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Toxicity of butene-fipronil, in comparison with seven other insecticides, in *Leptinotarsa decemlineata* and *Drosophila melanogaster*

Muhammad Shahid Arain · Pin-Jun Wan · Muhammad Shakeel · Muhammad Farooq · Xing-Xing Hu · Syed Ali Haider Shah · Muhammad Esmail Abdalla Elzaki · Guo-Qing Li

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Abstract The speed of toxic action of an insecticide is an indicator for control efficacy and has considerable practical importance. For agricultural pest control, fast-acting is an important feature for an insecticide to consistently reduce the amount of feeding damage. Butene-fipronil is a novel compound obtained via the structural modification of fipronil. However, information about the toxicity and speed of toxic action is still limited. In the present paper, we compared the toxic feature of butene-fipronil with seven other insecticides, of which imidacloprid and abamectin are slow-acting insecticides, and acephate, endosulfan, methomyl, α-cypermethrin and spinosad are fast-acting insecticides. We found that the contact and stomach toxicities of butene-fipronil were among the highest ever estimated to *Leptinotarsa decemlineata* and *Drosophila melanogaster*. The speed of toxic action of butene-fipronil was determined using median lethal time (LT_{50}) at a dose (concentration) equivalent to LD_{80} values. For *L. decemlineata*, the values for butene-fipronil, imidacloprid, abamectin, acephate, endosulfan, methomyl, cypermethrin and spinosad were calculated to be 39.9, 36.5, 37.5, 20.2, 22.4, 23.8, 16.4 and 23.1 h, respectively. Those for *D. melanogaster* were 29.8, 31.5, 29.4, 14.0, 20.3, 18.1, 13.5, and 20.1 h, respectively. ANOVA analysis showed that butene-fipronil, imidacloprid, abamectin had similar LT_{50} values, whereas acephate, endosulfan, methomyl, spinosad and cypermethrin had comparable LT_{50} values. Thus, butene-fipronil belongs to slow-acting insecticides. Our results provide more empirical information for butene-fipronil potential application.

Keywords Butene-fipronil · *Leptinotarsa decemlineata* · *Drosophila melanogaster* · Toxicity · Speed of toxic action

M. S. Arain (✉) · P.-J. Wan · X.-X. Hu · M. E. A. Elzaki · G.-Q. Li
Education Ministry Key Laboratory of Integrated Management of Crop Diseases and Pests, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
e-mail: shahid786_a@yahoo.com

M. S. Arain
Research center for Resource Insect and Biochemistry, Gunagdong Institute of Applied Biological Resources, 105 Xingang Road W., 510260 Guangzhou, China

M. Shakeel (✉)
Key Laboratory of Bio-Pesticide Innovation and Application of Guangdong Province, College of Agriculture, South China Agricultural University, Guangzhou, China
e-mail: sanjum2005@gmail.com

M. Farooq
Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan

S. A. H. Shah
Sindh Agricultural University, Tandojam, Pakistan

Introduction

In general, insecticides are classified as fast-, moderate- and slow-acting (Hannig et al. 2009) based on a most common criterion 'time from exposure to mortality' (Campbell 1926; Chang 1952; Bettini et al. 1958; Clinch and Ross 1970). Some insecticides cause the lethality within approximate 1 day (fast-acting), whereas some kill the pest several days later (slow-acting). The speed of toxic action of an insecticide is an indicator for control efficacy and has considerable practical importance (Cory et al. 1994; Sun et al. 2004). For agricultural pest control, fast-acting, especially the speed to cause cessation of feeding, is an important feature for an insecticide to consistently reduce the amount of feeding damage (Hannig et al. 2009). Baculoviruses, for example, exhibit slow speed of action in insect pest control (Moscardi 1999). Crop damage after application of a baculovirus spray can therefore be substantial, even if mortality in target insects is eventually high (Bianchi et al. 2000). Genetically modified baculoviruses show faster speed of action (Stewart et al. 1991; Inceoglu et al. 2006), and subsequently can provide improved protection of crops in comparison with wild type baculoviruses (Cory et al. 1994; Sun et al. 2004). In contrast, German cockroach (*Blattella germanica*) foraging individuals can translocate a slow-acting insecticide to other members of their aggregation, and conspecific individuals then contact the insecticide, or ingest insecticide-laden feces (coprophagy), other excretions, or dead and dying insects (necrophagy and cannibalism). Thus, slow-acting insecticides in palatable baits are most effective. For example, coprophagy by first instars is an important mechanism underlying the horizontal transmission of hydramethylnon, a slow-acting insecticide (Silverman et al. 1991; Kopanic and Schal 1997, 1999; Buczkowski et al. 2001). Similarly, termiticides are required to be slow-acting and non-repellent in order to achieve greater colony suppression or even elimination (Su et al. 1982, 1987, 1991, 1994; Su 1994; Su and Scheffrahn 1991; Yeoh and Lee 2007).

Butene-fipronil is a novel compound obtained via the structural modification of fipronil, and has a relatively low toxicity to fish (Niu et al. 2007a, c). Since butene-fipronil is a GABA-gated chloride channel blocking insecticide (Zhao and Salgado 2010), it has no obvious cross-resistance to other insecticides with different modes of action (Liu et al. 2009; Niu et al. 2007b, 2008). Up to now, butene-fipronil has been proven to exhibit high toxicity to some insect species in Lepidoptera, Hemiptera, Coleoptera, Orthoptera and Diptera (Niu et al. 2007a, c; Liu et al. 2009; Yuan et al. 2009; Li et al. 2009; Arain et al. 2014). However, the speed of action of butene-fipronil remains undetermined.

Colorado potato beetle *Leptinotarsa decemlineata* is a notorious defoliator of potato throughout most of the northern Xinjiang Uygur autonomous region in China, and it often causes extremely large potato yield loss each year (Jiang et al. 2010, 2011, 2012; Shi et al. 2012, 2013; Lu et al. 2011). The control of this pest has mainly relied on insecticides (Alyokhin 2009; Alyokhin et al. 2008). This inevitably leads to the development of resistance to various insecticides such as organophosphates, carbamates, pyrethroids, and neonicotinoids (Alyokhin 2009; Alyokhin et al. 2008; Zichová et al. 2010; Mohamadi et al. 2010; Jiang et al. 2010, 2011, 2012; Shi et al. 2012; Lu et al. 2011; Malekmohammadi et al. 2012; Rinkevich et al. 2012; Szendrei et al. 2012). Moreover, *Drosophila melanogaster* is easy to care for, breeds quickly, and lays many eggs, it is an excellent model system to assess toxicities of insecticides (Arain et al. 2014). In order to test the speed of action of butene-fipronil in different representative insects and under different mode of actions, we selected a coleopteran and a dipteran, and determined the contact toxicity in *L. decemlineata* and stomach toxicity in *D. melanogaster*, in comparison to seven other insecticides. Among them acephate, endosulfan, methomyl, α-cypermethrin and spinosad were fast-acting compounds, whereas abamectin and imidacloprid were slow-acting chemicals (Hannig et al. 2009; Franc and Bouhsira 2009).

Material and methods

Experimental animals

Post-diapause *L. decemlineata* adults were collected from potato fields in the spring at Urumqi (43.82 N, 87.61E), Xinjiang Uygur autonomous region in China. Insects were routinely reared in an insectary at 28 ± 1 °C under a 14 h:10 h light–dark photoperiod and 50–60% relative humidity, with potato foliage at vegetative growth or young tuber stages in order to assure sufficient nutrition. The newly-eclosed fourth-instar larvae and adults at least 7 days after emergence were used in the experiments.

D. melanogaster Canton-S (CS), w^{1118} and Oregan flies were raised on standard cornmeal/molasses/agar medium under controlled temperature (25 ± 1 °C), photoperiod (12 h light/12 h dark) and relative humidity (about 50%). The 5 day-old adults were used in the experiments.

Insecticides

Technical grade butene-fipronil, abamectin, acephate, chlorpyrifos, methomyl, α-cypermethrin, imidacloprid and spinosad were selected (Table 1). These chemicals were kept in a refrigerator between the experimental sessions.

Bioassays

For *L. decemlineata*, a topical application was used to assess the contact toxicity in the fourth instar larvae and the adults. Insecticides were dissolved individually in analytical-grade acetone, and five concentrations within a mortality range of 0–100% based on preliminary assays were used. Larvae were treated individually with 0.22 μL of insecticide solution, which was applied to the dorsal abdominal segment using a 10-μL microsyringe connected to a microapplicator (Hamilton Company, Reno, NV). Similarly, adults were treated individually with 1.1 μL of insecticide solution, which was applied to the ventral area of the abdomen using a 50-μL microsyringe. Each control larva or adult received 0.22 or 1.1 μL of acetone. Three replications of 10 individuals per concentration were performed. After treatment, the test insects were placed in Petri dishes (9 cm in diameter and 1.5 cm in height) containing fresh potato foliage and kept under environmental conditions outlined for beetle rearing (Shi et al. 2012).

For *D. melanogaster*, a method described previously (Wang et al. 2013) was used to determine insecticide stomach toxicity to the adults. Insecticides were dissolved in and serially diluted two fold with acetone to obtain several solutions. An aliquot of 200 μL of test solution (treatment) was mixed with 10 g of hot conventional artificial diet to prepare artificial diets demonstrating five different concentrations within a mortality range of approximately 0–100% based on preliminary assays. Acetone was used as negative control. Thirty newly emerged flies were housed in a rearing vial (3 cm in diameter and 12 cm in height) containing 10 g of standard cornmeal-sucrose-yeast agar diet for a period of 4 days. And then, 90 adults in three replicates were transferred into 3 vials (3 cm in diameter and 12 cm in height) containing the same quantity of one of the toxic diets or acetone control diet prepared above. The adults were kept under environmental conditions outlined for the fly rearing.

To compare the speed of toxic action of the eight insecticides, the median lethal time (LT_{50}) of these insecticides at a dose (concentration) equivalent to LD_{80} values were calculated.

Data analysis

The mortalities were assessed daily after treatment. Control mortality was typically less than 10%. Abbott's formula was used to correct the data for control mortality (Abbott 1925). Probit analysis was used to calculate the concentrations needed to cause 50% mortality, their 95% fiducial limits and the slope of the line relating probit mortality to the log dose by POLO Plus logit probit software (LeOra Software Company, Petaluma, CA, USA). For median lethal time (LT_{50}) values, we gave the data as means ± SD, and were analyzed by ANOVA followed by the Tukey-Kramer test, using SPSS for Windows (SPSS, Chicago, IL, USA).

Table 1 Detailed information of insecticides used in this study

Insecticide Butene-fipronil	Technical grade (AI) 90.0%	Manufacturer Daliang Ruize Agro-chemical Co., Ltd	Speed of toxic action
Acephate	96.5%	Jiangsu Nantong Agro-chemical Co., Ltd	Fast-acting
Endosulfan	92.5%	Nanjing Red-sun Chemical Co., Ltd	Fast-acting
Methomyl	95.0%	Jiangsu Changlong Chemical Co., Ltd	Fast-acting
α-Cypermethrin	94.3%	Nanjing Red-sun Chemical Co., Ltd	Fast-acting
Imidacloprid	96.4%	Jiangsu Changlong Chemical Co., Ltd	Slow-acting
Abamectin	93% (B1a)	Hebei Weiyuan Biochemical Co., Ltd	Slow-acting
Spinosad	94.2%	Hebei Sannong Chemical Co., Ltd.	Fast-acting

Results

Toxicity of eight insecticides to *L. decemlineata* and *D. melanogaster*

Ninety six hours after insecticide treatment to *L. decemlineata* by the topical application, the LD_{50} values for the eight selected insecticides in the fourth instars ranged from 0.002(0.002-0.003) to 0.691(0.371–1.014) μg/individual. According to the relative toxicity index (RTI, determined by comparing butene-fipronil LD_{50} value with the LD50 value of other insecticide), the eight insecticides could be classified into three groups with relatively least (RTI > 10.0), medium (5.0 < RTI < 10.0) and high (RTI < 5.0) toxicity to the larvae. Acephate, methomyl, and endosulfan fell into the first category; α-cypermethrin and spinosad belonged to the second group; whereas butene-fipronil, abamectin, and imidacloprid were placed into the third group (Table 2).

The toxicities of insecticides to the *L. decemlineata* adults also differ widely among the eight insecticides. The LD_{50} values ranged from 0.010 (0.008–0.014) to 2.046 (0.766–4.504) μg/individual. Butene-fipronil, abamectin, imidacloprid, and α-cypermethrin were most toxic, with the RTI less than 5, spinosad showed a moderate toxic effect, with the RTI of 7.5. In contrast, acephate, methomyl, and endosulfan were least toxic, with the RTI more than 10 (Table 2).

For the *D. melanogaster* adults, the LC_{50} values for the eight insecticides ranged from 0.05(0.04-0.06) to 20.88(18.31–23.21) μg/g. From the mean LC_{50} values in CS, w^{1118} and Oregan strains, the order of toxicities from the most toxic to the least toxic was spinosad, α-cypermethrin, abamectin, butene-fipronil, imidacloprid, endosulfan, methomyl and acephate (Table 3).

Table 2 Susceptibilities of *Leptinotarsa decemlineata* to several insecticides 96 h after treatment

Developing stage	Insecticide	N[b]	X^2	df[c]	Slope (±SE)	LD_{50}(μg/pest) (95%FL)	RTI[d]
The 4th instars	Butene-fipronil	241	3.3	7	5.061(±0.212)	0.002(0.002–0.003)	1.0
	Acephate	300	3.8	9	1.974(±0.112)	0.691(0.371–1.014)	345.5
	Endosulfan	210	2.7	6	2.310(±0.124)	0.109(0.094–0.147)	54.5
	Methomyl	181	2.1	5	1.875(±0.107)	0.111(0.051–0.221)	55.5
	α-Cypermethrin	210	3.1	6	2.153(±0.207)	0.018(0.011–0.026)	9.0
	Imidacloprid	240	3.5	7	2.834(±0.224)	0.009(0.006–0.014)	4.5
	Abamectin	239	3.7	7	2.179(±0.186)	0.002(0.002–0.003)	1.0
	Spinosad	184	2.5	5	3.132(±0.256)	0.018(0.011–0.026)	9.0
Adults	Butene-fipronil	240	0.8	7	3.342(±0.134)	0.017(0.011–0.025)	1.0
	Acephate	210	2.8	6	2.374(±0.218)	2.046(0.766–4.504)	120.3
	Endosulfan	239	1.9	7	1.957(±0.141)	0.326(0.167–0.647)	19.2
	Methomyl	180	2.9	5	3.014(±0.301)	0.331(0.302–0.513)	19.5
	α-Cypermethrin	240	5.0	7	2.168(±0.313)	0.060(0.038–0.105)	3.5
	Imidacloprid	181	2.6	5	1.842(±0.157)	0.024(0.011–0.032)	1.4
	Abamectin	240	3.1	7	1.888(±0.213)	0.010(0.008–0.014)	0.6
	Spinosad	240	2.2	7	2.168(±0.233)	0.128(0.053–0.299)	7.5

[a] Number of total tested individuals from 5 concentrations. Butene-fipronil was at the doses of 0.001, 0.002, 0.004, 0.008 and 0.016 μg/larva, and 0.004, 0.008, 0.016, 0.032 and 0.064 μg/adult. Acephate was 0.15, 0.30, 0.60, 1.20 and 2.40 μg/larva, and 0.5, 1.0, 2.0, 4.0 and 8.0 μg/adult. Endosulfan was 0.03, 0.05, 0.10, 0.20 and 0.40 μg/larva, and 0.1, 0.2, 0.4, 0.8 and 1.6 μg/adult. Methomyl was 0.05, 0.10, 0.20, 0.40 and 0.80 μg/larva, and 0.1, 0.2, 0.4, 0.8 and 1.6 μg/adult. Cypermethrin was 0.005, 0.010, 0.020, 0.040 and 0.080 μg/larva, and 0.015, 0.030, 0.060, 0.120 and 0.240 μg/adult. Imidacloprid was 0.003, 0.005, 0.010, 0.020 and 0.040 μg/larva, and 0.005, 0.010, 0.020, 0.040 and 0.080 μg/adult. Abamectin was 0.0005, 0.0010, 0.0020, 0.0040 and 0.0080 μg/larva, and 0.005, 0.010, 0.020, 0.040 and 0.080 μg/adult. Spinosad was 0.005, 0.010, 0.020, 0.040 and 0.080 μg/larva, and 0.05, 0.10, 0.20, 0.40 and 0.80 μg/adult

[b] Degree of freedom

[c] Relative toxicity index (RTI) was determined by comparing butene-fipronil LD50 value with the LD50 value of other insecticide. The same as Table 3

Comparison of LT_{50} values

In order to determine the speed of toxic action of the eight insecticides, the LT_{50} values of butene-fipronil, imidacloprid, abamectin, acephate, endosulfan, methomyl, cypermethrin and spinosad at a dose (concentration) equivalent to LD_{80} values were compared.

For *L. decemlineata*, the values for butene-fipronil, imidacloprid, abamectin, acephate, endosulfan, methomyl, cypermethrin and spinosad were calculated to be 39.9, 36.5, 37.5, 20.2, 22.4, 23.8, 16.4 and 23.1 h, respectively. ANOVA analysis showed that butene-fipronil, imidacloprid, abamectin had similar LT_{50} values, whereas acephate, endosulfan, methomyl, spinosad and cypermethrin had comparable LT_{50} values (Figs. 1 and 2).

For *D. melanogaster*, the LT_{50} values for butene-fipronil, imidacloprid, abamectin, acephate, endosulfan, methomyl, cypermethrin and spinosad were respectively 29.8, 31.5, 29.4, 14.0, 20.3, 18.1, 13.5, and 20.1 h. ANOVA analysis revealed that the insecticides could be classified into three groups. Butene-fipronil, imidacloprid, abamectin showed the largest LT_{50} values, followed by acephate, endosulfan, methomyl, and spinosad, whereas cypermethrin exhibited the shortest LT_{50} value (Figs. 1 and 2).

Thus, butene-fipronil belongs to slow-acting insecticides.

Discussion

Insects are major biotic factors that cause losses in agricultural production. Continuous development of new insecticides (Hainzl and Casida 1996) with

Table 3 Susceptibilities of *D. melanogaster* adults to several insecticides 96 h after treatment

Strain	Insecticide	N[b]	X^2	df[e]	Slope (±SE)	LC_{50}(μg/g) (95%FL)	RTI[d]
CS	Butene-fipronil	630	4.6	6	2.309(±0281)	0.841(0.632–1.024)	1.0
	Acephate	450	4.4	4	2.761(±0.122)	17.343(15.657–19.413)	3.6
	Endosulfan	540	4.4	5	2.342(±0.234)	2.760(2.371–3.215)	3.3
	Methomyl	450	4.0	4	3.103(±0157)	2.556(2.203–2.987)	3.0
	α-Cypermethrin	540	5.1	5	1.051(±0.091)	0.310(0.224–0.458)	0.4
	Imidacloprid	540	4.8	5	2.254(±0.092)	1.789(1.489–2.143)	2.1
	Abamectin	540	3.7	5	2.522(±0.10)	0.903(0.724–1.146)	1.1
	Spinosad	630	5.3	6	3.521(±0.37)	0.053(0.042–0.067)	0.1
w^{1118}	Butene-fipronil	540	3.9	5	1.917(±0.124)	1.334(1.115–1.926)	1.0
	Acephate	540	4.1	5	1.943(±0.164)	18.132(16.316–19.172)	14.3
	Endosulfan	450	3.7	4	2.426(±0.201)	3.424(2.857–3.978)	2.6
	Methomyl	540	3.4	5	3.241(±0.223)	4.043(3.011–4.178)	3.0
	α-Cypermethrin	630	4.8	6	1.255(±0.181)	0.506(0.397–0.624)	0.4
	Imidacloprid	630	5.0	6	2.132(±0.214)	1.845(1.432–2.541)	1.4
	Abamectin	450	4.3	4	2.22(±0.17)	1.02(0.83–1.26)	0.8
	Spinosad	540	4.1	5	2.141(±0.223)	0.074(0.060–0.086)	0.1
Oregan	Butene-fipronil	630	4.7	6	2.143(±0.189)	0.914(0.750–1.241)	1.0
	Acephate	630	3.7	6	1.567(±0.165)	20.878(18.341–23.281)	14.3
	Endosulfan	450	4.3	4	1.943(±0.187)	3.024(2.551–3.545)	3.3
	Methomyl	540	5.0	5	1.987(±0.184)	3.415(2.851–4.119)	3.7
	α-Cypermethrin	630	4.0	6	1.401(±0.121)	0.458(0.331–0.679)	0.5
	Imidacloprid	450	3.9	4	2.145(±0.182)	1.534(1.346–1.978)	1.7
	Abamectin	540	3.7	5	1.956(±0.135)	1.065(0.851–1.346)	1.1
	Spinosad	450	4.2	4	2.753(±0.212)	0.061(0.052–0.088)	0.1

[a] Butene-fipronil was at the concentrations of 0.2, 0.4, 0.8, 0.16 and 0.32 μg/g; acephate was 5, 10, 20, 40 and 80 μg/g; endosulfan and methomyl were 1, 2, 4, 8 and 16 μg/g; cypermethrin was 0.13, 0.25, 0.50, 1.00 and 2.00 μg/g; imidacloprid and abamectin were 0.5, 1.0, 2.0, 4.0 and 8.0 μg/g; spinosad was 0.02, 0.03, 0.06, 0.12 and 0.24 μg/g.

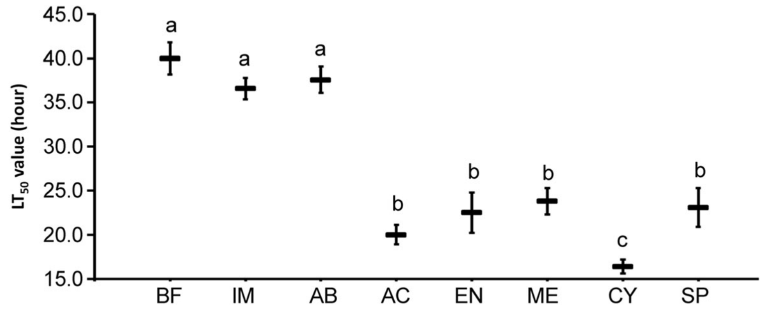

Fig. 1 Median lethal time (LT_{50}) value (hour) of butene-fipronil, compared with seven insecticides in *L. decemlineata* adults. A topical application was used to assess the contact toxicity in the adults. Adults were treated individually with 1.1 μL of insecticide solution. The mortalities were assessed at an interval of 8 h. The value indicate LT50 (±SD). Different letters indicate significant difference at *P* value <0.05

selective properties and specific action (Brévault et al. 2013) is necessary for their control. Butene-fipronil is a novel compound that has a relatively low toxicity to fish (Niu et al. 2007a). In the present paper, the toxicity of butene-fipronil to the coleopteran *L. decemlineata* and the dipteran *D. melanogaster* was determined, along with seven other insecticides. Among the eight insecticides, butene-fipronil belongs to the phenylopyrazoles and endosulfan is a polychlorocycloalkane. Both of them block insect GABA-gated chloride channels (Zhao and Salgado 2010). Abamectin is 16-membered macrocyclic lactones produced during fermentation by the soil microorganism, *Streptomyces avermitilis* (Gouamene-Lamine et al. 2003). The mechanisms of action for abamectin are also involved in GABA- and glutamate-gated chloride channel (Kass et al. 1980; Wolstenholme and Rogers 2005; Crump and Omura 2011; Moreno et al. 2010; Fritz et al. 1979). Neonicotinoid imidacloprid is a selective nicotinic acetylcholine receptor (nAchR) agonist (Casida and Durkin 2013). Spinosad is a macrocyclic lactone derived from the fungus *Saccharopolyspora spinosa* (Mertz and Yao 1990). It is an allosteric activator of insect nAChRs (Thompson et al. 2000; Kirst 2010). Acephate is an organophosphate and methomyl is a methylcarbamate. The toxicity of acephate and methomyl is attributable to inhibition of acetylcholinesterase (AChE) (Casida and Durkin 2013). Cypermethrin is a pyrethroid. It acts on axonal neurotransmission at insect voltage-gated sodium channel to block sodium transport (Casida and Durkin 2013).

Our results showed that the contact and stomach toxicities of butene-fipronil were among the highest ever estimated to *L. decemlineata* and *D. melanogaster*. The LD_{50} values of butene-fipronil for contact toxicity were 0.002 and 0.017 μg/individual to the *L. decemlineata* fourth instars and adults respectively. Comparably, the average LD_{50} values for fipronil in the *L. decemlineata* fourth instars and the adults were 0.012 and 0.023 μg/individual (Shi et al. 2012). Consistent with our results, butene-fipronil has been proven to exhibit high toxic to insect species in Lepidoptera, Hemiptera, Coleoptera and Orthopteran (Niu et al. 2007a, c; Liu et al. 2009; Yuan et al. 2009; Li et al. 2009). Thus, butene-fipronil is a powerful insecticide for the control of *L. decemlineata*.

In order to test the speed of toxic action of butene-fipronil, we selected five fast-acting compounds

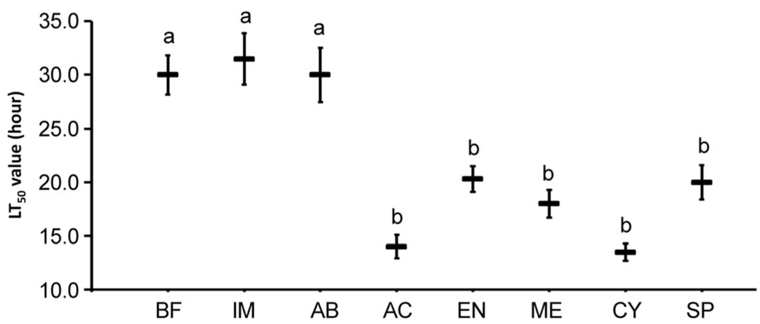

Fig. 2 Median lethal time (LT_{50}) value (hour) of butene-fipronil, compared with seven insecticides in the *D. melanogaster* adults. A diet exposure bioassay was used to determine stomach toxicities of insecticides. See the legend in Fig. 1 for further explanation

acephate, endosulfan, methomyl, α-cypermethrin and spinosad, and two slow-acting chemicals abamectin and imidacloprid (Hannig et al. 2009; Franc and Bouhsira 2009). We evaluated the speed of toxic action based on the most common criterion 'time from exposure to mortality' (Hannig et al. 2009). Our results revealed that butene-fipronil belongs to slow-acting insecticides.

It is well known that *L. decemlineata* consumes a small amount of foliage before fourth-instar. However, approximately 40 cm^2 of potato leaves are consumed mainly by fourth instars. Moreover, close to 10 cm^2 of foliage per day are consumed during the adult stage (Ferro et al. 1985). In the summer, the *L. decemlineata* larvae progressed through four distinct instars, with approximate periods of the first-, second-, third-, and fourth-instar stages of 2, 2, 2 and 4 days, respectively. According to our results, butene-fipronil should be used at the neonate or the second-instar larval stage. The slow-acting butene-fipronil then kills the larvae before the fourth-instar larval stage, to effectively protect potato foliage from *L. decemlineata*.

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