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Species diversity of fireflies in the carbamate contaminated areas in the lower Northern region, Thailand

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Abstract

Carbamate is one of the most commonly used insecticides in Thailand, which has raised public concern about their toxicity and effect on the environment. The use of carbamate insecticides could potentially cause a decline in beneficial arthropod biodiversity. Fireflies have been recognized as environmentally sensitive arthropods and labeled as a biological indicator. However, recent discoveries show that they may change their preferred habitat. Therefore, this research examined whether firefly species might adapt to living in insecticide-affected habitats, particularly those polluted with carbamate insecticides. Six spots in the lower part Thailand's northern region were studied to determine their firefly population and insecticide levels. Soil samples for each study area were collected to quantify the carbamate insecticides levels. Taxonomy of both adult and larval stages of fireflies were performed at each study area and the individual species were identified. Four species of fireflies (*Lamprigera tenebrosa*, *Pyrocoelia praetexta*, *Asymmetricata circumdata*, and *Mediopteryx* sp.) were identified in areas contaminated with two carbamate insecticides; oxamyl (detected in 5 sample sites), was found as the most common carbamate insecticides followed by methomyl (detected in 1 sample site). The level of oxamyl in soil samples ranged from 0.08 - 0.11 mg/kg, and the level of methomyl was 0.01 mg/kg. A low amount of carbamate pesticides discovered in this study, together with the presence of fireflies, suggested that certain firefly species can survive in such contaminated habitats.

Keywords: Firefly distribution, Insecticide contamination habitats, Carbamate insecticide residues, Oxamyl residue, Methomyl residue

1. Introduction

The global use of agricultural pesticides is estimated to be approximately 2 million tons per year [1], with Thailand using nearly ten thousand tons [2]. Pesticides, in theory, lower the negative impact of insect infestations, increasing crop production. However, environmental contamination of the pesticides is a major concern in many countries, including Thailand, due to ecological damage and negative impacts on non-target species. Pesticides' unintended consequences on non-target creatures have been debated for decades [3]. Organochlorines (chlorinated hydrocarbons), organophosphates, carbamates, and synthetic pyrethroids are some of the insecticides that have been introduced for insect pest management. Depending on their chemical compositions, insecticides have a long or short half-life. As a result, pesticide residues are likely to accumulate in the soil, water, and the environment. The environmental impact of insecticides has been examined, to study their ecological impacts on other environmentally important arthropods.

Carbamate insecticides are frequently used in Thailand to control insect pests in commercial crops. Carbamates are carbamic acid compounds that are highly effective broad-spectrum insecticides, that have been utilized in agriculture [4]. The toxicity of carbamates has been connected to problems in soil, surface, and groundwater contaminations which are potentially linked to ecological and human health risks [5-7]. Several factors influence carbamate degradation in the soil, including volatility, soil type, soil moisture, adsorption, pH, temperature, and photodecomposition [8,9]. As a result, carbamate toxicity is concentrated in benthic aquatic and epibenthic invertebrates, posing a threat to their survival, growth, development, and reproduction. However, nothing is known regarding carbamate toxicity in benthic invertebrates. Carbamate groups such as methomyl and oxamyl were one of the most commonly used insecticides [6,10,11]. In Thailand, these pesticides have long been employed to treat insect and nematode pests in a variety of agricultural practices [6,12]. These insecticides can be applied in a wide variety of ways, including foliar sprays and drip chemigation. Because of the chemical properties of oxamyl and methomyl, repeated dosages are required for nematode pest control, resulting in significant amounts of these pesticides in the soil [13]. Methomyl is a broad-spectrum pesticide that is widely used on vegetables and fruits. In agricultural products such as fresh vegetables, grains, oils, and meat, the maximum residue limits (MRL) for methomyl are 0.02–2.00 mg/kg [14]. Another carbamate insecticide, oxyamyl, has already been classed as a highly hazardous pesticide [15], with MRLs ranging from 0.01 to 5 mg/kg in vegetables, fruits, nuts, dairy products, and meat [16].

When insecticides are used to manage insect pests on foliage or root infestations, a significant amount of the pesticides may end up in the soil. Toxins are extensively distributed among soil-dwelling invertebrates such as earthworms, arthropods, and snails. The widespread use of broad-spectrum insecticides has a direct impact on a wide range of non-insect species [17]. This is also true in fireflies, which has been recognized as being threatened by pesticides usage [18]. Fireflies are a group of beetles belonging to the order Coleoptera which are recognized as glowworms due to their ability to emit light. Because several species of fireflies reside in the soil, soil conditions have a direct impact on their survival and growth. Pesticide exposure on fireflies, in laboratory circumstances, has been described in the Japanese firefly species, *Luciola cruciata*. Except for fenthion, an emulsion of 5% organophosphate insecticides (fenitrothion and difenphos) had little toxicity in both *Luciola cruciata* larvae and their snail preys [19]. Organophosphates (e.g., fenitrothion (MEP), phenthoate (PAP), Acephate, Fenthion, and Diazinon) mixed with certain neonicotinoids caused 80-100% mortality in both larvae and adults of fireflies in the aquatic genus of *Aquatica* (*Aquatica lateralis*). Furthermore, certain insecticides caused a decrease in egg hatching [20]. Many areas have observed a reduction in the number of fireflies. Firefly population declines may be caused by habitat alteration, such as deforestation for agricultural purposes, light pollution from tourism industry, and water pollution from chemical contamination [18]. In some parts of Europe, the expansion of urbanization and industrialization, as well as increased agricultural productivity, has led in a reduction in firefly abundance [21]. The increased usage of agricultural pesticides aimed at boosting agricultural productivity has been recognized as a key contributor to the depletion of insect populations [22]. Not only does direct application of pesticides to plantations impair larval stages of both aquatic and semi-aquatic fireflies, but indirect impacts from runoff waters from agricultural plantations contaminated with insecticides also harm larval stages of those fireflies. [23]. revealed that numerous pesticide residues from oil palm plantations accumulated in Malaysia's Selangor River, which is home to several firefly species. A field test of clothianidin-treated maize seed in the United States revealed a 70.4% drop in adult firefly abundance when compared to the control [24]. This clearly demonstrates that pesticide residue in the environment has a negative impact on firefly survival.

Fireflies have been recognized as an environmentally sensitive insect species whose preferred habitats are uncontaminated or undisturbed environments, making them a biological indicator. However, based on earlier studies of the diversity of fireflies in Northern Thailand, we discovered that certain species of fireflies populated unfavorable environments, such as agricultural regions, human-inhabited areas, and disturbed areas [25-27]. Our study was first to attempted to determine whether any firefly species might adapt to living in insecticide-affected habitats, particularly those polluted with carbamate insecticides. As a result, this study will be beneficial in anticipating what we can do to maintain the diversity of fireflies in nature.

2. Materials and methods

2.1 Sample collections and firefly identification

We conducted surveys in Thailand's lower northern regions. When fireflies were discovered, soil and firefly samples were collected. Grid sampling was used to obtain soil samples from six study sites. Briefly, the sampling sites were overlayed with a square grid in the field (each sampling site was ~ 1600 to 2400 square meters). Ten grid cells were laid out on the field with equal distant spacing between the intersection points. The core soil samples were randomly collected within a radius of the grid cell [27,28]. Before sampling, topsoil layers were removed, and each point was collected at a depth of 25-50 cm. Following collection, all soil samples from those ten grid cells conducted from each study site were homogeneously mixed and brought to the laboratory, where they were held at 4oC until analysis. Firefly samples were obtained in both adult and larval stages at each sampling site. Adult samples obtained in the surrounding regions were collected and kept in 70% ethanol for subsequent identification. Larval samples were placed in separate plastic containers and returned to an insect raising chamber. The larvae were maintained in a temperature-controlled chamber at around 25 ± 2^{o}C with a 12:12 light: dark photoperiod. They were fed by terrestrial snails as food and water was provided in a small plastic box. When they reach adulthood, they were identified using the criteria from these references.

1. Systematics and phylogenetics of Indo-Pacific Luciolinae fireflies (Coleoptera: Lampyridae) and the description of new Genera. (Ballantyne LA and Lambkin CL, 2013) [30]

2. Taxonomy and species distribution of fireflies (Coleoptera: Lampyridae) in the north of Thailand. (Nak-eiam, 2015) [26]

3. The Luciolinae of S. E. Asia and the Australopacific region: a revisionary checklist (Coleoptera: Lampyridae) including description of three new genera and 13 new species. (Ballantyne LA, Lambkin CL, Ho JZ, Jusoh WFA, Nada B, Nak-Eiam S, Thancharoen A, Wattanachaiyingcharoen W. and Yiu V, 2019) [31]

2.2 Analysis of insecticide residues in soil samples

2.2.1 Materials

A triphenyl phosphate (TPP) solution was used as an internal standard, and a standard mixture of Carbamate Insecticides (CIs) (catalog number: M-531M) were aldicarb sulfoxide, oxamyl, methomyl, carbofuran-3-hydroxy, propoxur, and carbofuran were all purchased from AccuStandard® (New Haven, CT, USA), and the standard stock solution was stored at 0 to 5^{o}C. The calibration standards and working standards were prepared by dilution with water: acetonitrile (10:90) on the day of analysis.

2.2.2 Extraction and clean-up

The extraction and cleanup of insecticides were conducted according to the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Save) method as previously described [11]. Soils were dried and sieved through a 2 mm sieve to eliminate contaminated materials, like stones, and then 5 grams were weighed into a polyethylene tube. Five grams of homogenized soil were mixed with 5 mL of water and stored at 4^{o}C overnight prior to insecticide analysis. Ten milligrams of acetonitrile were mixed with the prepared soil. To separate the organic phase, the internal standard solution (TPP at 100 µg/mL, 10 µL) was added to all samples, followed by 4 g of anhydrous magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate, and 0.5 g of disodium citrate sesquihydrate, then the mixture was shaken and centrifuged. The organic phase and the proteins were isolated using dispersive solid-phase extraction (D-SPE) through the displacement of the supernatant into another tube, comprising 150 mg of primary secondary amine (PSA), and 900 mg of magnesium sulfate. After shaking and centrifugation, the extracted samples were transferred into other tubes and evaporated until dry. The samples were reconstituted with 1 mL of 10% acetonitrile, then filtered through a 0.45 µm syringe filter prior to the insecticide residues being determined via high performance liquid chromatography (HPLC) - quadrupole time-of-flight (QTOF) - mass spectrometer (MS).

2.2.3 Apparatus and insecticide residues analysis

The determination of the insecticide residues was performed using an Agilent series 1260 Infinity HPLC instrument coupled to an Agilent 6540 QTOF mass spectrometer equipped with an electrospray ionization (ESI) interface. Details of the HPLC-QTOF-MS determination and apparatus were modified according to previously described methods [11,32]. The calibration curve was prepared from a stock solution of the CIs standard mixtures at 5 different concentrations, from 0.05-1.00 mg/mL, prepared by diluting them with acetonitrile/water (10/90) with a correlation coefficient of ≥ 0.998.

2.2.4 Quality assurance and quality control

To validate the recovery methods, studies were performed by adding CIs standard mixture to blank soil samples, at concentrations of 0.10 mg/kg, and then the extract protocol was run on those samples. The results for the 6 insecticides are given in (Table 1), and the limits of detection were ≤ 0.0096 mg/kg for each of them.

Table 1 Recovery and standard deviation of the carbamate insecticide residues.

Compounds	Spiked level (mg/kg)	Recovery (%)	CV (%)
aldicarb sulfoxide	0.10	97.086	5.744
oxamyl	0.10	93.409	1.208
methomyl	0.10	97.461	0.252
carbofuran-3-hydroxy	0.10	101.035	0.687
propoxur	0.10	109.131	2.975
carbofuran	0.10	116.408	8.187

2.2.5 Statistical analysis

Data analysis was carried out with statistical software. The Shapiro-Wilk test was used to evaluate the normality of the quantitative variables. The equality of variance was determined using Levene's test. The differences in the level of oxamyl in the soil from the various locations was determined using a one-way analysis of variance (ANOVA). A p-value less than 0.05 was considered statistical significance.

3. Results and discussion

Soil and firefly samples were obtained from six different locations. Some sampling locations were currently being used for agricultural proposes, while other were previously used for agriculture, and included two mixed orchards, one para rubber plantation, two vegetable gardens, and one banana plantation (Figure 1). Firefly occurrences were observed in all sampling sites and our results showed that all were contaminated with insecticides. Four terrestrial firefly species from two subfamilies, Lampyrinae and Luciolinae, were identified in the six different sample sites (Table 2).

Figure 1 Examples of the sampling locations (A) mix orchard No 1 (Location 1), (B) banana plantation (Location 6).

Table 2 The study sites and the distribution of the detected firefly species.

Locations	Habitats	Firefly species
1	mixed orchard No. 1	*Lamprigera tenebrosa, Pyrocoelia praetexta*
2	mixed orchard No. 2	*Lamprigera tenebrosa, Pyrocoelia praetexta*
3	para rubber plantation	*Lamprigera tenebrosa, Pyrocoelia praetexta, Asymmetricata circumdata, Medeopteryx* sp.
4	vegetable garden No. 1	*Lamprigera tenebrosa*
5	vegetable garden No. 2	*Lamprigera tenebrosa, Asymmetricata circumdata*
6	banana plantation	*Pyrocoelia praetexta*

Four species of fireflies, i.e., *Lamprigera tenebrosa*, *Pyrocoelia praetexta*, *Asymmetricata circumdata*, and *Mediopteryx* sp. were collected and identified (Figure 2). The para rubber plantation was the most suitable dwelling site for fireflies since it accommodated all four firefly species. Despite the fact that many different species of firefly were obtained from various sample sites, some of them appear to be limited to a specific habitat. *L. tenebrosa* was the notable firefly species that was found in all the sampling sites except the banana plantation. This is a common terrestrial firefly species, with larvae living on soil surfaces and adults sitting on the ground or surrounding plants. Except for the 2 vegetable gardens, *P. praetexta* was detected in the other four sample sites. Previous findings revealed that *P. praetexta* is a widespread species found in lowland settings across northern Thailand at elevations ranging from 80 to 500 meters above sea level. They were discovered in both natural and human exploited habitats, including dry evergreen forests, mixed deciduous forests, forest parks, public parks, and paddy fields [26]. In the banana plantation, we observed these firefly species larvae moving inside of the old banana stalks searching for their prey, such as snails and earthworms. The firefly species, *A. circumdata* was observed in the para rubber plantation at an elevation of approximately 600 mean sea level (MSL), and in the vegetable garden No.2 in the highland habitat in Rom Khlao Botanical Garden's at a height of around 1,000 MSL. This common firefly species has also been identified as one of the most prolific and extensively distributed firefly species in northern Thailand. Their distribution ranges from lowland to highland, with elevations up to 1,250 MSL of the lower montane forests, and they have been found in both natural and human-exploited locations [26,29]. *Mediopteryx* sp. was the rarest firefly species discovered in this study, and it was only found in the para rubber plantation, which was located at around 650 MSL and bordered by fruit orchards. This species was originally documented to be exclusively found in lowland areas with elevations less than 600 MSL [26]. However, the findings may suggest that those two firefly species, *L. tenebrosa* and *P. praetexta*, may adapt to living in polluted environment better than other species.

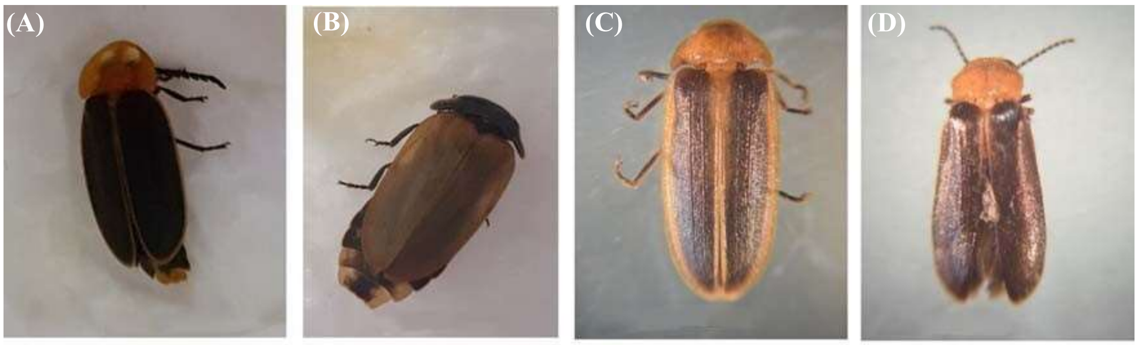

Figure 2 Examples of the collected firefly samples in this study (A) *Pyrocoelia praetext,* (B) *Lamprigera tenebrosa,* (C) *Asymmetricata circumdata,* (D) *Medeopteryx* sp.

The para-rubber plantation we studied had a lot of leaf litter on the ground, which created a lot of dampness for firefly larvae to live in which could be responsible for it having the most diverse set of firefly species in this study. Despite the fact that this plantation did not use insecticide, a significant quantity of oxamyl was detected. This might be due to insecticide residues leaching from surrounding orchards. Both *L. tenebrosa* and *P. praetexta* found acceptable dwelling places in the mixed orchards, but no other species were detected in this environment. *L. tenebrosa* was the sole occupant of vegetable garden No.1, which was surrounded by human residences. Similarly, fireflies were less abundant in the banana plantation, with only *P. praetexta* larvae detected on the ground or in the old banana stalks. The interference from human activities, such as light pollution from neighboring households and chemical leftovers from other agricultural operations, resulted in fewer firefly species occupying these places.

Carbamate pesticides were detected in soil samples obtained from all of the sample sites. The detections revealed two carbamate residues, methomyl, and oxamyl (Table 3). Methomyl was discovered only in the soil sample from the mixed orchard No. 1 (0.011±0.005 mg/kg), which was lower than the standard MRL. Five soil samples contained oxamyl, although there were no significant differences among them. The greatest amount of oxamyl residue was found in vegetable garden No.2 (0.110±0.024 mg/kg), while the lowest level was found in the banana plantation (0.082±0.010 mg/kg). Even though the para rubber plantation was identified as the finest habitat for fireflies, oxamyl residues were detected. The pesticide levels at all of the sampling sites were within the normal MRL range.

Table 3 The distribution of carbamate residues (mg/kg) in soil form the study samples.

Insecticides	methomyl (mg/kg) (n=3)	oxamyl (mg/kg) (n=3)
Sample 1 (mixed orchard No. 1)	0.011±0.005	nd
Sample 2 (mixed orchard No. 2)	nd	0.105±0.005
Sample 3 (para rubber plantation)	nd	0.102±0.023
Sample 4 (vegetable garden No. 1)	nd	0.090±0.005
Sample 5 (vegetable garden No. 2)	nd	0.110±0.024
Sample 6 (banana plantation)	nd	0.082±0.010

The values are expressed as mean ± SD. nd: non-detectable. n: represents number of samples in each group.

Methomyl : MRL = 0.02-2 mg/kg in agricultural products including fresh vegetables, grains, oils and meats referred from Thai Agricultural Standard TAS 9002-2556; Pesticide Residues (National Bureau of Agricultural Commodity and Food Standards, 2013).

Oxamyl: MRL = 0.01-5 mg/kg in agricultural products including fresh vegetables, fruits, nuts, meat, giblet and offal, and milk (Codex Alimentarius International Food Standards, 2021).

Oxamyl is a widespread insecticide/nematicide, whereas methomyl is used to control insect pests. As a result, oxamyl and methomyl deposition has been documented [11,33]. Our findings revealed varying levels of oxamyl and methomyl contamination in soils from various sample locations, which might be attributed to farmer activities and soil types. Insecticide residues can affect non-target organisms, particularly soil-dwelling invertebrates that serve an important role as firefly prey [18]. Terrestrial firefly larvae spend most of their life on soil and feed on snails, earthworms, and other soft-bodied invertebrates [34].

Residual insecticides can indirectly affect firefly populations by decreasing the availability of their prey. This has been demonstrated in highly poisonous imidacloprid and other neonicotinoids in earthworms [17], which are the primary prey of the *Photinus* fireflies in North America [35]. Carbamate pesticide residues discovered in this study, together with the presence of fireflies in all study sites, suggested that certain firefly species are capable of surviving in lightly contaminated habitats. Because carbamate insecticides degrade gradually, farmers apply them frequently to get the maximum results in controlling insect pests. Long-term exposure of fireflies to insecticide toxicity induced by long-term application of insecticides, as well as contaminants leaking from adjacent areas, may generate a gradual biochemical transformation resulting in firefly tolerance via physical and/or behavioral changes. The chemical-based agriculture production system consequently leads to environmental problems that result in the loss of natural habitats and changes in biodiversity. Therefore, at the policy level, reducing the application of carbamate insecticide residue via promoting agroecology-based agriculture, such as applying ecological and social concepts to the design and management of food and agricultural process may establish long-term sustainability in the ecosystem [36]. At the farm level, using the proper amount and timing of carbamate, along with the spray boom height, and droplet size should be optimized to increase the insecticides effectiveness while minimizing pesticide use. To reduce the harmful effects of insecticides on non-target organisms, including vulnerable creatures, alternative environmentally friendly methods for controlling agricultural pests should be taken into consideration.

4. Conclusion

This study revealed the presence of fireflies in carbamate insecticide contaminated areas. Six separate agricultural regions, including sampling sites which not directly sprayed with insecticides, but were contaminated from surrounding orchards. Four species of fireflies were collected and identified from these habitats; *L. tenebrosa*, *P. praetexta*, *A. circumdata*, and *Mediopteryx* sp. *L. tenebrosa* were found to be highly adapted to surviving in 5 of the 6 contaminated sites, followed by *P. praetexta* which exists in 4 of the sampling sites. Common species, *A. circumdata*, was observed in the para rubber plantation and the upland vegetable garden. Meanwhile *Mediopteryx* sp. was only observed in the para rubber plantation, suggesting it is more sensitive to pollution. The para rubber plantation was a suitable environment since the ground supplied ample moisture for both fireflies and their prey. However, the detection of oxamyl and methomyl insecticide residues in all sampling sites indicated that those firefly species were well adapted to living in insecticide polluted habitats.

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