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Toxicity of Insecticides to *Neochrysocharis okazakii*, a Parasitoid *Liriomyza* Leafminers on Vegetables

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Compatible use of biocontrol agents with insecticides is often essential to integrated pest management because the targets of each agent are normally restricted to a single or only a few species of pests and because the agent does not always provide satisfactory pest control. Selective use of chemicals least harmful to biological control agents is then ideal. In the present study, five insecticides, namely imidacloprid, pymetrozine, lufenuron, ethofenprox and clothianidin, were tested to determine their toxicity to *Neochrysocharis okazakii* (Hymenoptera: Eulophidae), a larval parasitoid of pest *Liriomyza* leafminers. Individual adult parasitoids were placed in the grass vials whose internal surface was coated with the insecticides. The insecticides tested showed different degrees of toxicity to the parasitoid. The LC_{50} values were 0.0035, 8.779, 0.0508, 0.0085 and 0.0231mg a.i l^{-1} for imidacloprid, pymetrozine, lufenuron, ethofenprox and clothianidin, respectively. Based on risk quotient, imidacloprid and enthofenprox are highly toxic. Pymetrozine was harmless to *N. okazakii* while lufenuron and clothianidin were slightly to moderately to toxic to the parasitoid. Since *N. okazakii* is an important natural enemy of *Liriomyza* leafminers, use of imidacloprid or enthofenprox should be avoided. Combination use of augmented *N. okazakii* with pymetrozine or lufenuron may be counterproductive in a vegetable IPM program.

Key words: biological control, IPM, leafminer pests, onion

INTRODUCTION

In Southeast Asia, several polyphagous *Liriomyza* species have been becoming major pests in vegetable growing areas (Shepard *et al*., 1998; Sivapragasam and Syed, 1999; Rauf *et al*., 2000). The most important species in the world are *L. sativae, L. huidobrensis* and *L. trifolii* (Murphy and LaSalle, 1999, Andersen *et al*., 2002) whereas, in Vietnam, *L. sativae, L. chinensis, L. bryoniae* and *L. huidobrensis* are widely serious pests. *Liriomyza sativae* was the most abundant *Liriomyza* infesting many vegetable crops, being the most important vegetable pest in the whole country (Andersen *et al*., 2002; Tran, 2009).

Neochrysocharis okazakii Kamijo (Hymenoptera: Eulophidae) is a major parasitoid of *Liriomyza* leafminers in Vietnam (Tran *et al*., 2006; Tran, 2009). This endoparasitoid is capable of developing on several *Liriomyza* leafminer species, including *L. trifolii, L. sativae, L. chinensis, L. brassicae* and *L. chinensis* (Saito *et al*., 1996; Arakaki and Kinjo, 1998; Konishi, 2004; Bjorksten *et al*., 2005, Tran *et al*., 2006). Although this wasp species is likely to be useful for *Liriomyza* leafminer control in Vietnam (Tran *et al*., 2006), many aspects of its ecology, behavior and life history still remain unclear.

Because of the rapid increase and spread of the leafminer and other pests, vegetable growers in Vietnam have frequently applied large quantities of insecticides, including imidacloprid, lufenuron, dimethoate, fenitrothion, phenthoate, trichlorfon, fenobucarb, permethrin, ethofenprox, cypermethrin, thiamethoxam, cartap and abamectin (Tran and Takagi, 2005; Ueno, 2006). The frequent use of insecticides to manage the pests may negatively influence the effectiveness of biological control agents. Insecticides can exert two different types of effects on natural enemies. Lethal effects are expressed as acute or chronic mortality arising from contact with a pesticide. Sublethal effects, in contast, are often chronic and are expressed as negative changes in the insect's life history attributes, such as its fecundity, longevity, developmental time, egg viability, food consumption rates, behavior, and so forth (Ruberson *et al*., 1998). Lethal effects are usually manifested as short–term mortality and often have the greatest impact on natural enemies (Johnson and Tabashnik, 1999).

Natural enemies and pesticides can be effectively integrated with adequate knowledge of the pesticides to be used and their effects on natural enemy populations (Jepson, 1989; Croft, 1990, Greathead, 1995). Understanding the impact of pesticides usually requires a variety of bioassays to determine the selectivity of pesticides against the natural enemies, and their role in the ecology of pest management programs (Croft, 1990; Hassan, 1989). Pioneering work has been carried out in Europe to develop standardized tests for measuring the toxicity of pesticides to beneficial arthropods using a

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sequential procedure progressing from exposure in the laboratory to field trials (Hassan, 1989).

Although many kinds of insecticides are used in Vietnamese vegetable production, the toxicity of the chemicals to *N. okazakii* has seldom been highlighted despite its potential importance in leafminer control. Accordingly, in the present study, a series of tests were conducted with adult *N. okazakii* to determine their sensitivities to seven insecticides that are most frequently used for vegetable production in the country. Basing on the results, we determine which insecticide is least toxic to the parasitoid, and therefore best suited for use in an IPM program.

MATERIALS AND METHODS

Insect rearing

Liriomyza sativae was reared on the kidney bean *Phaseolus vulgaris* L. A single seed of this plant was sown in a plastic pot (7.5 cm in diameter) and kept in the condition of 25˚C and 60–70% humidity under constant light. One week after germination, a tray (32 cm× 44 cm×6 cm) containing 24 potted plants was placed in a shelf covered with a fine nylon mesh. Leafminer adults were released into the shelf and allowed to oviposit on the plants for 24 h. Thereafter, the potted plants were maintained under the same condition until all leafminer larvae feeding on the plants reached the last instars. The leaves containing final–instar larvae were cut off and kept in a PET bottle (1.5*l* in volume) to gain adult leafminers.

The colony of *N. okazakii* was originated from wild parasitoids collected at Hue City, Vietnam. This parasitoid was maintained with the final instars of *L. sativae* in MIR–253 Sanyo incubator chambers at the condition of 25±0.5˚C, 60–70% humidity and 16L: 8D. For parasitization, six host–infested potted plants were placed in a plastic cage (35 cm×20 cm×25 cm) covered with a fine nylon mesh. Each leaf of kidney bean plants (15–20 cm in height) contained 30–50 second and third instars *L. sativae*. A piece of tissue paper (2×2 cm) saturated with a honey solution was placed in the cage to give food for the parasitoids. About 100–300 parasitoids were introduced into the cage. After an exposure for 24 h, the potted plants were transferred into a vented plastic container (60×50×40 cm) until pupation of the parasitoids (approximately 6 days after parasitization). The kidney bean leaves with parasitoid pupae were removed from the plant stems and transferred into a polyethylene terephthalate (PET) bottle (1.5 litres) for parasitoid emergence. Emergence of parasitoids was checked daily. The parasitoids collected from the bottle were placed in grass vials (28×60 mm diameter) and provided with honey immediately after emergence.

Insecticides

We tested the insecticides listed in Table 1. They were selected on the basis of their current and potential use for the management of key insect pests on vegetable crops.

Table 1. Insecticides tested for toxicity to *Neochrysocharis okazakii*

Common name	Trade name[a]	Recommended field rate (g a.i. ha^{-1})	Main targets
Imidacloprid	Admire 050EC	25	Aphids, leafhopper, flea beetle, whitefly, leafminer, thrips.
Pymetrozine	Chess 50WG	62.5	Aphids, whitefly, leafhopper,
Lufenuron	Match 050EC	6.25	Lepidopteran, thrips, rust mites.
Ethofenprox	Trebon10EC	50	Leaffolder, thrips, leafhopper, leafminer, cutworm, army worm, aphids
Clothianidin	Dantotsu 16 WSG	25	Thrips, leafminer, leafhopper

[a] EC: emulsifiable; WG: wettable granules; WSG: water soluble granules.

Table 2. Range of insecticide doses (mg a.i. l^{-1}) used to determine the LC_{50}

Imidacloprid	Pymetrozine	Lufenuron	Ethofenprox	Clothianidin
0.0	0.0	0.0	0.0	0.0
0.0025	0.25	0.075	0.0025	0.0001
0.0050	0.50	0.125	0.005	0.0005
0.0100	0.75	0.175	0.01	0.001
0.0250	1.00	0.25	0.05	0.005
0.0275	1.25	0.5	0.1	0.01
0.0350	1.50	0.75	0.5	0.05
0.0425	1.75	1.25	0.75	0.1
0.0500	2.00	1.5	1.0	0.5

Bioassays

Toxicity measurements were made by exposing parasitoids to insecticide coats on the inner surfaces on grass vials (28×60 mm in diameter). The coats were prepared by pipetting 0.5 *ml* insecticide solution with acetone into the vials, and manually rotating the vials on their sides until the solvent evaporated. The vials coated only acetone were used for control. Adult *N. okazakii* were individually placed in each vial along with a 1 cm square of cotton soaked in 30% honey in water. Exposed wasps were kept at 25°C, 60–70% humidity and 16L : 8D light period.

A range test was used to obtain the approximate LC_{50} for each insecticide (Table 2). A 50 *ml* stock solution was prepared for each insecticide with concentration reflecting recommended field rates by the producers (Table 1). The solution was made by diluting insecticides with Acetone 300, 99.5 +% (GC). A series of concentrations for each insecticide were made by adding acetone to a 1 *ml* stock solution. The ranges of doses tested for each insecticide were: imidacloprid, 0.0025 to 0.05 mg a.i. l^{-1}; pymetrozine, 0.25 to 2.0 mg a.i. l^{-1}; lufenuron, 0.075 to 1.5mg a.i. l^{-1}; ethofenprox, 0.0025 to 1.0 mg a.i. l^{-1} and clothianidin, 0.0001 to 0.5 mg a.i. l^{-1}. Mortality determinations were made 24 h after initial exposure. Twenty female parasitoids were tested at each insecticide concentration.

Data analyses

Dose–response data were subjected to probit analysis in order to obtain log concentration/probit mortality lines. The analysis was carried out using PriProbit Version. 1.63 (Sakuma, 1998). Risk quotients for the insecticides were calculated by dividing the recommmended field rate (g a.i ha^{-1}) by the LC_{50} values (mg a.i. l^{-1}). Insecticides were classified on the basis of risk quotient values: <50 = harmless, 50–2500 = slightly to moderately toxic, >2500 = dangerous (Preetha *et al.*, 2009).

RESULTS

Results of the probit analysis for dose–response data (LC_{50}, slopes and intercepts of the dosage–mortality lines) for *N. okazakii* are given in Table 3. The data showed a wide range of response to different insecticides. LC_{50}s for *N. okazakii* were 0.0035, 8.7790, 0.0508, 0.0085 and 0.0231 mg a.i. l^{-1} for imidacloprid, pymetrozine, lufenuron, ethofenprox and clothianidin, respectively. Thus, among 5 insecticides tested, imidacloprid was the most toxic, followed by ethofenprox, lufenuron, clothianidin and pymetrozine. The difference between the most toxic (imidacloprid) and least toxic (pymetrozine) insecticides were 2508–fold at the LC_{50} level.

The risk quotients between the recommended field rate and the LC_{50} value (risk quotient) for each insecticide were presented in Table 4. The quotients in increasing order were 7142.85, 5882.35, 1082.25 and 12.30 for imidacloprid, ethofenprox, clothianidin, lufenuron and pymetrozine, respectively. The insecticides were finally classified on the basis of the risk quotient values. Imidacloprid and ethofenprox were categorized as "dangerous" to *N. okazakii*, whereas clothianidin was slightly to moderately toxic. The remain two chemicals, i.e., lufenuron and pymetrozine were classified as "harmless" insecticides to *N. okazakii*.

Table 3. Median lethal concentration (LC_{50}) of insecticides to *Neochrysocharis okazakii*

Insecticide	LC_{50} (mg a.i l^{-1})	95% fiducial limits of LC_{50}	Regression equation ($Y = a+bx$)	χ^{2a}
Imidacloprid	0.0035	0.0240 – 0.0540	$Y=1.79+2.33x$	7.75
Pymetrozine	8.7790	7.2020 – 11.1850	$Y=3.59–3.56x$	8.33
Lufenuron	0.0508	0.0286 – 0.0671	$Y=3.16+0.98x$	3.55
Ethofenprox	0.0085	0.0044 – 0.0125	$Y=2.83+0.82x$	2.45
Clothianidin	0.0231	0.0096 – 0.0389	$Y=3.13+0.78x$	4.78

[a]All log concentration/probit mortality lines are a significantly good fit to the data (P<0.05).

Table 4. Risk quotients of insecticides to *Neochrysocharis okazakii*

Insecticide	Recommended field rate (g a.i. ha^{-1})	LC_{50} (mg a.i l^{-1})	Risk quotient	Category
Imidacloprid	25	0.0035	7142.85	dangerous
Pymetrozine	62.5	8.7790	7.12	harmless
Lufenuron	6.25	0.0508	12.30	harmless
Ethofenprox	50	0.0085	5882.35	dangerous
Clothianidin	25	0.0231	1082.25	slightly to moderately toxic

DISCUSSION

Beneficial arthropods can be exposed to pesticides by direct contact, by indirect contact with residues on plant surfaces, or by the ingestion of pesticide–contaminated prey or host (Jepson, 1989). In most studies, pesticide effects have been evaluated by exposure of the natural enemy to a range of pesticide concentrations (Desneux *et al*., 2004; Youn *et al*., 2003; Sanon *et al*., 2002; Tran *et al*., 2005, Preetha *et al*., 2009).

The adults of *N. okazakii* were highly susceptible to imidaclopid, and its LC_{50} value was very low (0.0035 mg a.i. l^{-1}). Our previous study has also indicated that imidacloprid are highly toxic to *N. formosa*, a congeneric parasitoid, with the LC50 of 0.033 $\mu g/0.5\,ml$, the value of which was 757.5 times lower than the recommended concentration (Tran *et al*., 2005). Similarly, high toxicity of imidacloprid has been reported for the egg parasitoids *Trichogramma pretiosum* Riley (Williams and Price, 2004), *T. platneri* Nagarkatti (Brunner *et al*., 2001) and *T. chilonis* (Ishii) (Preetha *et al*., 2009). Imidacloprid may, therefore, be unsuitable for compatible use of chemical and biological control.

Among the insecticides tested in the present study, clothianidin was found to be moderatetly toxic to *N. okazakii*, with the LC_{50} of 0.0231 mg a.i. l^{-1}. However, clothianidin is known seriously harmful to other parasitoids, such as *T. chilonis* (Ishii) (Preetha *et al*., 2009), *Eretmocerus mundus* (Mercet), *E. eremicus* Rose and *Encarsia formosa* Gahan (Sugiyama *et al*., 2011). Thus, it is concluded that neonicotinoid insecticides including imidacloprid and clothianidin can seriuosly be harmful to many hynemopteran parasitoids (Cloyd and Bethke, 2011).

Next to the neonicotinoids, ethofenprox (a synthetic pyrethroid) was found to be fairly toxic to *N. okazakii* with the LC_{50} of 0.0085 mg a.i. l^{-1}. Preetha *et al*. (2009) also reported ethofenprox was a highly toxic chemical to *T. chilonis*. Likewise, Takada *et al*. (2001) demonstrated that among the six insecticides, i.e., acephate, methomyl, ethofenprox, cartap, chlorfluazuron, and *Bacillus thuringiensis* (Bt), etofenprox showed the highest toxicity to the egg parasitoid *T. dendrolimi*.

In our study, lufenuron showed comparatively low toxicity to *N. okazakii*, in agreement with Tran *et al*. (2005) who reported the LC_{50} value for *N. formosa* was lower than recommended field rate for lufenuron. In the present study, pymetrozine was the safest among the five insecticides, with the LC_{50} of 8.7790 mg a.i. l^{-1}. The LC_{50} values obtained in our study allow to rank the insecticides in order of increasing toxicity: pymetrozine, clothianidin, lufenuron, ethofenprox and imidacloprid.

The recommended field rate should also to be taken into consideration when studying the safety of insecticides (Preetha *et al*., 2009). When testing commercial products, the quotient between the field recommended rate and LD_{50} (or LC_{50}) gives an indication of the risk (Youn *et al*., 2003; Desneux *et al*., 2004; Preetha *et al*., 2009). Risk quotient is therefore an important measure of risk to beneficials under field conditions because it takes the field recommended rates into consideration (Stark *et al*., 1995). In fact, the quotient was used for evaluating the risk of pesticides to an aphid parasitoid, *Aphidius ervi* (Desneux *et al*., 2004) and to , an egg parasitoid *T. chilonis* (Preetha *et al*., 2009), and was concluded to be useful. Similarly, risk quotient would allow comparison of the risk to *N. okazakii* among the five insecticides concerned in the present study.

Based on risk quotients, both imidacloprid and enthofenprox were dangerous. Since *N. okazakii* is an important larval parasitoid of *Liriomyza* leafminers, use of imidacloprid and enthofenprox should be avoided in the vegetable IPM system. The chemical clothianidin that fall under the category of slightly to moderately to toxic should be tested for its toxicity to the parasitoid in a higher tier risk assessment system under a sermi–field condition. Pymetrozine and lufenuron were found to be harmless to *N. okazakii*. Pymetrozine, an insect growth regulator, represents a novel type of insecticides, being the first and only compound out of the group of pyridine azomethines, and is widely used for controlling sucking pests such as aphids, whiteflies and planthoppers (Sechser *et al*., 2002). Lufenuron is also an insect growth regulator that acts by ingestion with less contact activity and provides a good degree of selectivity; it is mainly effective to the larvae of Lepidoptera (Javaid *et al*., 1999). These pests and the leafminers often co–exist in fields of various vegetables and ornamental crops, including tomato, melon, cucumbers, eggplants, green pepper, peach, chrysanthemum, apple and strawberry (Sibanda *et al*., 2000; van Lenteren, 2000; Marquini *et al*., 2002). It has been recommended that pymetrozine and lufenuron are effective for control of the target pests in conjunction with IPM on vegetables and ornamental crops where leafminers often co–exist with the pests. Since these chemicals are supposedly safe for *N. okazakii*, compatible use of augmented *N. okazakii* and those insecticides may be counterproductive in a vegetable IPM program.

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