicity of indoxacarb to *S. exigua* and *A. ipsolon*, with synergistic ratios ranging from 1.27 to 11.59 and 1.68 to 3.45, respectively. Among them, DDBAC and TDBAC presented the highest synergistic ratios to indoxacarb in controlling two Lepidoptera pests. Besides, the synergistic ratios to *S. exigua* is much higher than that to *A. ipsilon* with the synergistic ratios being greater than 6.5, while the synergistic ratios of the latter are only around 3.0. ODDAC combined with indoxacarb was more effective in controlling *S. exigua* than *A. ipsilon*.

4. Discussion

The overuse of pesticides has received extensive attention in the last few decades for the sake of its side effect on animals, environment and ecosystem. People then carried out various strategies to cut down its consumption in order to deal with the severe issue. As a powerful solution to reduce pesticide consumption, Ministry of Agriculture of the People's Republic of China had formulated a normalized file named "The implementation of a zero-growth action of pesticides by 2020" in January 2015 (MoA of China, 2015). As a result, agricultural researcher, producer, seller and user of pesticide gave more attention to the scientific and efficient application of pesticides. Synergistic technique tends to be one of the most promising strategies among the numerous proposed projects in the special file. Synergistic techniques in agricultural pest control always referred to use a mixture of chemicals at synergistically effective amounts or employ a synergistic agent to promote the wettability, retention and penetration of active ingredients, and thus yielded enhanced validity or bioactivity (Chang et al., 2015). To develop and assess potential chemicals with obvious synergistic effect would play an important role in the plan.

Liu et al. (2011) reported that DDBAC can be used as a synergist for insecticides to control *Spodoptera exigua* Hübner. In the current study, we demonstrated that all of the five tested QACs could increase the toxicity of indoxacarb to both *S. exigua* and *A. ipsilon*, especially TDBAC and DDBAC, which 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. This increase was very closely related to the molecular geometry of surfactants, especially the alkyl chain length and the alkyl chain number linked to the nitrogen atom. As previously reported (Liu et al., 2011), the contact toxicity of DDBAC and ODDAC as synergists to abamectin and chlorfenapyr presented a synergism on *S. exigua*, and the ODDAC with two alkyl chains was weaker than the DDBAC with only one alkyl chain in the synergistic effect. The results were consistent with those of on indoxacarb to *S. exigua* and *A. ipsilon* in present study. However, the opposite results were shared for the chlorpyrifos and the beta-cypermethrin to *S. exigua* (Liu et al., 2011). The alkyl chain length played a decisive role in changing the synergism to insecticides for the single alkyl chain benzyl ammonium chloride and the single alkyl chain trimethyl ammonium chloride. With the increased carbon chain length, the synergism was gradually enhanced.

Compared with the other two series of QACs, single alkyl chain benzyl ammonium chloride showed the highest synergistic ratios to indoxacarb on *S. exigua*. Research indicates the possibility of QACs being toxic to microorganisms by way of the QACs molecule being

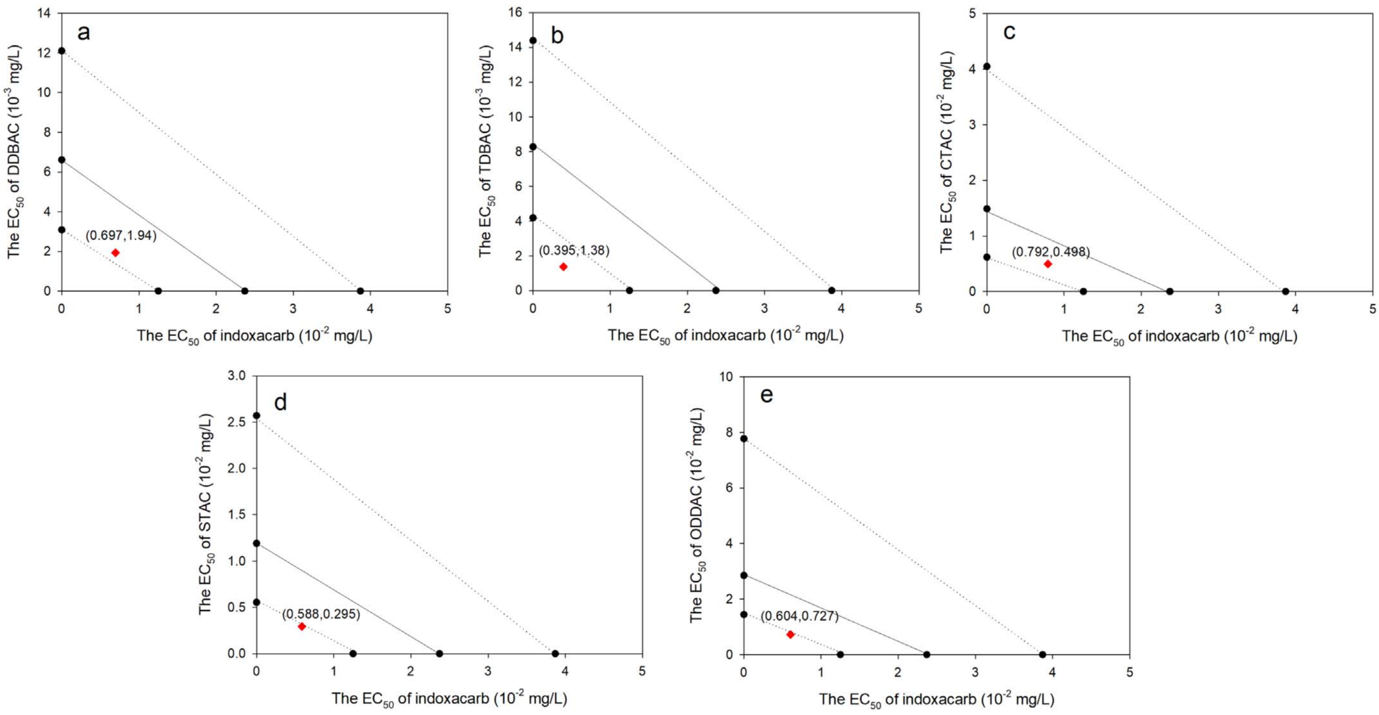

Fig. 3. The joint toxic effects (at EC_{50} level) predicted by the equivalent curve method for binary mixture of indoxacarb and the five QACs. DDBAC is benzododecinium chloride; TDBAC is benzyldimethyltetradecylammonium chloride; ODDAC is octyl-decyl dimethyl ammonium chloride; CTAC is N-Hexadecyltrimethylammonium chloride; and STAC is stearyl-trimethylammonium chloride. The upper and lower dotted lines represent the 95% confidence intervals, and that the labelled red dots (coordinates were described in brackets) represent where the joint toxicities of these two chemicals in combination fall.

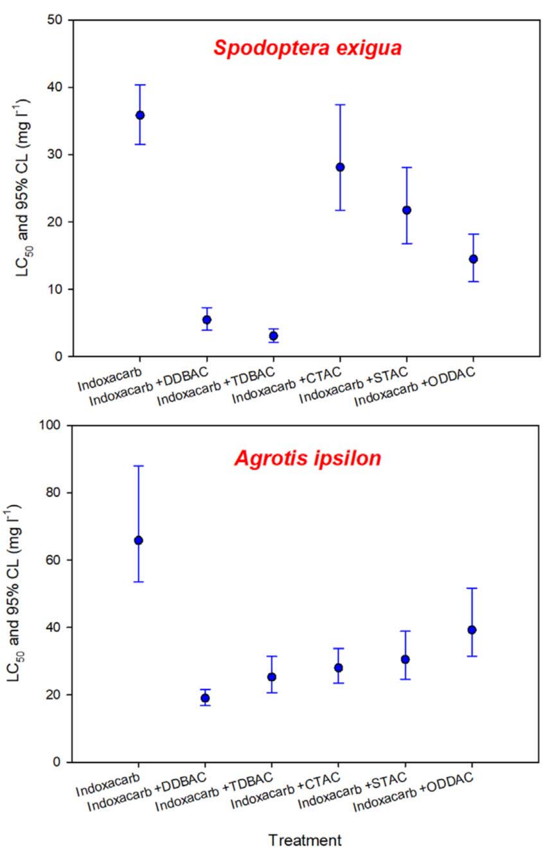

Fig. 4. LC_{50}s and 95% confidence limits of indoxacarb with or without an addition of QACs for beet armyworm (*Spodoptera exigua*) and black cutworm (*Agrotis ipsilon*).

combined with the cell membrane and then changing the cell membrane permeability to lead to exosmic cell content. Girling et al. (2000) reported that different failure modes and sites of action of surfactants on a lipid bilayer structure can result from different alkyl chain lengths of the surfactant. Therefore, we deduced that the higher toxicity of single alkyl chain benzyl ammonium chloride on pests and aquatic organisms is related to the benzyl in the molecule of these QACs. The benzyl in the molecule will improve the liposolubility and thus make it easier to damage the lipid bilayer and accelerate the leakage of the cell content or the combination with the structural protein, which may be directly toxic or enhance the toxicity of the insecticides by increasing their penetration. As Wei et al. (2013) reported, the inhibition of single alkyl chain trimethyl ammonium chloride and single alkyl chain benzyl ammonium chloride on cell proliferation will be enhanced as the alkyl chain length increases.

The QACs with a longer carbon chain showed a stronger synergism in the experiment of *S. exigua*, while those with a shorter carbon chain length had a higher synergic ratio for *A. ipsilon*. Gong et al. (1986, 1988) found a special characteristic of the integument structure of *A. ipsilon*. Only a small number of wax channels can be connected to the body surface caused by the structure of the thicker epidermis and heterogeneous epicuticle. Meanwhile, with the rare channels in the chitin lamella, the penetration resistance of hydrophobic chemicals to the epidermis was produced. The water solubility of indoxacarb was very low, with a solubility of 0.2 mg/L in water at 20 °C. Therefore, we inferred that the lower toxicity of indoxacarb on *A. ipsilon* than *S. exigua* was related to the penetration resistance ability of the integument structure for the former. This point can be supported by the results that TDBAC, STAC and ODDAC with longer alkyl chains in the molecule showed lower synergisms than DDBAC and CTAC did when combined with indoxacarb.

Utsunomiya et al. (1997) and Lewis (1990) reported a lower toxicity of double alkyl chain QACs than the single alkyl chain QACs on algae. Studies showed that in acute toxicity tests, the toxicity of QACs on the *Photobacterium* decreased significantly with the increased number of alkyl chains linked to the nitrogen atom (Cheng and Jiang, 2006), similar to *Chlorella pyrenoidosa* and *Scenedesmus quadricauda* (Jing et al., 2012). In our study, ODDAC with two alkyl chain also showed a lower single toxicity to *D. magna* compared with other surfactants.

Delicate difference could be seen in two different methods applied to assess the joint toxicities of mixtures of indoxacarb and five QACs on *D. magna*. Overall, equivalent curve method is more conservative than the additive index method in evaluating the synergism of QACs on *D. magna*. As the range of additive effect in AI method was limited, all five QACs displayed synergistic effect on *D. magna*. However in the equivalent curve method, CTAC and DDBAC with the weaker synergism in the former method were additive effect here. But the TDBAC revealed the most significant synergistic effect, with the STAC and ODDAC followed in both methods. The high potential risks of QACs to aquatic lives were consistent with previous reports on *Aliivibrio fischeri* (Carbajo et al., 2015; Di Nica et al., 2017). In addition, our results indicated that the independent toxicity and joint toxicity were closely related to the carbon chain length which is the decisive factor in improving the joint toxicity. The toxicity of QACs on *D. magna* decreased with the increased alkyl chain number linked to the nitrogen atom; the same result was generated for *Photobacterium*, as Cheng and Jiang (2006) reported. In 1989, the concentrations of free and complex linear alkylbenzene-sulfonates in the influent sewage and the river water in Japan had already reached 0.37–4.1 mg/L and 0.09–0.58 mg/L, while the total annually production of cationic surfactants was approximately seventy thousand tons (Utsunomiya et al., 1989, 1997). In 2004, worldwide annual consumption of QACs was reported to be as large as 500,000 t and now its consumption may continue to increase (Zhu et al., 2010). Thus, tremendous threats brought by QACs should not be neglected.

5. Conclusions

In conclusion, the present research demonstrated that the most effective QACs that were applied to indoxacarb as synergists provided the highest single toxicity and joint toxicity on *D. magna*. Equivalent curve method is more conservative than the additive index method in evaluating the synergism of QACs on *D. magna*. When we seek for synergists to promote control efficacy in managing pests, their environmental risks, especially risks to aquatic organisms should also not be neglected.

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Supporting information

Table S1 displayed the CAS numbers and chemical structural formulas of five QACs and indoxacarb. Detailed information about chemical analyses of indoxacarb and five QACs were listed as Table S2. Residues of the lowest and highest concentrations of five QACs and indoxacarb before and after toxicity test were listed in Table S3.

Declaration of interest statement

The authors declare no competing financial interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.11.038.

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