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Article

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A specific synergist for neonicotinoid insecticides: IPPA08, a *cis*-neonicotinoid compound with unique oxabridged substructure

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Abstract

Insecticide synergists are key components to increase the control efficacy and reduce active ingredient use. Here we describe a novel insecticide synergist with activity specific for insecticidal neonicotinoids. The synergist IPPA08, a *cis*-configuration neonicotinoid compound with unique oxabridged substructure, could increase the toxicity of most neonicotinoid insecticides belonging to IRAC 4A subgroup against a range of insect species, although IPPA08 itself was almost inactive to insects at synergistic concentrations. Unfortunately, similar effects were observed on the honey bee (*Apis mellifera*) and the brown planthopper (*Nilaparvata lugens*) resistant to imidacloprid. IPPA08 did not show any effects on toxicity of insecticides with different targets, which made us to define it as a neonicotinoid specific synergist. Unlike most insecticide synergists by inhibiting activities of detoxification enzymes, IPPA08 showed no effects on enzyme activities. The results revealed that IPPA08 worked as a synergist through a distinct way. Although the modulating insect nicotinic acetylcholine receptors (nAChRs, targets of neonicotinoid insecticides) was supposed a possible mode of action for IPPA08 as a neonicotinoid specific synergist, direct evidences are needed in further studies. In insect pest control, IPPA08 acts as a target synergist to increase neonicotinoid toxicity and reduce the amount of neonicotinoid used. Combinations of IPPA08 and insecticidal neonicotinoids may be developed into new insecticide formulations. In summary, combining an active ingredient with a ‘custom’ synergist appears to be a very promising approach for the development of effective new insecticide products.

Key words: neonicotinoid, insecticide synergist, detoxification enzyme, nicotinic acetylcholine receptors

Introduction

Insect pests threaten human welfare through food destruction and disease transmission. Insecticides are the primary means used to control most of insect pests. Neonicotinoid is the largest insecticide now and have been playing an important role in crop protection and public health since the introduction of imidacloprid in 1990s[1, 2]. The emergence of imidacloprid initiated the splendid era of neonicotinoids with other six neonicotinoids commercialized[3]. Currently, neonicotinoids have been used in more than 120 countries and areas[3], sharing more than 24% of total insecticide market in 2014[4]. However, the superiority of neonicotinoids is also challenged by the development of resistance resulting from their frequent and irrational use[5]. Another major challenge for neonicotinoids is from the toxicity to honey bees, which has led to a re-evaluation of the overall biological safety of neonicotinoids in Europe and elsewhere[6].

To deal with these challenges on neonicotinoids, the first strategy is insecticide structure modification. Neonicotinoid insecticides possess either an electron withdrawing nitro (-NO_2) or cyano (-CN) group, which have been postulated to contribute directly to their selectivity[7]. The -NO_2 or -CN group in all commercial neonicotinoids is in *trans*-configuration. However, some *cis*-configuration neonicotinoid compounds also show good insecticidal activities, which may provide substitutes for imidacloprid and other neonicotinoids, especially in the control of insect pests with high resistance to these *trans*-configuration insecticides[8-10]. Cycloxaprid, discovered by our group, is a *cis*-neonicotinoid with unique oxabridged

47 substructure[10, 11], outstanding activity[12] and good safety profiles[13, 14], which has been
48 newly registrated in China. Insecticide synergists may provide another important
49 choice to deal with problems in insecticide applications, such as decreasing the
50 application amount of insecticide active ingredient, and lowering the resistant levels
51 to insecticides. As a representative example, piperonyl butoxide (PBO) is the most
52 common synergist of insecticides. Since its invention in 1940s[15], PBO makes a great
53 contribution to the public health and the control of household insect pests in the past
54 several decades[16]. Triphenyl phosphate (TPP), diethyl maleate (DEM) and PBO are
55 now the most important synergists on insecticides through inhibiting activities of
56 detoxification enzymes[17-19].

57 In the development of cycloxaprid, its eight-membered homolog, IPPA08 (Fig S1),
58 was synthesized as a comparison. IPPA08 is almost inactive to insects but has the
59 unexpected synergistic effects on traditional *cis*-neonicotinoids. Here we described
60 the finding of IPPA08 as a specific synergist on neonicotinoids, which works in a
61 distinct mode from the synergists inhibiting activities of detoxification enzymes.

62

63 **Materials and Methods**

64 **Chemicals**

65 Piperonyl butoxide (PBO), triphenyl phosphate (TPP) and diethyl maleate (DEM)
66 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dinotefuran was
67 generously provided by Bayer CropSciences K.K. (Yuki, Ibaraki, Japan). Sulfoxaflor
68 (97.0%) and flupyradifurone (92.5%) were generously provided by Wuhan

JINGDING Chemicals Co. Ltd. (Wuhan, China). The representative oxabridged neonicotinoids, cycloxaprid and IPPA08, were synthesized and purified as previous description[11] (Fig. S1). Other insecticides were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solubility of IPPA08 in room temperature is greater than 1 mM (much higher if emulsifier was added) in water and greater than 15 mM in acetone.

Insects and Bioassay

Three field populations of the brown planthopper (*Nilaparvata lugens*) were collected in Guilin (Guangxi, China), Nanjing (Jiangsu, China) and Chainat (Thailand) in September 2011. The bioassay was carried out using the topical application method[20]. If not be specially noted, insects from Nanjing population were used as the material source. The American cockroach (*Periplaneta americana*) was provided by Feitian Medicinal Animal Co. Ltd (Danyang, Jiangsu, China) in September 2011 and bioassay was performed using the topical application method[21]. *Aphis gossypii* was collected in Hangzhou (Zhejiang, China) in September 2013 and bioassay was performed using the aphid-dip bioassay[22]. *Bemisia tabaci* was collected in Dezhou (Shandong, China) in September 2013 and bioassay was performed using the leaf-dipping method[23]. *Musca domestica* was collected in Zhenjiang (Jiangsu, China) in September 2013 and bioassay was performed using the artificial diet method[24]. *Apis mellifera* were purchased from Xishan apiary (Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China) in September 2014 and bioassay was performed following OECD

method for the acute oral toxicity test on honeybees[25]. As the insecticidal sensitivities of tested insect species are different, the concentration of IPPA08 was adjusted according to the insecticides used in the test. The mortality was recorded in 48 h. The data obtained were analyzed using Polo software (LeOra Software Inc., Cary, NC) to determine LD_{50} or LC_{50} values based on the bioassay methods.

Determination of IPPA08 stability in PBS with different pH

Phosphate buffer saline (PBS) was prepared with pH values of 4.0, 7.2 and 9.0. IPPA08 was dissolved in PBS buffer with the final concentrations of 0.1 mM and 1.0 mM, and incubated at room temperature (28±2 °C) for more than 48 h. Chromatographic analysis was performed using an Agilent 1260 series HPLC System (Agilent Technologies, USA) equipped with a photodiode array detector. Zobarx Extend-C18 Column (250 mm × 4.6 mm; 5 μm particle size) was used with column temperature of 30°C. UV detection wavelength was 345 nm. The mobile phase consisted of methanol and H_2O with isocratic elution at a ratio of 50:50 (v/v). The flow rate was held constant (1.0 ml/min) throughout the process and 5 μl of samples were injected. The analysis time was 6 min and the retention time of IPPA08 was 3.8 min.

Activity determination for three detoxification enzymes

Females (50-56 mg) were homogenized in 500-560 μl extraction buffer (0.1 M Tris-HCl, 0.5% Triton X-100, pH 7.8) and the homogenate was centrifuged at 12,000 g for 15 min. The supernatant was used as the enzyme source for the determination of O-demethylase or N-demethylase activity of P450 monooxygenases,

activity of esterases (ESTs) and Glutathione S-transferases (GSTs). Protein concentrations were determined using the bicinchoninic acid (BCA) kit (St. Louis, MO, USA) with bovine serum albumin (BSA) as the standard protein. Enzyme activities were then tested following methods reported before[26, 27].

To determine the influence of IPPA08 on enzyme activity, enzymes from female adults were treated in two different ways. In the first one, each female was treated by IPPA08 at dose of 2.5 ng/female, and the survival insects after 48 h were collected for the preparation of enzyme source. Then the enzyme activities were determined and compared to that of untreated insects. In the second one, the enzyme source was first prepared from untreated females and then incubated with IPPA08 at final concentration of 0.5 mM for 2 h. Then the enzyme activities were determined and compared to that of enzyme source without incubation with IPPA08.

Results

IPPA08 toxicity and its effects on imidacloprid toxicities

IPPA08 toxicity was tested on several insect species and compared to imidacloprid and cycloxaprid, two neonicotinoids. IPPA08 had significantly lower toxicity to all tested insects and only caused mortality at relatively high doses, such as above 4.25 ng/female for *N. lugens* (Fig. 1A) and 25 μg/female for *P. americana* (Fig. 1B). The calculated LD_{50}/LC_{50} values for IPPA08 against tested insect species were at least 10 times greater than that of imidacloprid and cycloxaprid (Table 1).

Although IPPA08 did not show toxicity at low concentrations/doses, it increased

imidacloprid toxicity. For *N. lugens*, 23% mortality by 2 ng/female imidacloprid was increased to 81% when co-applied with IPPA08 at low doses (1.0–4.0 ng/female), at which IPPA08 alone showed no insecticidal activity (Fig. 1A). Similarly in *P. americana*, 19% mortality by 2 μg/female imidacloprid was increased to 74% when co-applied with IPPA08 at low doses (3.0–15.0 ng/female) at which IPPA08 itself caused no mortality (Fig. 1B).

The effects of IPPA08 on imidacloprid toxicities were also tested on other insect species, including *A. gossypii*, *B. tabaci*, *M. domestica* and *A. mellifera*. Similar increases in imidacloprid insecticidal activity were also observed in these species, when imidacloprid was co-applied with IPPA08 at concentrations without direct toxicity (Table 2). Unfortunately, IPPA08 did not show selectivity in its effects on imidacloprid activity against the honey bee (*A. mellifera*).

When testing the influence of IPPA08 on imidacloprid toxicities in *N. lugens* populations with different resistance levels to imidacloprid, it was found that IPPA08 had similar synergistic effects on imidacloprid toxicities (Table 3).

Influence of IPPA08 on toxicities of insecticides from different classes

The influence of IPPA08 on the toxicities of other neonicotinoids was tested, including acetamiprid, thiacloprid, clothianidin, dinotefuran and cycloxaprid. For *N. lugens* (Fig. 2A) and *P. Americana* (Fig. 2B), IPPA08 at low doses (causing no toxicity by itself) increased the mortalities caused by fixed doses (2 ng/female for *N. lugens*, and 2 μg/female for *P. Americana*) of acetamiprid, thiacloprid and clothianidin, which was similar to that on imidacloprid. However, IPPA08 showed

157 little influence on toxicities of dinotefuran and cycloxaprid against both insect
158 species.

159 Based on IRAC Mode of Action Classification Scheme (version 8.1, 2016,
160 http://www.irac-online.org), the above tested insecticides belong to IRAC 4A (Group
161 4, nicotinic acetylcholine receptor competitive modulators). Recently in Group 4,
162 several insecticides were developed belonging to different subgroups, such as
163 sulfoxaflor in Sulfoximines (4C), flupyradifurone in butenolides (4D) and
164 triflumezopyrim in mesoionics (4E). Here the influence of IPPA08 on the toxicities
165 of sulfoxaflor and flupyradifurone was tested, but not including triflumezopyrim
166 because it is difficult to obtain this compound within a short time. In three insect
167 species, *N. lugens* (Fig. 3A), *A. gossypii* (Fig. 3B) and *B. tabaci* (Fig. 3C), IPPA08
168 did not show obvious synergistic effects on sulfoxaflor and flupyradifurone,
169 although the influence on imidacloprid toxicities was significant in these insects.

170 The influence of IPPA08 on the toxicities of insecticides from other classes was
171 also evaluated. IPPA08 had no synergistic effects on insecticides with different
172 targets, such as organophosphates and carbamates acting on insect
173 acetylcholinesterases (AChEs), pyrethroids acting on sodium ionchannels and
174 cyclopentadienes acting on γ-aminobutyric acid (GABA) receptors (Fig. 4).

175

176 **Key properties of IPPA08 as a specific synergist**

177 To exclude the possibility that the synergistic effects were caused by metabolites
178 of IPPA08, such as glutaraldehyde or (nitromethylene)imidazole (NMI), its

hydrolysis was studied. IPPA08 showed the good stability at all test conditions, although a small proportion (1.85-2.10%) was degraded in 51 h at acid condition with pH of 4.0 (Table 4).

Many synergists shows synergistic effects on insecticide toxicities, and the synergistic mode of most synergists is through inhibitiing the activity of detoxification eznymes, such as PBO inhibiting activities of P450 monooxygenases, TPP inhibiting activities of esterases (ESTs), and DEM inhibiting activities of Glutathione S-transferases (GSTs)[17-19]. In order to find out whether IPPA08 had inhibition effects on the acitivities of three important detoxification enzymes, IPPA08 was either applied to insects or incubated with enzyme solutions, and then the changes in enzyme activities were determined. On female adults treated with IPPA08 at dose of 2.5 ng/female, no significant differences in the activities of P450s, ESTs and GSTs were found when compared to untreated control (Table 5). Incubation of enzyme solution with IPPA08 at final concentration of 0.5 mM did not cause obvious changes in enzyme activities too. The results clearly revealed that IPPA08 had distinct mode of action as a synergist.

Discussion

There is an ongoing dilemma between the necessity for chemical insecticide use and the risks from potential adverse effects. Ways to cope with this dilemma include reducing pesticide use, increasing unit activity and selectivity, and reducing the rate of insecticide resistance development[28]. An important strategy is the development of

synergists to increase insecticide toxicity and reduce the amount of active ingredient. Here, we found that IPPA08, a *cis*-neonicotinoid with unique oxabridged substructure, showed significantly synergistic effects on neonicotinoid insecticides, currently the most important class of insecticides. IPPA08 significantly increased toxicities of several neonicotinoid insecticides against a range of insect species, although IPPA08 itself was almost inactive to insects at synergistic concentrations. Unfortunately, the synergistic effects of IPPA08 showed little selectivity among insect species and IPPA08 increased imidacloprid toxicity against honey bees at a level similar to other insect species. IPPA08 also produced similar effects in combination with imidacloprid on several *N. lugens* populations with different imidacloprid resistance levels suggesting that IPPA08 cannot increase susceptibility to neonicotinoids if resistance has already developed.

IPPA08 only showed its synergistic effects on neonicotinoids specifically, but was without any effects on the toxicity of insecticides from other classes, such as organophosphates, carbamates, pyrethroids and cyclopentadienes. Until now, most successful synergists act as inhibitors of metabolic enzymes, such as P450 monooxygenases and other detoxification enzymes[17, 29]. PBO is the most commonly used insecticide synergist[15], which has made a great contribution to the control of malaria mosquito vectors and insect vectors of other diseases. PBO is also commonly used in formulations made for the control of urban pest insects[16]. However, IPPA08 did not show any inhibition on the activity of three important detoxification enzymes, in either treating insects directly by IPPA08 or incubating

223 enzymes with IPPA08. The results indicated that IPPA08 worked as a synergist in a
224 way different from inhibiting activities of detoxification enzymes, and did not
225 obtained its specificity on neonicotinoids through inhibiting activity of some specific
226 enzymes which happens to only metabolize neonicotinoids. Detoxification enzymes
227 play important roles in insecticide resistances, such as P450s monooxygenases in
228 imidacloprid resistance in *N. lugens*[30, 31]. In *N. lugens* with different imidacloprid
229 resistance conferred by the enhance activity of detoxification enzymes, IPPA08
230 showed similar effects to synergize imidacloprid, which also supported the supposal
231 that IPPA08 did not exert its synergism by inhibiting enzyme activities.

232 Neonicotinoids act on insect nicotinic acetylcholine receptors (nAChRs), which
233 play crucial roles in chemical-to-electrical transduction by mediating fast cholinergic
234 synaptic transmission[32]. As a prototypical member of Cys-loop receptors, nAChRs
235 mediate fast cholinergic synaptic transmission in both vertebrate and invertebrate
236 nervous systems and have been investigated as targets of many clinical drugs and
237 insecticides[32]. On mammalian nAChRs, two types of allosteric modulators, NS1738
238 as the representative member of type I modulator and PNU-120596 as type II
239 modulator, can changes the pharmacological properties through modulating the
240 receptor protein, which could be recognized as synergists for the agonist drugs on
241 mammalian nAChRs[33, 34]. IPPA08 may also possess its synergistic effects through
242 modulating insect nAChRs, which conferred its specificity for neonicotinoids acting
243 on insect nAChRs. Of course, such supposal needed direct evidences, which will be
244 in further studies, and the present study only provided some implicit cues for such

supposal. IPPA08 likely only showed obvious effects on neonicotinoids belonging to 4A subgroup in Group 4 of IRAC Mode of Action Classification Scheme (version 8.1, 2016, http://www.irac-online.org), but not significantly synergize neonicotinoids belonging to other subgroups, such as 4C and 4D. Even among neonicotinoids in 4A subgroup, IPPA08 showed little effects on the toxicity of dinotefuran, which may be because of the difference in chemical structures and acting sites on insect nAChRs. In contrast to dinotefuran, all of the other commercial neonicotinoid insecticides in 4A subgroup contain a chlorinated heterocyclic (chloropyridyl or chlorothiazolyl) group[35], and dinotefuran was also thought to act on the distinct site(s) from other neonicotinoids[36]. IPPA08 also showed little effects on the toxicity of cycloxaprid, whose mode of action is still unknown. The -NO_2 or -CN group in all commercial neonicotinoids is in *trans*-configuration, but cycloxaprid has the group in *cis*-configuration and was with unique oxabridged substructure, which may give it possibility to act on distinct site(s) of insect nAChRs[10, 11]. If really through modulating insect nAChRs, IPPA08 may modify the microstructure of binding site(s) for most commercial neonicotinoids in 4A subgroup, which consequently increase the binding affinity of these neonicotinoids on insect nAChRs. However, dinotefuran, cycloxaprid and neonicotinoids belonging to non-4A subgroups may have the distinct site(s) from these neonicotinoids, and IPPA08 modulation may not change the microstructure of this distinct binding site(s).

Conflict of interest

The authors declare no competing financial interest.

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Figure captions

Figure 1. IPPA08 toxicity and its synergistic effects on imidacloprid toxicity. (A) The IPPA08 toxicity and its effects on imidacloprid (2 ng/female) toxicity against *Nilaparvata lugens*. (B) IPPA08 toxicity and its effects on imidacloprid (2 μg/female) toxicity against *Periplaneta americana.* Data are the means of at least six independent experiments ± SEM.

Figure 2. Effects of IPPA08 on the toxicity of neonicotinoid insecticides. (A) The effects in *Nilaparvata lugens*. The dose for each neonicotinoid insecticide was 2 ng/female, and IPPA08 doses were listed in the figure. (B) The effects in *Periplaneta americana*. The dose for each neonicotinoid insecticide was 2 μg/female, and IPPA08 doses were listed in the figure. Data are means of at least six independent experiments ± SEM.

Figure 3. Effects of IPPA08 on the toxicity of sulfoxaflor (Sul) and flupyradifurone (Flu) compared with imidacloprid (Imi). (A) The effects in *Nilaparvata lugens*. The doses for imidacloprid, sulfoxaflor and flupyradifurone were 2.00, 0.25 and 0.25 ng/female, and IPPA08 dose was 2.00 ng/female. (B) The effects in *Aphis gossypii*. The concenrations for three insecticides were 0.50, 0.50 and 0.25 mg/L, and IPPA08 concenration was 0.50 mg/L. (C) The effects in *Bemisia tabaci*. The concenrations for three insecticides were 100, 20 and 20 mg/L, and IPPA08 concenration was 100 mg/L. Data are means of at least six independent experiments ± SEM.

Figure 4. Effects of IPPA08 on the toxicity of insecticides from different classes against *Nilaparvata lugens*. The doses for test insecticides are shown under the

insecticide name. IPPA08 doses were listed in the figure. Data are means of at least six independent experiments ± SEM.

Figure S1. Chemical structures of imidacloprid, cycloxaprid and IPPA08.

Tables

Table 1. Insecticidal activities of three compounds against different insect species

Insect species	Testing method	Neonicotinoids	Slope±SE	LD_{50}/LC_{50} (95% FL)[a]
Nilaparvata lugens	Topical application (ng/female)	Imidacloprid	1.742	7.545 (6.103-9.628)
		Cycloxaprid	1.879	1.016 (0.905-1.143)
		IPPA08		>100
Periplaneta americana	Topical application (μg/female)	Imidacloprid	2.315	12.477 (10.514-15.302)
		Cycloxaprid	1.898	17.003 (15.427-19.138)
		IPPA08		>250
Aphis gossypii	Insect Dipping (mg/L)	Imidacloprid	1.821	1.272 (1.102-1.534)
		Cycloxaprid	1.574	0.913 (0.797-1.068)
		IPPA08	1.328	65.160 (50.395-86.683)
Bemisia tabaci	Leaf Dipping (mg/L)	Imidacloprid	1.433	635.518 (488.361-827.046)
		Cycloxaprid	1.690	94.557 (81.362-110.502)
		IPPA08		>10000
Musca domestica	Artificial diet (g/L)	Imidacloprid	1.646	2.679 (2.375-3.214)
		Cycloxaprid	1.596	1.133 (0.984-1.320)
		IPPA08		>100
Apis mellifera	Oral feeding ($\times10^{-2}$ μg/ worker)	Imidacloprid	2.513	1.542 (1.393-1.726)
		Cycloxaprid	2.408	3.116 (2.872-3.423)
		IPPA08	1.704	36.227 (32.526-41.708)

Data are the means of at least six independent experiments ± SEM. [a] LD_{50} values are provided for *Nilaparvata lugens*, *Periplaneta Americana* and *Apis mellifera*, and LC_{50} values are provided for other insect species, which is the same in the following tables.

Table 2. The synergistic effects of IPPA08 on imidacloprid in different insect species

Insect species	Test method	IPPA08 Con.[a]	Imidacloprid LD_{50}/LC_{50}	95% CI	Slope	SR[b]
Nilaparvata lugens	Topical application ng/female	0	7.545	6.103-9.628	1.742	1.00
		1.25	1.696	1.344-2.157	1.622	4.45
		2.5	1.032	0.854-1.203	1.475	7.31
Periplaneta americana	Topical application μg/female	0	12.477	10.514-15.302	2.315	1.00
		2.5	3.851	3.307-4.572	1.836	3.24
		3.75	2.244	1.956-2.718	1.724	5.56
Aphis gossypii	Insect dipping mg/L	0	1.272	1.102-1.534	1.821	1.00
		1.5	0.361	0.310-0.418	1.633	3.52
		3.5	0.166	0.131-0.215	1.474	7.63
Bemisia tabaci	Leaf dipping mg/L	0	635.518	488.361-827.046	1.433	1.00
		200	164.642	125.271-209.832	1.276	3.86
		600	91.971	73.468-118.570	1.562	6.91
Musca domestica	Artificial diet g/L	0	2.679	2.375-3.214	1.646	1.00
		0.5	0.632	0.542-0.780	1.411	4.24
		1.25	0.445	0.376-0.529	1.358	6.02
Apis mellifera	Oral feeding ($\times10^{-2}$ μg /worker)	0	1.542	1.393-1.726	2.513	1.00
		0.1	0.366	0.323-0.444	1.972	4.21
		0.25	0.231	0.198-0.272	1.765	6.67

[a]IPPA08 at the concentrations showed no insecticidal activities against the test insects. [b]SR, synergism ratio. Data are the means of at least six independent experiments ± SEM.

Table 3. The synergistic effects of IPPA08 on imidacloprid in *Nilaparvata lugens* with different resistance levels to imidacloprid

Population	IPPA08 Con.	Imidacloprid LD_{50}	95% CI	Slope	SR[b]
Guilin (RR[a]=23.25)	0	3.209	2.854-3.706	2.227	1.00
	1.25	0.669	0.581-0.795	1.741	4.80
	2.5	0.425	0.374-0.480	1.790	7.55
Nanjing (RR=54.67)	0	7.545	6.103-9.628	1.742	1.00
	1.25	1.696	1.344-2.157	1.622	4.45
	2.5	1.032	0.854-1.203	1.475	7.31
Chainat (RR=115.38)	0	15.922	14.111-18.294	2.354	1.00
	1.25	3.951	3.326-4.817	1.663	4.03
	2.5	2.095	1.714-2.503	1.527	7.60

[a]RR, resistance ratio; [b]SR, synergism ratio. The test method is the topical application and the unit for LD_{50} values is ng/female. Data are the means of at least six independent experiments ± SEM.

Table 4. The stability of IPPA08 in PBS buffer at different pH

Treatment	pH	Con. (mM)	Peak area I[a] (mAU·s)	Peak area II[b] (mAU·s)	Detection time	Degradation rate (%)
T1	4.0	0.1	425.50±4.59	417.61±4.45	51 h	1.85
T2	4.0	1.0	3500.51±37.55	3427.11±36.72	51 h	2.10
T3	7.2	0.1	437.17±4.63	431.43±4.74	35 d	1.31
T4	7.2	1.0	4133.73±44.54	4094.71±44.51	35 d	0.94
T5	9.0	0.1	447.47±5.03	447.35±4.78	119 h	0.03
T6	9.0	1.0	3629.62±39.24	3613.41±39.17	118 h	0.45

[a]Detection at 0 h after the dissolution of IPPA08 in the buffer. [b]Detection of IPPA08 in the buffer at the time mentioned in the column of Detection time.

Table 5. The influences of IPPA08 on the activities of detoxification enzymes

Enzyme	Insects treated with IPPA08		Enzymes incubated with IPPA08	
	CK	Treatment	CK	Treatment
P450-OD (pmol·min^{-1}·mg^{-1})	7.33±0.66	8.21±1.02	6.84±1.02	6.39±0.86
P450-ND (pmol·min^{-1}·mg^{-1})	13.16±1.65	12.74±2.18	11.47±1.37	11.23±1.23
CarE (μmol·min^{-1}·mg^{-1})	1.83±0.22	1.69±0.17	1.66±0.21	1.71±0.28
GSTs (μmol·min^{-1}·mg^{-1})	104.47±11.82	113.80±13.05	92.15±10.82	97.50±18.35

P450-OD/P450-ND, O-demethylase/N-demethylase activity of P450 monooxygenases; ESTs, esterases; GSTs, glutathione S-transferases. Data are means of at least six independent experiments ± SEM.

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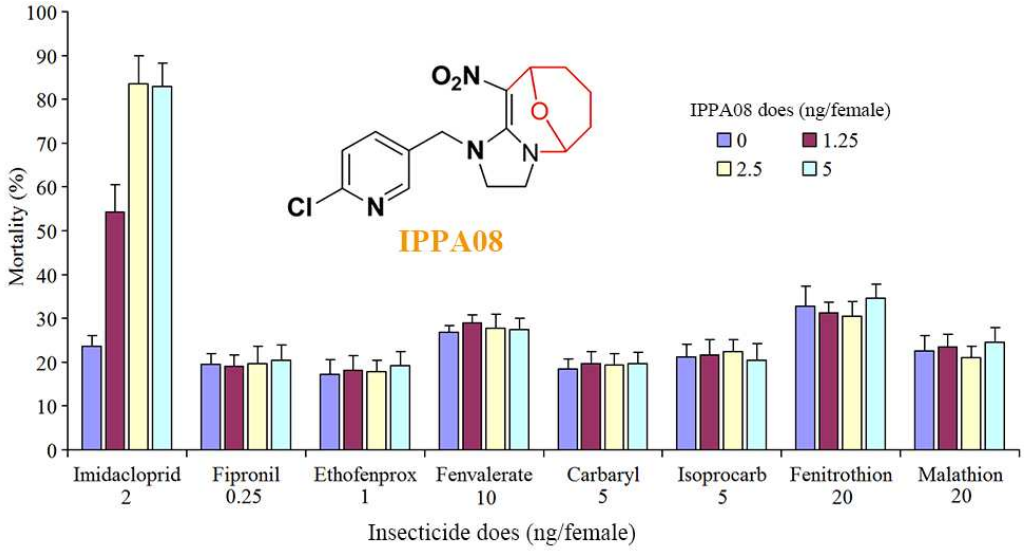

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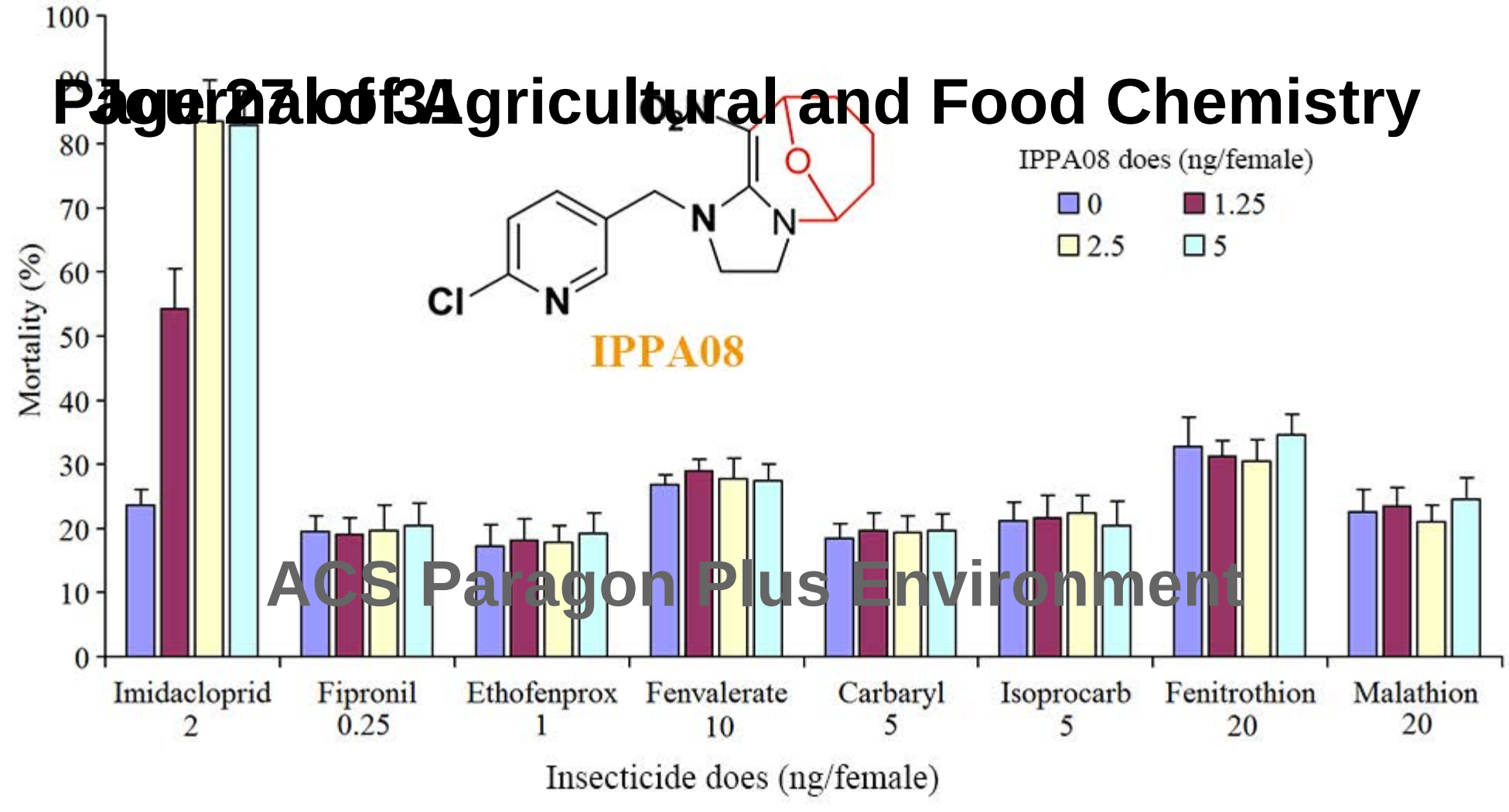

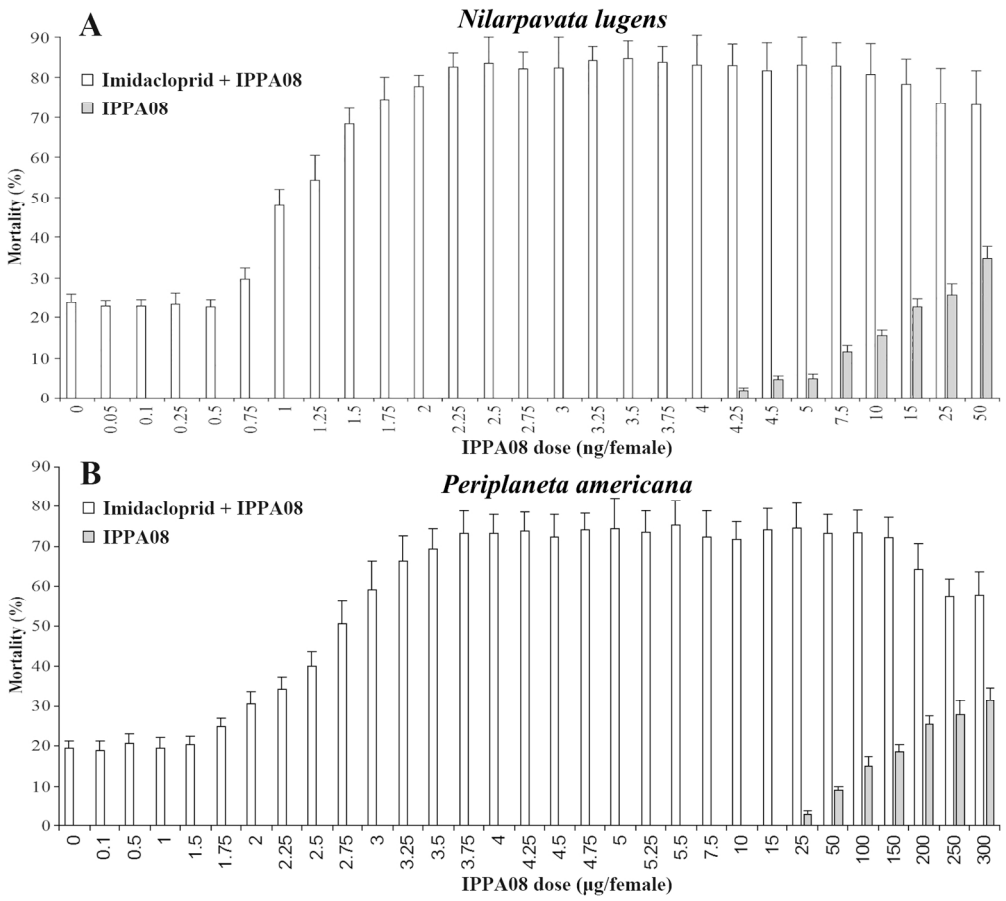

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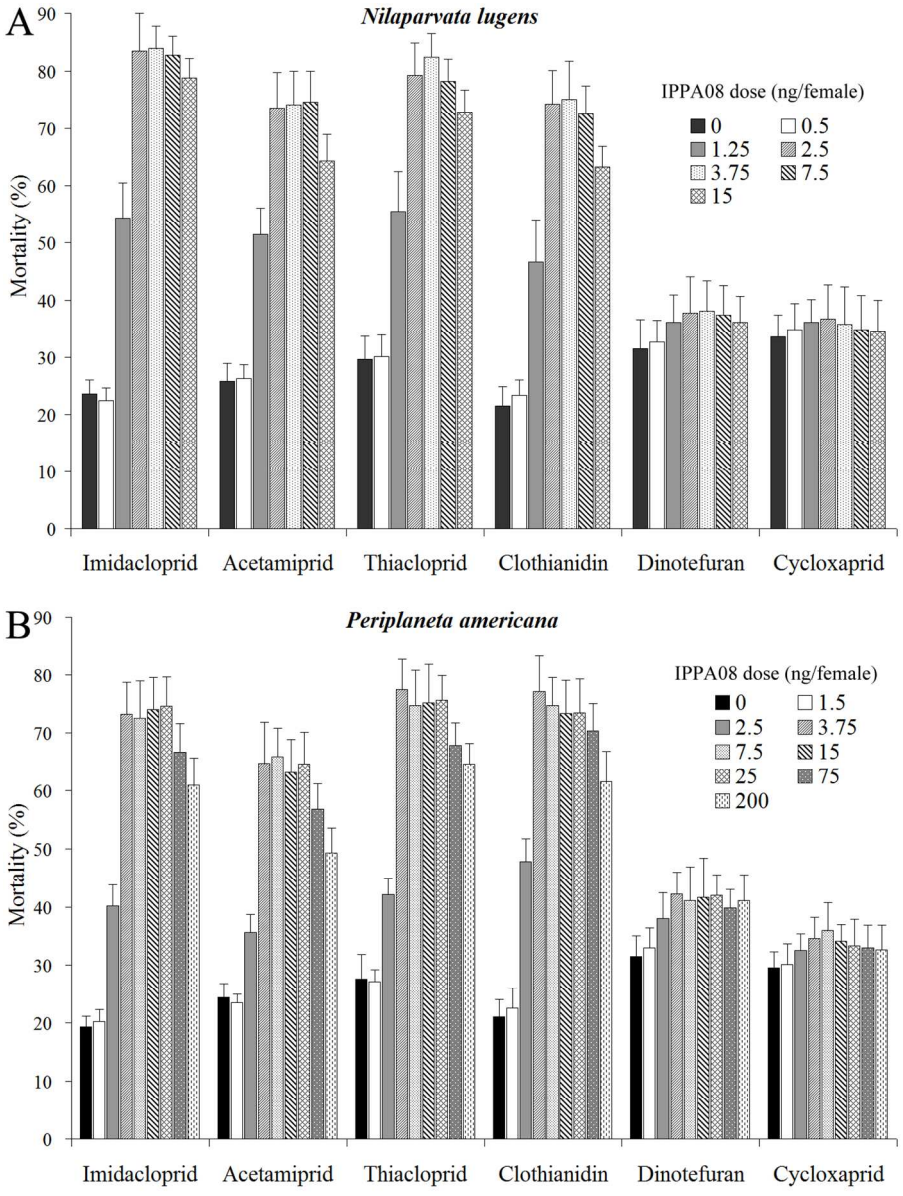

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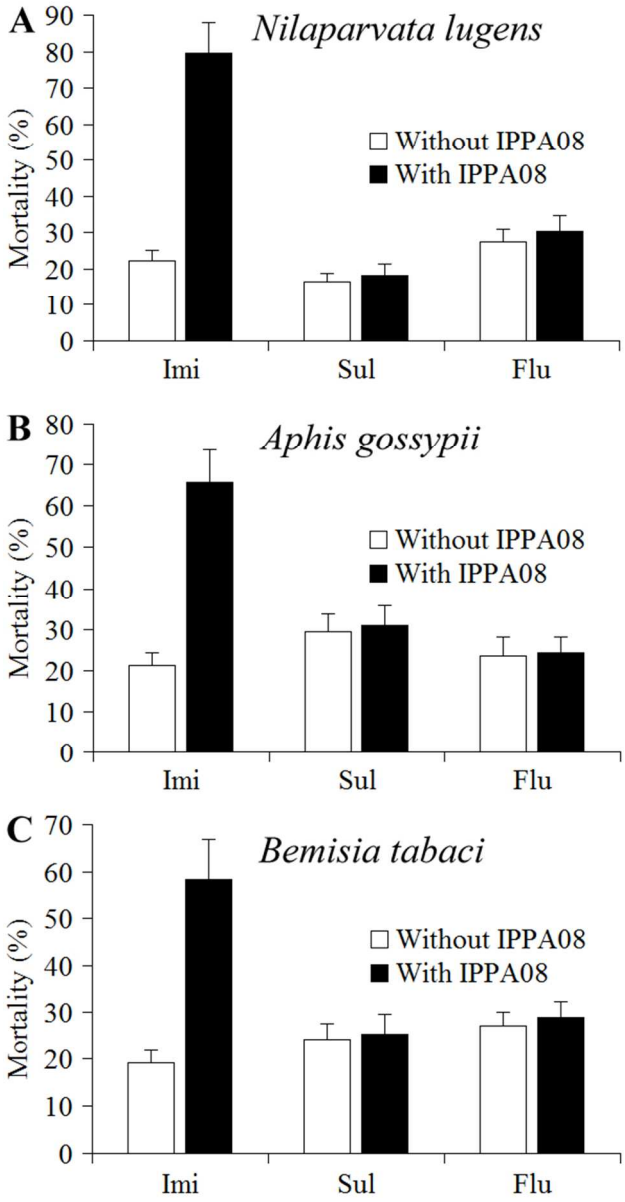

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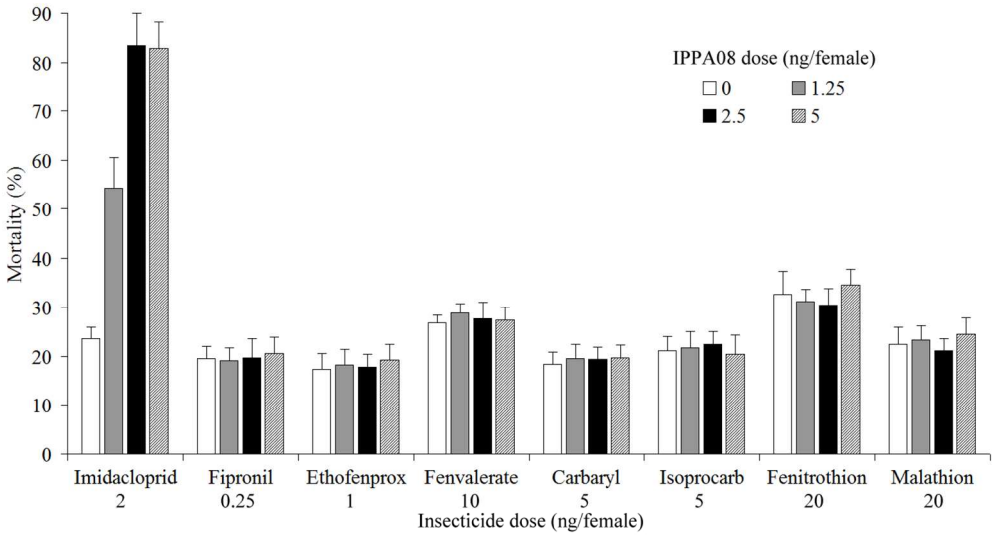

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