International Journal of Pest Management
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ttpm20

Synergistic effect of imidacloprid combined with synergistic agents (Beichuang, Jiexiaoli) on Myzus persicae (Hemiptera: Aphididae)

Haitao Chen[ab], Yongqiang Zhang[a], Hecui Zhang[c] & Wei Ding[ab]
[a] College of Plant Protection, Southwest University, Chongqing 400715, P.R. China
[b] Chongqing Institute of Tobacco Science, Chongqing 400715, P.R. China
[c] College of Agriculture and Biotechnology, Southwest University, Chongqing 400715, P.R. China
Published online: 24 Sep 2014.

International Journal of Pest Management, 2014
Vol. 60, No. 3, 201–207, http://dx.doi.org/10.1080/09670874.2014.956355

Synergistic effect of imidacloprid combined with synergistic agents (Beichuang, Jiexiaoli) on *Myzus persicae* (Hemiptera: Aphididae)

Haitao Chen[a,b], Yongqiang Zhang[a], Hecui Zhang[c] and Wei Ding[a,b]*,[1]

[a]*College of Plant Protection, Southwest University, Chongqing 400715, P.R. China;* [b]*Chongqing Institute of Tobacco Science, Chongqing 400715, P.R. China;* [c]*College of Agriculture and Biotechnology, Southwest University, Chongqing 400715, P.R. China*

(*Received 4 March 2014; final version received 24 July 2014*)

Myzus persicae is a well known aphid pest, which can transport plant viruses to plants of the nightshade/potato family, namely the Solanaceae, and other food crops. Our aim was to explore the effects of imidacloprid combined with synergistic agents (Beichuang and Jiexiaoli) on *Myzus persicae*. Different concentrations of imidacloprid combined with synergistic agents were used to treat *M. persicae*. Biological activity of *M. persicae* was analyzed under indoor conditions, and the control efficiency of the admixture was determined through field experiments. The penetration rate of the admixture on tobacco leaf and *M. persicae* was analyzed, and the liquid surface tension and contact angle was measured. Imidacloprid combined with Beichuang and Jiexiaoli showed significant synergistic effects with high control efficacy. Beichuang and Jiexiaoli significantly improved the penetration of imidacloprid into the cuticle of tobacco leaves and the insect body wall. The surface tension and contact angles were abated by synergists. The combination of imidacloprid with Beichuang and Jiexiaoli showed a significant synergistic effect, which can be used for decreasing the dosage of imidacloprid and improving its long-term control efficacy.

Keywords: Beichuang; detoxification enzyme; imidacloprid; Jiexiaoli; synergistic effect

1. Introduction

Myzus persicae Sulzer (Hemiptera: Aphididae) is an aphid pest that can cause damage on a wide variety of plants, such as those from the families Compositae, Cruciferae, and Solanaceae (Emden et al. 1969). *Myzus persicae* can reproduce asexually and can complete its lifecycle several times in a single season. Both the nymph and adult stages of this insect feed on plant sap, which can lead to a loss of plant productivity. Besides, aphids are able to transmit over 100 plant viruses (Perdikis et al. 2008) and have the potential to develop chemical resistance to insecticides. Therefore, improving methods for insecticide utilization to manage *M. perscae* populations are needed to acquire sustainable supression of this damaging pest.

Imidacloprid is a nicotine-derived insecticide which was first produced in 1984 by Bayer AG and Japanese pesticide companies. Imidacloprid is widely used because of its excellent control efficacy against homopteran pests such as *Nilaparvata lugens*, mealworms, and aphids by targeting acetylcholine receptors in the insect's nervous system (Rinkevich and Scott 2012; Subhani et al. 2013). Meanwhile, this insecticide is reported to have low toxicity to mammals (Kapoor et al. 2011). In tobacco production, imidacloprid is one of the major insecticidal agents used for preventing damage by *M. persicae*. However, the wide use of this insecticide has led to an increase in the development of resistance worldwide (Baker and Beveridge 2001; Moyses and Gfeller 2001; Armbrust and Peeler 2002; Paul et al. 2004) which, in turn, can lead to a decline in control efficacy in the long term. Therefore, much research effort has been assigned to analyze the resistance of pests to imidacloprid and to improve its rational use to ensure efficacy while retarding the development of resistance of target pests (Gorman et al. 2003; Perry et al. 2008; Yucong et al. 2009). The application of synergist was found to play a key role in supressing the degradation of imidacloprid (Zhao et al. 2000; Zewen et al. 2003). However, few reports have dealt with the synergistic effects of imidacloprid combined with synergistic agents against *M. persicae.*

Jiexiaoli (JXL) is a silicone surfactant which has been applied to paddy and cotton fields, and Beichuang (BC) has been commercialized as a synergistic agent but has rarely been reported on. In this study, the effect of imidacloprid with synergists BC and JXL on *M. persicae* was investigated through indoor toxicity and field control efficacy experiments. The penetration rate of imidacloprid combined with synergists into the tobacco leaf and *M. persicae* was analyzed. Besides, the activities of detoxification enzymes in *M. persicae* were also determined. This information may provide theoretical support for decreasing the dosage of imidacloprid and improving its long-term control efficacy.

2. Material and methods

2.1. Materials

Myzus persicae Sulzer individuals were originally collected from greenhouses holding cabbage crops, at the

*Corresponding author. Email: dingwei48t@gmail.com
[1]Address: 216 Tiansheng Road, College of Plant Protection, Southwest University, Beibei, Chongqing 400716, P.R. China

Institute of Entomology Southwest University in 1993, Chongqing, China. *Myzus persicae* was reared on *Nicotiana tabacum* (Linn.) plants in pots (8 cm diameter, 15 cm height) and maintained in a controlled environment, at 20°C and 16:8 (light:dark) photoperiod, with relative humidity of 70%. Stocks of *M. persicae* had been kept for 20 years with no chemical and acaricide exposure during this period.

JXL is a widely used silicone synergist (100% ethoxy modified trisiloxane), which was purchased from GE Organosilicone American Company. BC is a mixture adjuvant containing 35% N-methyl-2-pyrrolidone and 65% Tween-80, purchased from Shufeng Chemical Ltd., Sichuan Province, China.

2.2. Biological activity, indoor assays

Imidacloprid (Beijing KNXG Science and Technology Center) was dissolved in 0.1% (v/v) acetone. The 97% imidacloprid was used for indoor experiments. Different concentrations of imidacloprid; imidacloprid + BC and imidacloprid + JXL group were prepared at 0.0, 10, 20, 40, 80, 160, 320, 640 mg/L; 0.0, 8, 16, 32, 64, 128, 256, 512 mg/L and 0.0, 6, 12, 24, 48, 96, 192, 384 mg/L, respectively. At 1 h before the experiments, the BC and JXL were added to the imidacloprid solution at concentrations of 0.5 mg/L and 0.3 mg/L, respectively. The drug in the different groups was dripped on the chest and back of *M. persicae* separately with a 0.05-μL capillary microdropper under a microscope. For each experimental group, a total of 30, 2-day-old *M. persicae* adults were treated consecutively three times. The processed *M. persicae* specimens were placed in dactylethrae at 25°C to check the number of deaths after 2, 4 and 6 h. The number of deaths was used to calculate the toxicity regression equation, lethal concentration (LC_{50}) and 95% confidence limits. The synergistic effect of the agents was established by calculating the co-toxicity coefficient (CTC) under toxicity tests (Sun and Johnson 1960). The formula was as follows:

$$\mathrm{CTC} = \mathrm{LC}_{50}\ \text{of insecticide (alone)} / \mathrm{LC}_{50}\ \text{of} \times (\text{insecticide} + \text{synergist}).$$

2.3. Control efficiency in field experiments

Imidacloprid wettable powder (Qianger Biotechnology development Ltd., Heilongjiang Province, China) at 70% purity was used for the field experiments. All experiments were performed from 2011 to 2012 at Qianjiang tobacco-planting area (Chongqing), located at 108°36′E and 29°19′N at an altitude of 750 m. This planting area has a yellow acidic soil (pH 5.53) and small amounts of organic matter (18.68 g/kg). Soil pH was determined at a ratio of soil to calcium chloride suspension of 1:2.5 (w/v). The variety of tobacco "Yunyan 97" was used. Each treatment was replicated three times, in a completely randomized block design with 80 plants per plot. Commercial fertilizers were applied annually in the entire experimental area before the plants were transplanted. These fertilizers consisted of 750 kg of tobacco special compound fertilizer per square hectometer (hm^2), 450 kg of organic fertilizer (organic matter ≥ 30%, N-P_2O_5-K_2O ≥ 4.0%) per hm^2, 225 kg of potassium nitrate per hm^2, and 37.5 kg of ammonium nitrate/phosphorus (30/60) per hm^2. The area of the entire experimental field used in this study in each year was 1000 m^2. BC and JXL were added to 70% imidacloprid wettable powder. The dosage of imidacloprid was according to the conventional dosage and its decrements of 30% and 60%. The dosage of BC was 12% of the total pesticide, whereas the dosage of JXL was 0.33% according to the data from practical agricultural production and the manufacturer's instructions. The *M. persicae* population was recorded at 0, 1, 3, and 7 d after treatment. The mean number of *M. persicae* in one plant was calculated by randomly choosing 10 of 80 plants in each group. The total *M. persicae* population for each group was calculated as the mean number estimated ×80 (80 plants per plot).

2.4. Determination of tobacco leaf epidermal penetration

Leaf discs 8 cm in diameter were prepared from fresh leaves. The leaves were placed face-up on the back of a Petri dish. The drug liquid (0.3 μL) was dripped on the blade surface in each group. At 3, 6, 12, 24, and 48 h after administration, the drug was recovered by washing the leaves with 0.1% acetone and distilled water solution. The residual amount of the preparation was calculated after condensed in a constant volume. All measurement proceedures were done according to the pesticide residues detection protocols (i.e. by chromatography).

The leaf epidermal transmittance was calculated according to a previous method (Zeng and Zhao 1995). The formula is listed as follows:

$$X(\%) = \left(1 - \frac{\mathrm{C} \times \mathrm{V}}{\mathrm{M}}\right) \times 100,$$

where X is the pharmaceutical transmittance (%), C is the concentration of the eluted sample (mg/L), V is the sample volume (mL), and M is the drug quantity (mg/leaf).

2.5. Integument penetration of imidacloprid against Myzus persicae

Imidacloprid was compounded to a certain concentration with acetone. The LC_{50} value of the treatment with topical application was 0.5 μL per aphid. The treated aphids were incubated at 24 ± 1°C under 14 h per day illumination. A total of 20 aphids was collected at 1, 3, 6, 9, 12, and 24 h after the treatment and then rinsed three times, with 1 mL methanol. The pesticide residues on insect epidermis were collected. The eluent was placed in a 10-mL volumetric flask and then diluted to 10 mL with methanol. A total of 20 early third instar larvae of *M. persicae* were not subjected to any chemical treatment, serving as the control. Similarly, control specimens were rinsed with 1 mL

methanol, and the eluent was diluted to 10 mL. The residual rate of the epidermis was determined by high-performance liquid chromatography (HPLC), and the penetration ratio was calculated.

HPLC was performed under the following conditions: mobile phase, methanol: water = 55:45 (volume ratio); flow rate: 1.0 mL/min; column: C18, 5 μm, 250 mm × 4.6 mm (diameter); DAD detector wavelength: 254 nm; injection volume: 20 μL.

The penetration ratio was calculated as follows (Liu and Shen 2002):

$$X(\%) = \left(1 - \frac{\mathrm{C}\times\mathrm{V}}{\mathrm{N}\times\mathrm{M}}\right)\times 100,$$

where X is the pharmaceutical penetration rate (%), C is the concentration of the eluted sample (mg/L), V is the sample volume (mL), N is the number of insects leaching, and M is the drug quantity of each insect (mg/individual).

2.6. Measurement of liquid surface tension and contact angle

Imidacloprid combined with BC and JXL in series concentrations (1.5×10^{-4}, 1.5×10^{-3}, 1.5×10^{-2}, 0.15, 0.25, 0.5, 2, 4 g/L) was used for measurement. The interfacial tension of the liquid was measured by the JYW-200B automatic interface tensiometer (Shi peng testing equipment co., Ltd., Chengde, China) and the contact angles of tobacco leaves were measured by a contact angle apparatus (Shi peng testing equipment co., Ltd., Chengde, China) according to the manufacturer's instructions.

2.7. Determination of enzyme activity

Aphids were collected and homogenized with 1:4 (w/v) of a 0.1 M Tris buffer (pH 8.0). Samples were then centrifuged for 30 min at 10,000 × g and 4°C, and the supernatant fraction was collected, pooled in a 15-mL tube, and frozen at −80°C until use. Carboxlesterase was detected by measuring the product of the reaction between the thiol-derivative compound (produced by the action of the enzyme on the substrate) and DTNB at 412 nm and 25°C (Ellman et al. 1961). Acetylcholinesterase was measured using a substrate of acetylthiocholine iodide (Bradford 1976). Glutathione S-transferase was evaluated following the method described previously (Sun et al. 1987).

2.8. Statistical analysis

Differences in corrected mortality among different concentrations were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple tests using SPSS 16.0 (SPSS Inc., Chicago, USA). Enzyme activities as well as the relative quantity in aphids were tested using ANOVA.

3. Results

3.1. Effect of imidalcloprid with synergistic agents

The toxicity of imidacloprid combined with synergistic agents on *M. persicae* was analyzed first in indoor conditions. As shown in Table 1, imidacloprid combined with BC and JXL showed significant synergistic effects after treatment, with the CTC values between 303 and 495. Besides, the insecticidal efficacy was also detected in field. As shown in Table 2, a significant difference was found between treatments I and III after 1 d. Treatment I showed the highest control efficacy, whereas treatment III showed the lowest control efficacy. However, no significant difference was found among treatments I, II, and IV after 3 d and 7 d. The lowest control efficacy was exhibited in treatments III and V, which showed significant differences.

3.2. Penetration rate of imidacloprid combined with synergists applied to tobacco leaves and Myzus persicae

The pesticide tested was usually penetrating the leaf epidermis and entering the plant tissue when sprayed on plant leaves. Thus, the penetration of agents into the leaf epidermis directly affected the pesticide efficacy. As shown in Figure 1A, the cuticular penetration rate of imidacloprid combined with synergists BC and JXL gradually increased with time. The penetration rate of BC was

Table 1. Toxicity of imidacloprid combined with Beichuang (BC) and Jiexiaoli (JXL) against *Myzus persicae* determined indoors.

Treatment	Time (h)	Regression equation	LC_{50} (95% CL) (mg/L)	Correlation coefficient	CTC
Imidacloprid	2	$Y = -1.8756 + 2.7842x$	294.81 (259.8−334.53)	0.9970	–
Imidacloprid + BC	2	$Y = 0.7516 + 2.1366x$	97.37 (81.09−116.92)	0.9890	303
Imidacloprid +JXL	2	$Y = 2.6283 + 1.2816x$	70.89 (51.2−98.16)	0.9858	416
Imidacloprid	4	$Y = -2.2290 + 3.0677x$	227.24 (204.33−252.71)	0.9904	–
Imidacloprid +BC	4	$Y = 2.2447 + 1.6066x$	51.88 (37.11−72.53)	0.9794	438
Imidacloprid +JXL	4	$Y = 2.3147 + 1.4472x$	71.69 (53.43−96.2)	0.9862	317
Imidacloprid	6	$Y = -1.7006 + 2.9409x$	189.84 (170.38−211.53)	0.9928	–
Imidacloprid +BC	6	$Y = 2.8080 + 1.3834x$	38.41 (24.65−59.86)	0.9685	494
Imidacloprid +JXL	6	$Y = 3.0095 + 1.2568x$	38.35 (23.72−61.99)	0.9563	495

CTC = co-toxicity coefficient; CL = confidence limit.

Table 2. Control efficiency of imidacloprid combined with Beichuang (BC) and Jiexiaoli (JXL) against *Myzus persicae* in the field.

Treatment		Corrected mortality (%)		
		1 d	3 d	7 d
I	Imidacloprid 2 g/667 m^2	40.77 a	67.54 a	81.07 a
II	Imidacloprid 1.2 g/667 m^2+BC	32.43 ab	60.52 a	88.09 a
III	Imidacloprid 0.6 g/667 m^2+BC	17.91 b	38.77 b	68.47 b
IV	Imidacloprid 1.2 g/667 m^2+JXL	36.63 ab	64.22 a	90.00 a
V	Imidacloprid 0.6 g/667 m^2+JXL	32.57 ab	44.45 b	67.83 b

Values in the same column with different lowercase letters differ significantly at $P < 0.05$ by Duncan's multiple-range test.

slightly higher than that of imidacloprid from 0 h to 48 h. However, the penetration rate of JXL was initially lower than that of imidacloprid before 6 h, but it increased sharply after 24 h and then showed excellent penetrability.

Epidermal penetration of the insecticide in *M. persicae* was another focus of study. Therefore, the efficacy of imidacloprid combined with BC and JXL on the cuticule of aphids was also analyzed. As a result, the liquid penetration rate increased significantly after synergists BC and JXL were added. The penetration rate of imidacloprid with BC remained higher than that of imidacloprid alone from 0 h to 24 h. However, the penetration rate of imidacloprid with JXL was lower than that of imidacloprid alone from 0 h to 9 h, and its penetration rate began to exceed the imidacloprid group only after 9 h (Figure 1B).

3.3. *Changes in surface tension and contact angle of pesticide liquid on the tobacco leaf*

The surface tension of liquid and the contact angle can reflect the spreadability of pesticide droplets on plant leaves. As shown in Figure 2, the surface tension of the

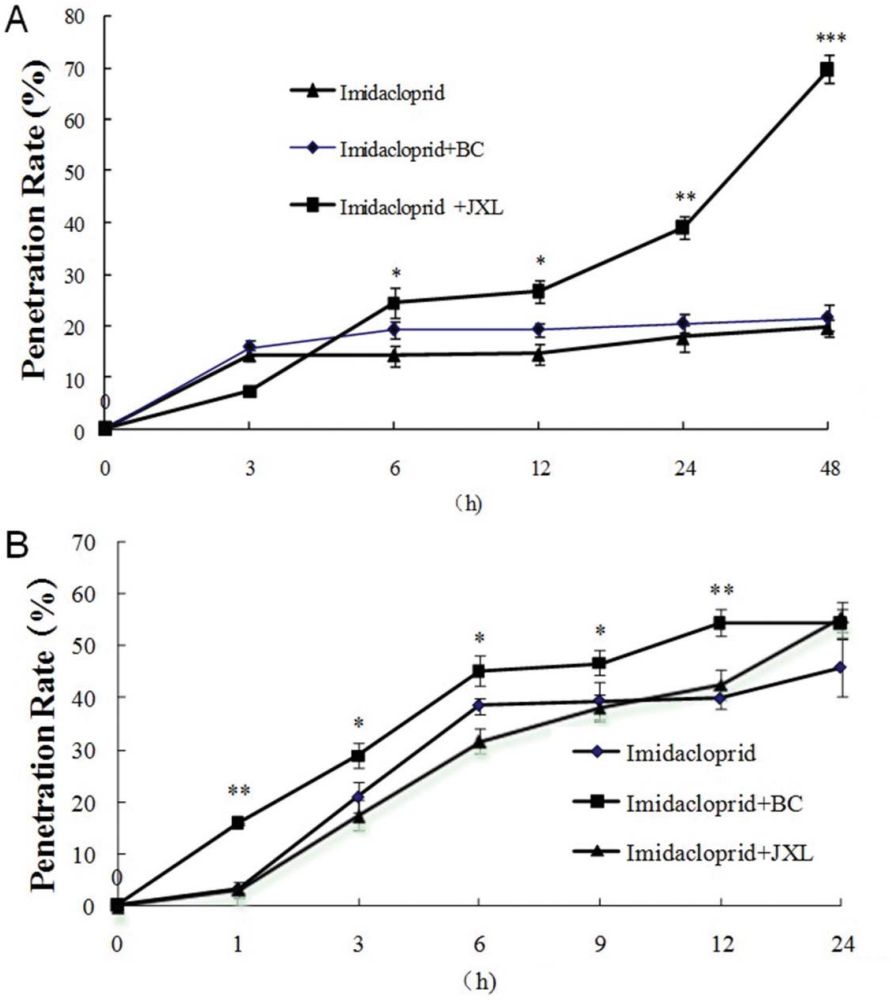

Figure 1. Penetration rate of imidacloprid combined with synergists Beichuang (BC) and Jiexiaoli (JXL). A, tobacco leaves; B, *Myzus persicae*. * represents $P < 0.05$, ** represents $P < 0.01$, *** represents $P < 0.001$.

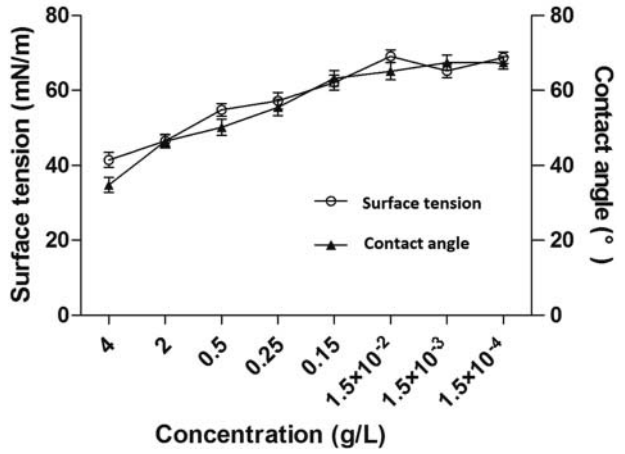

Figure 2. Changes of surface tension in pesticide liquid and contact angle on the tobacco leaf.

liquid and contact angle was gradually reduced with increasing synergist concentration.

3.4. *Activity of detoxification enzymes in Myzus persicae*

The effect of imidacloprid combined with synergists on the activity of acetylcholinesterase of *M. persicae* was analyzed first. As shown in Figure 3A, enzymatic activity was not changed significantly after adding BC and JXL. Besides, the activities of glutathione-S-transferase and carboxylesterase were also investigated. As shown in Figure 3B,C the enzymatic activities were suppressed by the combination of both synergists, differing significantly from imidacloprid alone.

4. Discussion

Imidacloprid, when administered as a systematic and contact insecticide, exhibits high efficacy and low mamalian toxicity. Imidacloprid has been widely used and management of resistance to this insecticide is highlighted worldwide (Mullins 1993). In this study, we demonstrated the synergistic effects of BC and JXL in terms of indoor toxicity and field control efficacy. The penetration of liquid insecticide into the cuticle of tobacco leaves and into the insect's body wall was significantly improved by the addition of JXL and BC, as were surface tension and contact angles abated. Besides, the combination of imidacloprid with BC and JXL decreased the activity of detoxifying enzymes, which weakened the detoxification ability of the target insects, improving the control efficacy of this pesticide.

The penetration of insecticides is an important aspect in pesticide toxicological research. As reported previously, surfactant can greatly improve the leaf cuticular penetration of insecticides in citrus leaves (Zeng and Zhao 1995). In this study, synergists (BC and JXL) not only significantly enhanced the penetration of imidacloprid into the cuticle of leaves, but also into the pest,

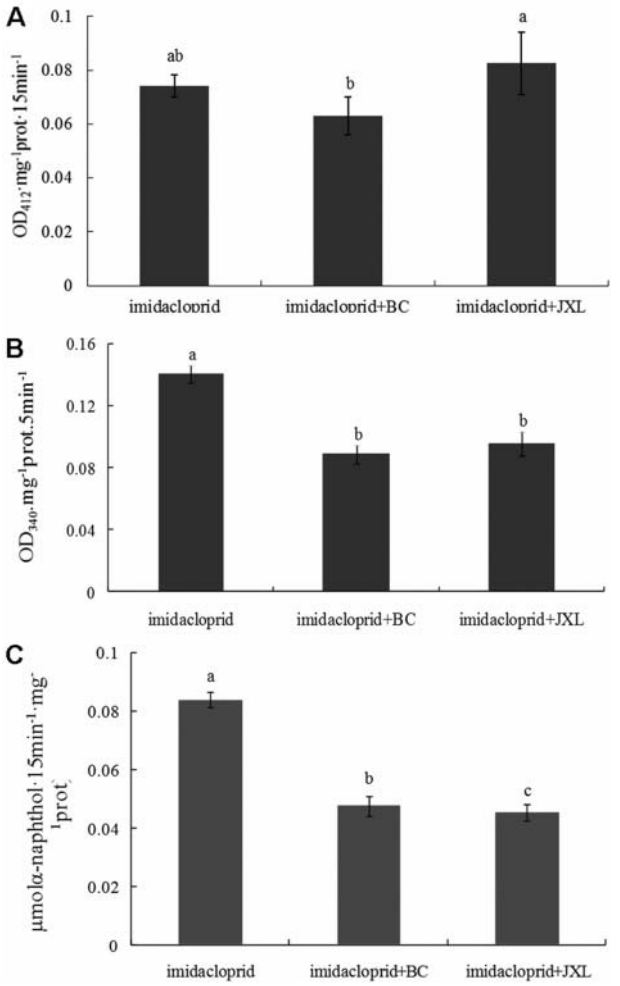

Figure 3. Effects of the imidacloprid combined with synergists Beichuang (BC) and Jiexiaoli (JXL) on the activity of detoxification enzymes in *Myzus persicae*. A, acetylcholinesterase; B, glutathione S-transferase; C, carboxylesterase. Different lowercase letters represent significant differences at $P < 0.05$.

namely *M. persicae*. As known, resistance will appear when the penetration of insecticide is low. In contrast, the effect of insecticides is promoted when the cuticular penetration is increased. Therefore, both BC and JXL can significantly improve the penetration of insecticides and reduce resistance in *M. persicae*.

The spreadability of pesticide droplets on plant leaves is the main quality index of a pesticide spray. The surface tension of liquid and the contact angle on leaves can reflect the spreadability of pesticide droplets on the plant leaves (Yu et al. 2009). The spreadability of droplets will increase with decreasing liquid surface tension and leaf contact angle (Hake et al. 2007). In this study, the surface tension and contact angle was decreased with increasing synergistic concentration. Thus, we suspected that the combination of imidacloprid with synergistic agents can lead to improving the spread of imidacloprid droplets on plant leaves.

Acetylcholinesterase is the important target enzyme of organophosphate and carbamate pesticides (Badiou et al. 2008; Li et al. 2010; Lai et al. 2013). The variation of acetylcholinesterase in the enzyme system can weaken the

inhibitory effect of pesticides and enhance resistance. As outlined in previous reports, the activity of acetylcholinesterase could be significantly decreased by the addition of specific synergist (He et al. 2000). Carboxylesterase and glutathione S-transferase are considered to be important detoxification enzymes in pests (Kostaropoulos et al. 2001; Haubruge et al. 2002; Wang et al. 2013). They play crucial roles in the mechanism of insecticide resistance and in the detoxification of exogenous mixtures (Kim and Yim 2013). In the past, researchers have found that organophosphorous and carbamate insecticides show synergistic effects in inhibiting the activity of carboxylesterase (Bingzong 1991). The toxicity of several organophosphorus insecticides is enhanced by simultaneous administration of diethyl maleate (Welling and De Vries 1985). In this study, the combination of imidacloprid with BC and JXL significantly suppressed the activities of glutathione-S-transferase and carboxylesterase, but only slightly affected the activity of acetylcholinesterase. These findings suggest that the toxicity of imidacloprid combined with BC and JXL was likely enhanced by inhibiting the detoxification ability of the insects.

In conclusion, the combination of imidacloprid with BC and JXL showed a significant synergistic effect on *M. persicae*. The application of imidacloprid with BC and JXL may have advantages in decreasing the dosage of imidacloprid used and improving its long-term control efficacy. In addition, the prevalence of pests can be prevented and the crop productivity can be controlled effectively.

Disclosure statement

The authors have declared that no competing interests exist.

Acknowledgements

This study was supported by National Nature Science Foundation (31272058). This study was partially supported by the project number 31272058 National Science Funding Committee, China.

References

Armbrust KL, Peeler HB. 2002. Effects of formulation on the run-off of imidacloprid from turf. Pest Manage Sci. 58:702–706.

Badiou A, Froment M, Fournier D, Masson P, Belzunces L. 2008. Hysteresis of insect acetylcholinesterase. Chem Biol Interact. 175:410–412.

Baker R, Beveridge I. 2001. Imidacloprid treatment of marsupials for fleas (*Pygiopsylla hoplia*). J Zoo Wildl Med. 32:391–392.

Bingzong GXZ. 1991. Synergism of inhibitors of carboxylesterase to pyrethroid insecticides in aphids. J China Agric Univ. 4:89–93.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248–254.

Ellman GL, Courtney KD, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7:88–95.

Emden H, Eastop V, Hughes R, Way M. 1969. The ecology of *Myzus persicae*. Annu Rev Entomol. 14:197–270.

Gorman K, Wren J, Devine G, Denholm I. 2003. Characterisation of neonicotinoid resistance in *Bemisia tabaci* from Spain. Proceedings of the The BCPC International Congress: Crop Science and Technology, Vols 1 and 2. Proceedings of an international congress held at the SECC, Glasgow, Scotland, UK, 10–12 November 2003. Brighton (UK): British Crop Protection Council.

Hake H, Ben-Zur R, Schechter I, Anders A. 2007. Fast optical assessment of pesticide coverage on plants. Anal Chim Acta. 596:1–8.

Haubruge E, Amichot M, Cuany A, Berge J-B, Arnaud L. 2002. Purification and characterization of a carboxylesterase involved in malathion-specific resistance from *Tribolium castaneum* (Coleoptera: Tenebrionidae). Insect Biochem Mol Biol. 32:1181–1190.

He YZ, Huang, L.M., He, F.Q., Feng G.L., Wang Y.C. 2000. Synergistic mechanism of NIA16388 to deltamethrin in deltamethrin-resistant housefly (*Musca domestica*). Chinese J Pestic. 1:19–24.

Kapoor U, Srivastava M, Srivastava L. 2011. Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. Food Chem Toxicol. 49:3086–3089.

Kim K, Yim J. 2013. Glutathione S-transferase omega suppresses the defective phenotypes caused by PINK1 loss-of-function in Drosophila. Biochem Biophys Res Commun. 437:615–619.

Kostaropoulos I, Papadopoulos AI, Metaxakis A, Boukouvala E, Papadopoulou-Mourkidou E. 2001. Glutathione S-transferase in the defence against pyrethroids in insects. Insect Biochem Mol Biol. 31:313–319.

Lai D-H, Yang Z-D, Xue W-W, Sheng J, Shi Y, Yao X-J. 2013. Isolation, characterization and acetylcholinesterase inhibitory activity of alkaloids from roots of *Stemona sessilifolia*. Fitoterapia. 89:257–264.

Li B, Wang Y, Liu H, Xu Y, Wei Z, Chen Y, Shen W. 2010. Genotyping of acetylcholinesterase in insects. Pestic Biochem Physiol. 98:19–25.

Liu Y, Shen J. 2002. Cuticular penetration mechanism of resistance to lambda–cyhalothrin in *Spodoptera exigua* (Hubner). Kun chong xue bao Acta Entomol Sinica. 46: 288–291.

Moyses EW, Gfeller FJ. 2001. Topical application as a method for comparing the effectiveness of insecticides against cat flea (Siphonaptera: Pulicidae). J Med Entomol. 38:193–196.

Mullins J. 1993. Imidacloprid. A new nitroguanidine insecticide. Proceedings of the ACS symposium series. Washington (DC): American Chemical Society; 1993.

Paul AJ, Hutchens DE, Firkins LD, Keehan CM. 2004. Effects of dermal application of 10.0% imidacloprid–0.08% ivermectin in ivermectin-sensitive Collies. Am J Vet Res. 65:277–278.

Perdikis D, Kapaxidi E, Papadoulis G. 2008. Biological control of insect and mite pests in greenhouse solanaceous crops. Eur J Plant Sci Biotechnol. 2:125–144.

Perry T, Heckel DG, McKenzie JA, Batterham P. 2008. Mutations in Dα1 or Dβ2 nicotinic acetylcholine receptor subunits can confer resistance to neonicotinoids in Drosophila melanogaster. Insect Biochem Mol Biol. 38:520–528.

Rinkevich FD, Scott JG. 2012. Reduction of dADAR activity affects the sensitivity of Drosophila melanogaster to spinosad and imidacloprid. Pestic Biochem Physiol. 104: 163–169.

Subhani Q, Huang Z, Zhu Z, Zhu Y. 2013. Simultaneous determination of imidacloprid and carbendazim in water samples by ion chromatography with fluorescence detector and post-column photochemical reactor. Talanta. 116: 127–132.

Sun Y-P, Johnson E. 1960. Analysis of joint action of insecticides against house flies. 1. J Econ Entomol. 53:887–892.

Sun Y, Feng G, Yuan J, Zhu P, Gong K. 1987. Biochemical mechanism of resistance of cotton aphids to organophosphorus insecticides. Acta Entomol Sinica. 30:13–20.

Wang Y, Gu Z, Wang J, Sun S, Wang B, Jin Y, Shen W, Li B. 2013. Changes in the activity and the expression of detoxification enzymes in silkworms (*Bombyx mori*) after phoxim feeding. Pestic Biochem Physiol. 105:13–17.

Welling W, De Vries J. 1985. Synergism of organophosphorus insecticides by diethyl maleate and related compounds in house flies. Pestic Biochem Physiol. 23:358–369.

Yu Y, Zhu H, Frantz J, Reding M, Chan K, Ozkan H. 2009. Evaporation and coverage area of pesticide droplets on hairy and waxy leaves. Bioprocess Biosyst. 104:324–334.

Yucong W, ZeWen L, HaiBo B, ZhaoJun H. 2009. Imidacloprid resistance and its mechanisms in field populations of brown planthopper, *Nilaparvata lugens* Stål in China. Pestic Biochem Physiol. 94:36–42.

Zeng XN, Zhao SH. 1995. Investigations on the penetration of insecticides into the cuticle of citrus leaf. South China Agric University. 16:25–29.

Zewen L, Zhaojun H, Yinchang W, Lingchun Z, Hongwei Z, Chengjun L. 2003. Selection for imidacloprid resistance in *Nilaparvata lugens*: cross-resistance patterns and possible mechanisms. Pest Manage Sci. 59:1355–1359.

Zhao J-Z, Bishop BA, Grafius EJ. 2000. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol. 93:1508–1514.