Ecotoxicological effect of insecticides on *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae) an egg parasitoid of *Riptortus pedestris* (Hemiptera: Alydidae)

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Abstract

Due to increased field occurrence of *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) on various crops including soybean, persimmon, and apple in recent years in Korea, demand for insecticide application to control the stink bug has become higher. Acute toxicity of eight pesticides on *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae), a major egg parasitoid of *R. pedestris*, was compared in the laboratory. Fenitrothion, spinosad, cyfluthrin, etofenprox, and carbosulfan caused 100% mortality of *O. nezarae* within 24 hours by topical application or exposure to residue. Fenitrothion was also highly toxic to the parasitoid when ingested orally. In a previous study, release of refrigerated non-viable eggs of *R. pedestris* was found to increase field parasitism, and thus we evaluated the sublethal effect of fenitrothion when *O. nezarae* parasitize refrigerated or unrefrigerated host eggs. Although parasitism rate on both kinds of eggs significantly decreased when *O. nezarae* were provided with host eggs sprayed with fenitrothion, no difference in parasitism rate, adult emergence, sex ratio, development time, and longevity of *O. nezarae* was found between the refrigerated or unrefrigerated host eggs when the insecticide was treated either before or after oviposition. There was no significant sublethal effect when parasitized host eggs were treated with the insecticide. From these results, all the insecticides tested showed high acute toxicity against *O. nezarae* with relatively lower sublethal effects. Also refrigeration of host eggs does not affect the susceptibility of *O. nezarae* to insecticide.

Introduction

Fig. 1. Adult and eggs of *R. pedestris*

Riptortus pedestris is an important pest of various crops in Korea and Japan.[1] Chemical control is a commonly used management tactic against the stink bug *R. pedestris* in soybean fields.[2,3]

O. nezarae are considered to be the most important natural enemies of *R. pedestris* in soybean fields.[4] Refrigeration of *R. pedestris* eggs can be good method for mass propagation of *G. japonicum* and it was supplemented in the field to increase the field parasitism.[5,6] The refrigerated eggs of *R. pedestris* become non-viable, thus introduction of the refrigerated eggs does not impose additional pest population in field.

Most growers rely on the use of chemical insecticides against insect pests including the soybean stink bugs in Korea. Potential harmful effects are inevitable in neighbor farms where biological control tactics like release of non-viable host eggs were adopted.

Objective of this study is to assess the lethal and sublethal effects of fenitrothion, one of the major insecticides used for the control of stink bugs in Korea were evaluated on *O. nezarae* parasitizing either refrigerated or unrefrigerated eggs of *R. pedestris*.

Materials and Methods

1. Rearing Insects

R. pedestris and egg parasitoid, *O. nezarae* were maintained in the laboratory according to the methods described Alim and Lim[7]. The eggs were collected daily and stored at 2.0±0.7˚C in a refrigerator (30 days refrigerated eggs) for the use in experiments.

2. Acute toxicity

2.1. Topical application : Five parasitoids were placed in a centrifugal tube (50ml cap.) and sprayed with 0.5ml of each pesticide. The parasitoids were then immediately moved into a new Petri dish (5.5D×1.3Hcm) individually and kept at 26.6ºC, 36.7% RH and 16L:8D hours of photoperiod in a growth chamber without food sources. Mortality of the parasitoids was recorded every 4h. These procedures were repeated using 35-74 parasitoids for each pesticide. Distilled water was used as control in this study.

2.2. Residue on substrate : The bottoms of Petri dishes (5.5D×1.3H cm) were sprayed with 0.8ml of each pesticide in field recommended dose and dried for 1h. One female adult of *O. nezarae* were then placed in each Petri dish with pesticide residue and incubated in the 26.6ºC a growth chamber. Mortality of *O. nezarae* were recorded every 4h. Total 35-40 parasitoids of each species were replicated for each pesticide. Distilled water was used as a control.

2.3. Oral ingestion : Females of *O. nezarae* were starved for 12 hours and then placed individually in the Petri dishes with a contaminated diet which was prepared by mixing with 5 ml of honey, 9.95 ml of distilled water, and 0.05 ml of each pesticide. The Petri dishes were kept in the 26.6 ºC growth chamber, and mortalities were recorded every 4 h. A total of 36-40 parasitoids of each species were tested for each pesticide. Fresh honey was used as a control.

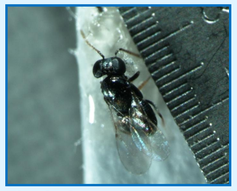

Fig. 2. *Ooencyrtus nezarae*

3. Sublethal effects of fenitrothion on unrefrigerated and refrigerated eggs of *R. pedestris*

3.1. Fenitrothion spray before oviposition : Unrefrigerated and refrigerated eggs of *R. pedestris* were submerged for 5 seconds in fenitrothion solution in field recommended concentration. The control groups were submerged in distilled water only.

After air drying for 1 hour, 15 each of unrefrigerated and refrigerated eggs were attached to a small piece of gauze by using non-toxic adhesive glue in a Petri dish. One mated 4 days old female *O. nezarae* was placed in each Petri dish and allowed to oviposit for 24 hours and were incubated in 26.9 ºC and 72% RH in the growth chamber. After 24 hours removed the parasitoids, and all eggs were separated individually in micro tube (2 ml capacity). Emergence of parasitoids was assessed daily. Number of parasitized eggs, emergence rate, sex ratio and developmental time of male and female were recorded. Host eggs from which parasitoids did not emerge were dissected and parasitization status was confirmed.

3.2. Fenitrothion spray after the oviposition : The effect of fenitrothion on *O. nezarae* developing inside the host eggs after oviposition was also examined. Unrefrigerated or refrigerated host eggs oviposited by *O. nezarae* and were dipped into fenitrothion at 0, 1, 4, 8, and 12 days after. The control groups were dipped in distilled water only. Eggs were allowed to dry and then placed in the 26.9 ºC growth chamber until adult emergence. Parasitism rate and biological attributes were assessed as describe above.

For the measurement of adult longevity of *O. nezarae,* first three females were selected from each group and placed individually in centrifugal tubes (50 ml capacity) with honey and a piece of moistened cotton in the bottom and were kept at 29.9 ºC, 43.5% RH, and 16 L: 8 D hours of photoperiod in the incubator. Fresh honey and moistened cotton were provided every three other days. The parasitoids were then transferred to new tubes as needed. Number of parasitoids dead was recorded daily.

Table 1. List of pesticides assayed

Use type	Chemical name	Class	Active ingredient (ppm)
Insecticides	Fenitrothion	Organophosphate	500
	Carbosulfan	Organocarbamate	200
	Cyfluthrin	Pyrethroid	25
	Etofenprox	Pyrethroid	200
	Thiamethoxam	Neonicotinyl	50
	Spinosad	Microbial	50
Fungicide	Metalaxyl	Xylylalanine	125
Herbicide	S-metolachlor	Chloroacetanilide	748

Results and Discussion

Fenitrothion was highly toxic to adult *O. nezarae* with 100% of mortality within 8 hours regardless of exposure method. Spinosad, thiamethoxam, etofenprox, cyfluthrin, carbosulfan caused 100% mortality of *O. nezarae* within 24 hours after exposure in the topical experiment (**Fig. 3A**).

Spinosad, thiamethoxam, etofenprox, cyfluthrin, carbosulfan caused 100% mortality of *O. nezarae* 12 hours after exposure in the residue experiment (**Fig. 3B**). In oral ingestion experiments of *O. nezarae,* fenitrothion killed all the parasitoid in 24 hours (**Fig. 3C**).

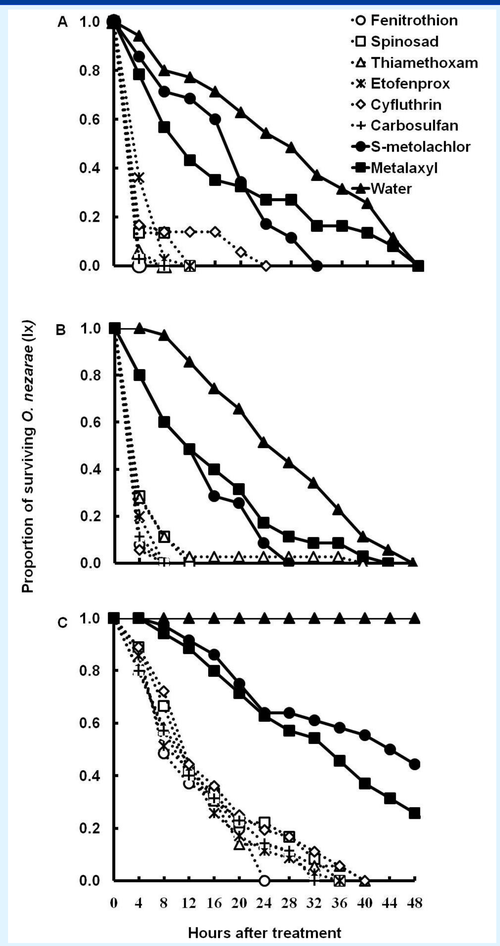

Fig. 3. Survivorship of adult female *O. nezarae* by topical application (A), residue (B), and oral ingestion (C) at 26.6 ºC.

Fenitrothion is a broad-spectrum insecticide and known to cause high acute toxicity to natural enemies.[8] *O. nezarae* were less susceptible to thiamethoxam, etofenprox and cyfluthrin in oral ingestion (**Fig. 3C**). *Trissolcus nigripedius* Nakagawa parasitoid of *Dolycoris baccarum* L. was less susceptible to thiamethoxam in oral ingestion.[9]

Table 2: Effect of fenitrothion application on unrefrigerated and refrigerated host eggs containing immature *O. nezarae*

Treatment	Days after parasitization	Egg status	Proportion of host eggs produced parasitoids	Parasitoid emerged /host egg	
				Male	Female
Water	0	Unrefrigerated	1.00	1.1	3.5
		Refrigerated	0.97	1.1	3.2
Fenitrothion	0	Unrefrigerated	0.91	1.3	3.5
		Refrigerated	0.90	1.1	3.6
	4	Unrefrigerated	0.94	1.2	2.9
		Refrigerated	0.97	1.4	3.3
	8	Unrefrigerated	0.90	1.3	3.1
		Refrigerated	0.97	1.1	3.0
	12	Unrefrigerated	1.00	1.2	3.6
		Refrigerated	0.94	1.4	3.9

Survivorship of *O. nezarae* inside host eggs measured as proportion of host eggs produced parasitoids were not significantly different between unrefrigerated and refrigerated *R. pedestris* eggs when fenitrothion was applied at different days after parasitization (0 days Z_c=0.306, P=0.758; 4 days Z_c=0.028, P=0.977; 8 days Z_c=0.055, P=0.955; 12 days Z_c=0.740, P=0.459) (**Table 2**).

From the analysis of refrigeration and fenitrothion application time, number of parasitoids emerged parasitoids per host eggs was also not different between unrefrigerated and refrigerated eggs (Male $F_{1,229}$=0.51, P=0.474; Female $F_{1,230}$=1.61, P=0.206). However, number of female *O. nezarae* was significantly affected by fenitrothion spraying time ($F_{3,230}$=7.99, P<0.001) (**Table 2**).

The lack of difference in parasitism and number of *O. nezarae* progeny between the unrefrigerated and refrigerated eggs indicate that *O. nezarae* accept refrigerated host eggs as presented in a previous study.[7]

Table 3: Biological attributes of *O. nezarae* from refrigerated and unrefrigerated eggs parasitized in different days after fenitrothion spray (provided 15 egg/female)

Treatment	Days after spray	Egg status	Host egg produced parasitoids	Emerged parasitoid/egg	
				Male	Female
Water	0	Unrefrigerated	6.4	0.8	2.8
		Refrigerated	6.4	0.6	2.5
Fenitrothion	0	Unrefrigerated	0.7	0.5	1.8
		Refrigerated	0.7	0.9	2.6
	1	Unrefrigerated	4.0	0.5	2.3
		Refrigerated	3.9	0.6	2.4
	2	Unrefrigerated	4.4	0.6	2.3
		Refrigerated	4.3	0.6	2.4
	4	Unrefrigerated	4.8	0.7	2.5
		Refrigerated	4.7	0.5	2.2

O. nezarae parasitized 11% (both refrigerated and unrefrigerated) of host eggs when parasitoids released 0 days after fenitrothion application compared to control (**Table 3**). Similar pattern also found in another scelionid parasitoid when they were exposed to host eggs treated with fenitrothion.[8] There was no significant reduction in the numbers of host egg produced parasitoids by the refrigeration ($F_{1,72}$=0.00, P=1.000), but by the fenitrothion application ($F_{3,72}$=81.59, P<0.001).

Mean number of male and female *O. nezarae* emerged from the host eggs were not affected by either host egg refrigeration (male $F_{1,264}$=0.06, P=0.809; female $F_{1,264}$=0.08, P=0.782) or fenitrothion application before parasitization (Male $F_{3,264}$=0.28, P=0.842; Female $F_{3,264}$=0.06, P=0.982) **(Table 3).** Development time of both males and females of *O. nezarae* was affected by host egg refrigeration (male $F_{1,153}$=20.00, P<0.001; female $F_{1,263}$=44.32, P<0.001) (**not showed in Table**). However, significant effect of fenitrothion application time before parasitization was found only in female *O. nezarae* ($F_{3,263}$=3.20, P=0.024) with significant interaction between refrigeration and the application time ($F_{3,263}$= 3.88, P=0.010).

Conclusions

- All the insecticides tested showed high acute toxicity against *O. nezarae* with relatively lower sublethal effects by fenitrothion.
- Generally refrigeration of host eggs does not affect the susceptibility of *O. nezarae* to insecticide.

Acknowledgement

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