รายงานวิจัยฉบับสมบูรณ์

โครงการ ประสิทธิภาพของไส้เดือนฝอยศัตรูแมลงในการควบคุมแมลงวันผลไม้ศัตรูพริก (*Bactrocera latifrons* Hendel) ในสภาพไร่

โดย นางสาวประกายจันทร์ นิ่มกิ่งรัตน์

มีนาคม พ.ศ. 2562

สัญญาเลขที่ MRG6080069

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CONTENTS PAGE

1. Abstract (ภาษาไทย)

ไส้เดือนฝอยศัตรูแมลง เป็นหนึ่งในศัตรูธรรมชาติที่มีประสิทธิภาพสูงในการนำมาใช้ควบคุมแมลงกลุ่มแมลงวันผลไม้ ถึงแม้ว่าแมลงวันผลไม้ศัตรูพริกจะเป็นแมลงศัตรูหลักที่สำคัญและสร้างความเสียหายให้กับผลผลิตพริกเป็นอย่างมาก แต่จากการศึกษาที่ผ่านมายังไม่มีรายงานการใช้ไส้เดือนฝอยศัตรูแมลงในแมลงวันผลไม้ศัตรูพริกมาก่อน ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของการใช้ไส้เดือนฝอยศัตรูแมลงเพื่อควบคุมแมลงวันผลไม้ศัตรูพริก การทดลองในสภาพห้องปฏิบัติการถูกแบ่งออกเป็น 3 หัวข้อหลัก ได้แก่ 1) การศึกษาค่าความเป็นพิษเฉียบพลันของไส้เดือนฝอยที่มีต่อหนอนแมลงวันผลไม้ศัตรูพริกวัยสุดท้าย ที่อัตราแตกต่างกัน 0 1,000 1,500 2,000 2,500 และ 3,000 ตัวต่อหนอน พบว่า ไส้เดือนฝอย *S. siamkayai* สามารถเข้าทำลายระยะหนอนวัยสุดท้ายของแมลงวันผลไม้ศัตรูพริกได้ดีที่สุดและแตกต่างกันทางสถิติกับไส้เดือนฝอย *S. carpocapsae* และ *H. bacteriophora* ในทุกอัตรา และพบอัตราการตายสูงสุดที่อัตราความเข้มข้น 3,000 ตัวต่อหนอน เท่ากับ 94.44% และมีค่าความเป็นพิษเฉียบพลัน (LD_{50}) และ (LD_{90}) เท่ากับ 1,246 และ 2,327 ตัวต่อหนอน ขณะที่ค่าความเป็นพิษเฉียบพลันของไส้เดือนฝอยที่มีต่อดักแด้ที่อัตราแตกต่างกัน คือ 0 2,000 2,500 3,000 3,500 และ 4,000 ตัวต่อดักแด้ พบว่า ไส้เดือนฝอย *S. siamkayai* สามารถเข้าทำลายระยะดักแด้ของแมลงวันผลไม้ศัตรูพริกได้ดีที่สุดและแตกต่างกันทางสถิติกับไส้เดือนฝอย *S. carpocapsae* และ *H. bacteriophora* ในทุกอัตราและพบอัตราการตายสูงสุดที่อัตราความเข้มข้น 4,000 ตัวต่อดักแด้ เท่ากับ 86.66% และมีค่าความเป็นพิษเฉียบพลัน (LD_{50}) และ (LD_{90}) เท่ากับ 2,292 และ 4,224 ตัวต่อดักแด้ 2) การทดสอบประสิทธิภาพการค้นหาเหยื่อของไส้เดือนฝอยศัตรูแมลงในดักแด้แมลงวันผลไม้ศัตรูพริกที่ระยะทางแตกต่างกันดังนี้ 0 2.5 5 7.5 10 12.5 และ 15 เซนติเมตร พบว่าไส้เดือนฝอย *S. siamkayai* มีความสามารถในการค้นหาดักแด้ในแนวราบได้ดีที่สุดช่วงระยะทาง 0 และ 2.5 เซนติเมตร โดยมีเปอร์เซ็นต์การตายของดักแด้เท่ากับ 96.25 และ 97.5% ตามลำดับ และยังสามารถเคลื่อนที่เข้าทำลายดักแด้ในระยะทางสูงสุดคือ 15 เซนติเมตร โดยมีเปอร์เซ็นต์การตายของดักแด้เท่ากับ 2.5% และ 3) การศึกษาผลของสารป้องกันกำจัดแมลงต่างชนิดที่มีต่อการมีชีวิตรอดของไส้เดือนฝอยศัตรูแมลง เมื่อผสมไส้เดือนฝอยร่วมกับสารอิมิดาโคลพริด (imidacloprid) สารไทอะมีโทแซม (thiamethoxam) สารไซเพอร์เมทริน (cypermethrin) และ สารคาร์โบซัลแฟน (carbosulfan) พบว่าไส้เดือนฝอยที่ผสมร่วมกับสารอิมิดาโคลพริดมีเปอร์เซ็นต์การรอดชีวิตของไส้เดือนฝอยสูงที่สุด และไม่มีความแตกต่างกันกับกรรมวิธีที่ผสมไส้เดือนฝอยกับน้ำกลั่น ซึ่งแตกต่างกับไส้เดือนฝอยที่ผสมร่วมกับสารไทอะมีโทแซม สารไซเพอร์เมทริน และ สารคาร์โบซัลแฟน โดยมีเปอร์เซ็นต์การรอดชีวิตเท่ากับ 56.5 15.5 และ 0.75% ตามลำดับ การศึกษาผลของสารป้องกันกำจัดแมลงต่างชนิดที่มีต่อการความรุนแรงของไส้เดือนฝอยศัตรูแมลงหลังผสมไส้เดือนฝอยกับสารไทอะมีโทแซมและสารอิมิดาโคลพริดทำให้ประสิทธิภาพการเข้าทำลายดักแด้ลดลงเหลือเพียง 52.50 และ 45% ตามลำดับ และมีความแตกต่างกันทางสถิตกับการใช้ไส้เดือยฝอยศัตรูแมลงเพียงอย่างเดียวที่สามารถทำให้ดักแด้ตายได้สูงถึง 87.50% ผลการทดสอบในสภาพโรงเรือน แสดงให้เห็นว่าประสิทธิภาพการควบคุมแมลงวันผลไม้ศัตรูพริกในโรงเรือนที่พ่นด้วยไส้เดือน

ฝอยนั้นมีสูงถึง 70.86% เมื่อเทียบกับโรงเรือนที่ไม่มีการจัดการ ขณะที่ผลการควบคุมในสภาพไร่ ไส้เดือนฝอยยังคงมีประสิทธิภาพในการควบคุมแมลงวันผลไมศัตรูพริกได้สูงถึง 65.04%

คำหลัก: ไส้เดือนฝอยศัตรูแมลง, แมลงวันผลไม้ศัตรูพริก, *Bactrocera latifrons*, การป้องกันกำจัดศัตรูพืชโดยชีววิธี

Abstract (English)

As part of Biological control method, entomopathogenic nematodes (EPNs) has been found to be a highly effective bio-agent against many species of fruit fly but none of them was reported on chilli fruit fly, though it is the most important key pest in chilli which can cause tremendous yield losses. Therefore, the objective of this study was to assess the control ability of EPNs against chili fruit fly. Laboratory experiment was divided into 3 phases as follows: 1) The median lethal dose of three different EPNs species on final instar larvae of chili fruit fly at different concentrations of 0, 1,000, 1,500, 2,000, 2,500 and 3,000 IJs/larva were performed. Result revealed that *S. siamkayai* was superior to *S. carpocapsae* and *H. bacteriophora* in all concentrations with the 94.44% control rate when applied 3,000 IJs/larva. The median lethal dose (LD_{50}) and (LD_{90}) of *S. siamkayai* were 1,246 and 2,327 IJs/larva, respectively. The median lethal dose at different concentrations of 0, 2,000, 2,500, 3,000, 3,500 and 4,000 IJs/pupa from different EPNs species were also obtained on chilli fruit fly pupal stage. *S. siamkayai* showed the highest control rate compared to *S. carpocapsae* and *H. bacteriophora* in all concentrations with the mortality rate at 86.66% after applied 4,000 IJs/pupa. The LD_{50} and LD_{90} on pupa were 2,292 and 4,224 IJs/pupa. 2) The nematode ability on host searching at different distances of 0, 2.5, 5, 7.5, 10, 12.5 and 15 cm. was tested. *S. siamkayai* can search for chili fruit fly pupa within 0 and 2.5 cm. with the mortality rate of pupa at 96.25 and 97.5%, respectively. The farest distance was measured at 15 cm. with the mortality rate of pupa at 2.5%. and 3) The interaction of *S. siamkayai*, and four common types of insecticides, namely imidacloprid, thiamethoxam, cypermethrin and carbosulfan were examined concerning to EPNs survival and infectivity. The results indicated that the survival rate of EPNs mixed with imidacloprid was not statistically significantly different to insecticide-free EPNs. The survival rate for other EPNs and insecticide mixtures, were significantly different from the insecticide-free EPNs [thiamethoxam (56.50%), cypermethrin (15.50%) and carbosulfan (0.75%)]. The surviving EPNs were then tested against chili fruit fly pupae compared to insecticide-free EPNs (control). The highest mortality rate was observed in the control (87.50%). Mortality was significantly low in EPNs treated with thiamethoxam (52.50%) and imidacloprid (45%) ($P \leq 0.05$). The net house and field experiments were conducted in order to compare the efficacy of EPNs and without management methods. The control efficacy from EPNs treated net house gave a better result compared to untreated net house at 70.86%. Similar result was found when applied EPNs under field condition. The control efficacy of EPNs under field condition can reach up to 65.04%.

Keywords: entomopathogenic nematodes, chili fruit fly, *Bactrocera latifrons,* biological control

2. Objectives

1. To monitor and evaluate a natural chili fruit fly population and its natural enemy
2. To investigate the effect of EPNs against chili fruit fly under laboratory, semi-filed and field conditions

3. Introduction to the research problem and its significance

Chili pepper is one of the most widely grown and important vegetables among other horticultural crops in Thailand. Each year farmers need to produce chili peppers to reach of both domestic and international market demands, resulting in the expansion of planting areas toward the country. Office of Agricultural Economics reported the total production in 2014 was 46,166 tons (1,900 Kilogram/ha) and the export volume of fresh and frozen chili fruit was 392 million Baht (11,229,210 Kilogram), which was obviously higher than 2013 (Office of Agricultural Economics, 2014; Ministry of Commerce, 2015). Mainly chili plantations located in the North East followed by the North and East of the country.

Although the entire country is able to produce high amount of chili peppers, but part of them were lost during production process by insect pest infestation. The insect infestation not only gives direct impact on production, but also on price and market effects, food security and nutrition and financial costs. More than 35 species of chili insect pests were reported including broad mite, whitefly, thrip, aphid, mealybug, cutworm and chili fruit fly (Siri et al., 2010). However, the only key pest which gives a serious damage during fruit developmental stage was chili fruit fly (*Bactrocera latifrons* Hendel (Diptera: Tephritidae)) while this insect was recently recorded as important insect pest in the world list. Vijaysegaran and Osman (1991) reported that chili fruit fly can tremendously increase yield loss up to 60-80 % and give direct impact on fruit's quality and quantity. Wingsanoi and Siri (2011a,b) reported the result of field survey in Thailand and found the number of damaged fruits and infested fruits by chili fruit fly per plant were 43% and 20 fruits/plant, respectively. Fruit fly infestation starts from adult female lay eggs below the skin or soft tissues of the fruit. Once the eggs hatch, the larvae feed within the fruit, the larval tunnels provide entry points for secondary microorganisms that cause the fruit to rot and drop to the soil (Siri et al., 2007; Stonehouse et al., 2004). Pupariation is in the soil under the chili plant and adults occur throughout the year. Initial fruit damage is hardly noticeable because larvae are very small and feed inside the fruit, and this can result in late control action. Study of Srikacha et al. (2008) showed that the final instar larvae will drop and turn into pupal stage in the ground. Therefore, one possibility to prevent the occurrence of adult before start new generation is to eliminate either last instar larvae or pupae in the soil.

In general, farmers often use insecticides to protect their crops from fruit fly. The misuse of insecticides for a long period of time leading to cross resistance, high production cost and toxic residues in product, human, natural enemies and environment. These chemicals, moreover, are unable to reach the larvae inside the fruits. In order to encourage farmers to reduce the use of insecticides in their crops and produce safe products through organic marketing channels, biological control agents such as entomopathogenic nematode are widely recommended.

The entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are highly effective biological control agents against mainly soil-dwelling insect pests. In many part of the world like Europe and North America, the entomopathogenic nematodes (EPNs) are now practically used under the field in many crops such as orchards, soft fruits, vegetables, production nurseries, greenhouses and turfgrass (Grewal et al., 2005). An effective mode of action of nematodes starts when a single free-living stage, the dauer juvenile (DJ), locates suitable insect host and penetrates through the mouth, anus or spiracle (Nimkingrat et al., 2014). After making way to haemocoel, the nematodes release a symbiotic bacterium from its anterior part of its intestine (Ehlers, 2001). The death of insect host occurs within 24-48 h after the invasion of nematode through haemolymph (Simoes and Rosa, 1996). The DJ continues to feed on bacteria and digests host tissues, develops to adult stage and then reproduces one or more generations depending on available resources. The last generation of nematodes is produced while the host nutrients are depleted, then nematode leave the host cadaver and seek new hosts (Poinar, 1990). Few studies are focused only on the use of EPN in other species of fruit fly ie. the Olive fruit fly (*Bactrocera oleae*), Peach fruit flies (*B. zonata*), Oriental fruit flies (*B. dorsalis*), Melon fruit fly (*B. cucurbitae*), Queensland fruit fly (*B. tryoni*) and Mediterranean fruit fly (*Ceratitis capitate*) (Lindegren, 1990; Lin et al., 2005; Soliman, 2014; Sirjani et al., 2009; Langford et al., 2014) but none of them were addressed on chili fruit fly, *Bactrocera latifrons.* Therefore, to provide baseline information of the possibility to use EPNs against chili fruit fly, study of the effect of EPNs alone and the combination of EPNs and other control agents on the mortality of chili fruit fly, *Bactrocera latifrons,* under laboratory, semi-field and field conditions must take urgent action.

4. Schedule for the entire project and expected outputs

Activity	**1st Year**		**2nd Year**	
	Q1	**Q2**	**Q1**	**Q3**
Monitoring and evaluation of chili fruit fly and its natural enemy in Khon Kaen province.	**X**	**X**		
Effect of EPNs against chili fruit fly under laboratory condition.				
- *Mass rearing*	**X**	**X**		
- *Virulence test against chilli fruit fly larva and pupa*	**X**			
- *Host finding ability*	**X**	**X**		
- *Effects of insecticides on movement of EPNs*		**X**		
Effect of EPNs against chili fruit fly under semi-field condition.			**X**	**X**
Effect of EPNs against chili fruit fly under field condition.				**X**
Data analysis				**X**
Final report and publication				**X**

5. Expected outputs

Expected manuscript title: Interaction between insecticides and Entomopathogenic nematode against chili fruit fly pupae (*Bactrocera latifrons* Hendel).

Expected journal name: BioControl

Impact factor: 1.767

ISI database: Q1

6. Content Research

6.1 Materials and methods

6.1.1 Monitoring and evaluation of chili fruit fly and its natural enemy in Khon Kaen province.

Study of fruit fly populations in 4 chili plantations (variety Superhot) as follows: 1) Ban Na Fai Nuea village (1 plot), Ban None village (2 plots) and Ban Mor village (1 Plot) in Khon Kaen Province (Table 1). Plot size from each plot was 15*12 m. and planting distance was 0.5* 0.5 m. Systemic sampling of insect population was used as shown in figure 1A. Individual plant (N=20) was randomly sampled Guard rows were not included in the sampling area. Data were collected starting from flowering to fruiting stages. Damaged fruits were carried back to laboratory for counting number of fruit fly. All data were analyzed by using XLSTAT 2006 (XLSTAT, New York, NY, USA) and the Tukey's HSD test program was used to to compare the differences between plots.

Table 1 Detail of chili plantation in Khon Kaen province

Plot No.	Farmer name	N°	E°	Location
1	Mrs. Boonyuen Donmuen	16°43'35.6"	103°00'21.1"	Ban Na Fai Nuea village, sub-district, Nam Pong district
2	Mrs. Ubon Jontong	16°32'13.2"	102°58'21.9	Ban None village, Ban None sub-district, Samsung district
3	Mr. Saap Noneting	16°31'24.1"	102°58'32.9	Ban None village, Ban None sub-district, Samsung district
4	Mrs. Jintana Lakornket	16°30'27.2"	102°59'09.9	Ban Mor, Kukam sub-district, Samsung district

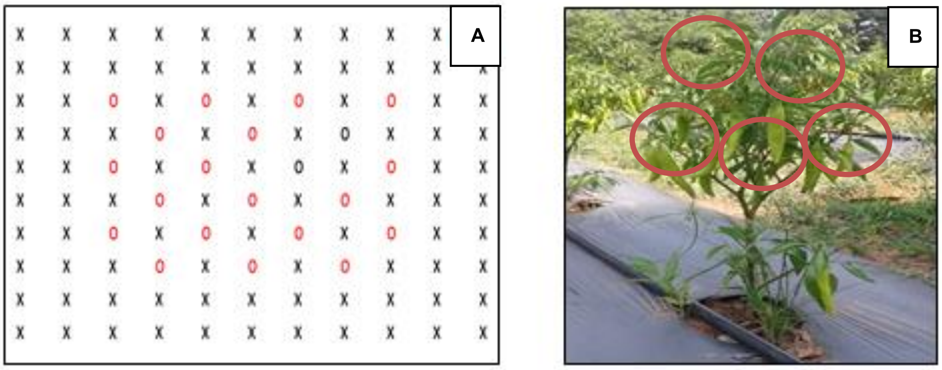

Figure 1 Sampling layout of insect pest and natural enemy populations in chili (A) and sampling site on the chilli plant (B)

6.1.2 Effect of EPNs against chili fruit fly under laboratory condition.

6.1.2.1 Mass rearing

Nematode

The EPNs were propagated on last instars of the wax moth *Galleria mellonella* L. as described by Kaya and Stock (1997). The Dauer Juveniles (DJs) were kept in storage solution (11.25 g NaCl, 0.525 g $CaCl_2$ x 2 H_2O, 0.315 g $MgSO_4$, 0.1 g ascorbic acid in 1 L. distilled water) at 25 °C and used within 1 week after harvest (Fig. 2).

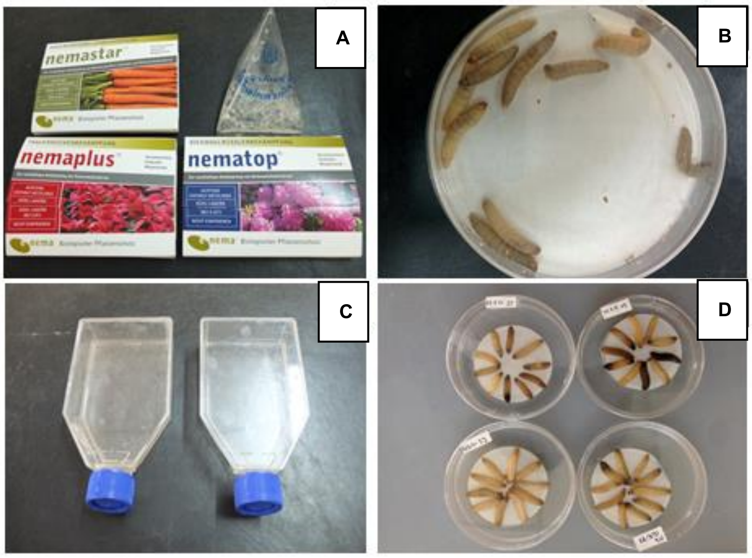

Figure 2 Entomopathogenic nematodes: entomopathogenic nematodes stock culture (A), Paper assay (B), entomopathogenic nematodes in storage bottle (C) and White trap (D)

Chili fruit fly

Gravid female fruit flies were placed inside the plastic cup size 7*9 cm. with 10% papaya juice and papaya slices as food and oviposition site (Fig. 3A). After oviposition, eggs werer then transferred into a new rearing box on artificial diet (Fig. 3B) and keep for pupation in sand. Soon after eclosion, male and female adults were transferred into a net cage for mating. Water and yeast extract mixed with honey were used to feed male and female adults. Offspring from F2 are then able to use for experiment (Fig. 3).

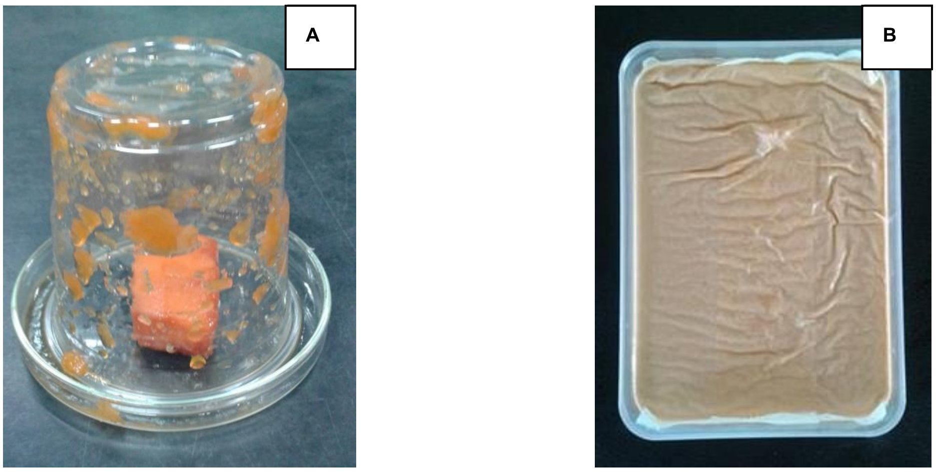

Figure 3 Oviposition site of female chili fruit flies (A) and artificial diet (B).

6.1.2.3 Virulence test of EPNs against final instar larva of chili fruit fly

Completely Randomized Design (CRD) was used in this experiment with 6 treatments and 4 replications for assess the median lethal dose: LD_{50} by using sand assay. Ten final instar larvae of chili fruit fly per replication were placed into petri dish which was filled with 10% moist sand. EPNs were sprayed as the following treatments:

Treatment 1	0 IJ/larva (Control)
Treatment 2	1,000 IJs/larva
Treatment 3	1,500 IJs/larva
Treatment 4	2,000 IJs/larva
Treatment 5	2,500 IJs/larva
Treatment 6	3,000 IJs/larva

Petri dishes were then transferred into dark room 25±2 ºC. Dead and alive larvae were counted for 5 days (Fig. 4). LD_{50} and LD_{90} were analyzed by using Probit analysis (XLSTAT, New York, NY, USA) and Tukey's HSD test ($P \leq 0.05$) was used to compare the differences between treatments.

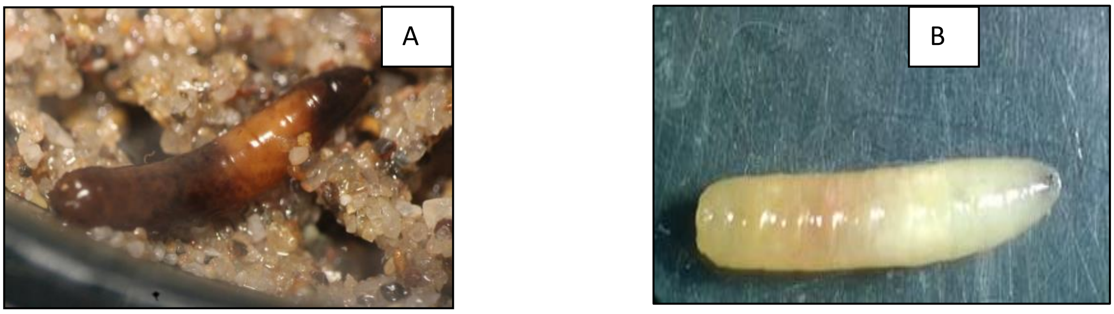

Figure 4 Dead (A) and alive larva of fruit fly (B)

6.1.2.4 Virulence test of EPNs against pupa of chili fruit fly

Completely Randomized Design (CRD) was used in this experiment with 6 treatments and 4 replications for assess the median lethal dose: LD_{50} by using sand assay. Ten pupae per replication were placed into petri dish which was filled with 10% moist sand. EPNs were sprayed as the following treatments:

Treatment 1 0 IJ/pupa (Control)

Treatment 2 2,000 IJs/pupa

Treatment 3 2,500 IJs/pupa

Treatment 4 3,000 IJs/pupa

Treatment 5 3,500 IJs/pupa

Treatment 6 4,000 IJs/pupa

Petri dishes were then transferred into dark room 25±2 ºC. Dead and alive pupae were counted for 5 days (Fig. 5). LD_{50} and LD_{90} were analyzed by using Probit analysis (XLSTAT, New York, NY, USA) and Tukey's HSD test ($P \leq 0.05$) was used to compare the differences between treatments.

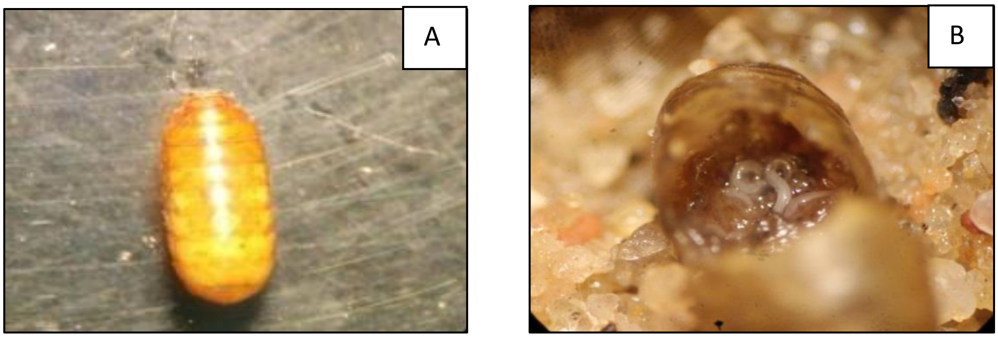

Figure 5 Dead (A) and alive pupa of fruit fly (B)

6.1.2.5 EPNs host finding ability

Four thousand EPNs per pupa were sprayed according to Completely Randomized Design (CRD) with 4 treatments and 4 replications by using sand assay. Ten pupae per replication were placed into plastic box size 30*20*8 cm., which was filled with 10% moist sand, far from the EPNs releasing spots as follows:

Treatment 1	0	cm. (Control)
Treatment 2	2.5	cm.
Treatment 3	5	cm.
Treatment 4	7.5	cm.
Treatment 5	10	cm.
Treatment 6	12.5	cm.
Treatment 7	15	cm.

Petri dishes were then transferred into dark room 25±2 ºC. Dead and alive pupae were counted for 5 days (Fig. 4). LD_{50} and LD_{90} were analyzed by using Probit analysis (XLSTAT, New York, NY, USA) and Tukey's HSD test ($P \leq 0.05$) was used to compare the differences between treatments.

6.1.2.6 Effect of insecticides on EPNs survival

Completely Randomized Design (CRD) was used in this experiment with 5 treatments and 4 replications. One hundred EPNs per replication were placed into petri dish which were mixed with different insecticides as follows:

Treatment 1	EPNs mixed with water (Control)
Treatment 2	EPNs mixed with imidacloprid (3g/ 20L. of water)
Treatment 3	EPNs mixed with carbosulfan (110 CC./ 20L. of water)
Treatment 4	EPNs mixed with thiamethoxam (2g/ 20L. of water)
Treatment 5	EPNs mixed with cypermethrin (10 CC/ 20L. of water)

Petri dishes were then transferred into dark room 25±2 ºC. Dead and alive EPNs were counted for 3 days. Tukey's HSD test ($P \leq 0.05$) was used to compare the differences between treatments.

6.1.2.7 Effect of insecticides on EPNs virulence

Completely Randomized Design (CRD) was used in this experiment with 3 treatments and 4 replications. One hundred EPNs, which survived more than 50% from previous studied, were first washed to remove insecticides residue on cuticle and then sprayed onto 10 pupae per replication by using sand assay as follows:

Treatment 1 EPNs mixed with water (Control)

Treatment 2 EPNs after mixed with imidacloprid

Treatment 3 EPNs after mixed with thiamethoxam

Petri dishes were then transferred into dark room 25±2 ºC. Dead and alive pupae were counted for 5 days. Tukey's HSD test (P≤ 0.05) was used to compare the differences between treatments.

6.1.3 Effect of EPNs against chili fruit fly under semi-field condition

Completely Randomized Design (CRD) was used in this experiment with 2 treatments and 3 replications. Thirty chilli plant (Var. Superhot) were grown inside plastic pot with 10 inch diameter under individual net house size 5*10*8 m.. Gravid females were released into net house when fruit setting for oviposition. Only 20 damaged fruits per plant were left. One week later, after final instar larvae dropped to the ground, EPNs were sprayed as follows:

Treatment 1 Water only (Control)

Treatment 2 EPNs (20 million IJs/ 5 L. of water)

Adults which were emerged from pupae were counted on sticky traps. Data were analyzed by using Tukey's HSD test (P≤ 0.05) to compare the differences between treatments.

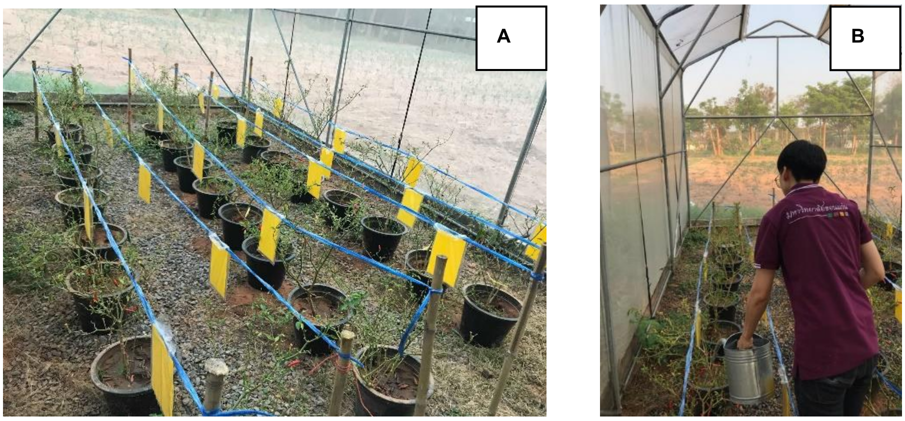

Figure 6 Net house experiment: Sticky traps were used to collect fruit fly adult (A) and EPNs spray (B)

6.1.4 Effect of EPNs against chili fruit fly population under field condition

Randomized Complete Block Design (RCBD) was used in this experiment with 2 treatments and 4 blocks. Fifty plants per treatment under an individual block (total 100 plants per block). Superhot variety was grown until fruiting stage. Twenty net cages per treatment per block were randomly covered chilli plants. Gravid female were then released into the cage for oviposition. Only 20 damaged fruits per plant were left. One week later, after final instar larvae dropped to the ground, EPNs were sprayed as follows:

Treatment 1 Water only (Control)

Treatment 2 EPNs (20 million IJs/ 5 L. of water)

Adults which were emerged from pupae were counted on sticky traps. Data were analyzed by using Tukey's HSD test (P≤ 0.05) to compare the differences between treatments.

Figure 7 Field experiment: Sticky traps were used to collect fruit fly adult (A) and chilli fruits were removed during experiment

6.2 Result and discussion

6.2.1 Monitoring of chili fruit fly and its natural enemy population in Khon Kaen province.

Numbers of damaged fruit from all plots were not significantly different with fruit number of 7.5 2.33 5.67 and 5.33 fruit/plant unlike undamaged fruit per plant from plot 2 which were significantly different from plot 1, 3 and 4 with fruit number of 24.33 57.00 58.00 and 48.67 fruit/plant, respectively (Table 2). Similar result to percentage of crop loss and number of fruit fly pupa per fruit, there were no significantly different among all plots at 13.15, 9.57, 9.83 and 10.87% and 1.11, 1.00, 1.12 and 1.06 pupa/fruit, respectively. Percentage of emergence of the adult gave no different in all plots at 75.72, 88.89, 87.50 and 88.89%, respectively. In addition, *Diachasmimorpha longicaudata* was the only parasitoid that could be found in all plots with the number of 1.00, 0.67, 0.33, and 0.33 parasitoid/fruit, respectively (Table 3). Plot number 2 was initially infested by thrips and aphids during the vegetative stage, which resulting in yield loss and fruit quality but it gave no effect on number of fruit fly and its natural enemy. Wingsanoi and Siri (2011a) report that pupa of fruit fly in Khee-noo hom KKU 40 was found only 2.5 pupa/fruit and percentage of emergence of adult was over 80% which is similar to our result in all surveyed plots. Poramacom (2000) suggested that shape and size of chili fruit are the main factors that attracted female fruit fly. It seemed that female fruit fly prefers to oviposit their eggs inside larger fruit.

Table 2 Number of damaged and undamaged fruit from farmer plots in Khon Kaen province

Plot No.	Undamaged fruit (fruit/plant)[1/]	Damaged fruit (fruit/plant)[1/]	Crop loss (%)[1/]
1	57.00 A	7.5 A	13.15 A
2	24.33 B	2.33 A	9.57 A
3	58.00 A	5.67 A	9.83 A
4	48.67 A	5.33 A	10.87 A

[/1] Means followed by the same letter are not significantly difference at 95% (P≤ 0.05)

Table 3 Number of fruit fly and its natural enemy which collected from Khon Kaen province

Plot No.	Number of pupa of fruit fly (pupa/fruit)[1/]	Number of emergence of adult (individual/fruit)[1/]	Number of *Diachasmimorpha longicaudata* (individual/fruit)[1/]
1	1.11 A	75.72 A	1.00 A
2	1.00 A	88.89 A	0.67 A
3	1.12 A	87.50 A	0.33 A
4	1.06 A	88.89 A	0.33 A

[/1] Means followed by the same letter are not significantly difference at 95% ($P \leq 0.05$)

6.2.2 Virulence test of Entomopathogenic nematode against final instar larva of chili fruit fly

Results after treated last instar larvae with three different species of EPNs at different doses of 0, 1,000, 1,500, 2,000, 2,500 and 3,000 IJs/larva showed that *S. siamkayai* gave the lowest LD_{50} and LD_{90} at 1,246 and 2,327 IJs/larva, respectively. Followed by *S. carpocapsae* and *H. bacteriophora* with LD_{50} and LD_{90} at 1,710, 3,428 and 2,074, 4,260 IJs/larva, respectively (Table 4). Gehan and Mona (2014) studies gave comparable result to our finding. They found that at 25 ºC *S. carpocapsae* was superior than *H. bacteriophora* in controlling *B. zonata* with the LC_{50} value of 2,040 and 2,562 IJs/larva, respectively. Slightly different result was addressed by the work of Fetoh et al. (2011) where LC_{50} on final instar larva of *B. zonata* after treated with *S. carpocapsae* and *H. bacteriophora* were lower than our result with the value of 325.3 and 540.2 IJs/larva, respectively. Drees et al. (1992) explained that species of EPNs, species of insect host, EPNs concentration and methodology can play an important role or it can be host finding behavior of EPNs that take part of it. Jame and Gaugler (1993) suggested that cruiser EPNs are suitable to the host that are less active such as pupa or grub while ambusher EPNs are more suitable to active host like maggot or caterpillar. As from our result, *S. carpocapsae* is ambusher EPNs so they can locate on maggot better than *H. bacteriophora* whose is cruise EPNs. However, there is no study on *S. siamkayai* movement behavior but it can imply from this result that they can have intermediate movement abilities.

Table 4 Median lethal dose of EPNs on final instar larva of fruit fly

EPNs species	LD_{50} [1/]	LD_{90} [1/]
Steinernema siamkayai	1,246 A	2,327 A
Steinernema carpocapsae	1,710 B	3,428 B
Heterorhabditis bacteriophora	2,074 C	4,260 C

[/1] Means followed by the same letter are not significantly difference at 95% ($P \leq 0.05$)

Moreover, *S. siamkayai* can kill the last instar larva of fruit fly better than *S. carpocapsae* and *H. bacteriophora* at all concentration with the mortality rate of 94, 85.55 and 67.77%, respectively (Table 5).

Table 5 Mortality rate of last instar larva after treated with EPNs

Concentration (IJs/larva)	Mortality rate of last instar larva of fruit fly (%) ±SD [1/]			F-test	CV (%)
	Steinernema siamkayai	***Steinernema carpocapsae***	***Heterorhabditis bacteriophora***		
0 (Control)	1.11±0.57 Ae	2.22±0.57 Ae	3.33±1.00 Ae	ns	9.18
1,000	47.77±1.52 Ad	41.33±1.52 ABd	35.55±1.15 BCd	*	11.86
1,500	62.22±1.15 Ac	48.88±0.57 Bcd	44.44±1.52 Bcd	*	10.22
2,000	82.22±2.30 Ab	53.33±1.00 Bc	48.88±2.08 Bbc	*	9.47
2,500	93.33±1.57 Aa	64.44±0.57 Bb	54.44±1.52 Cb	*	6.40
3,000	94.44±0.57 Aa	85.55±1.52 Ba	67.77±1.52 Ca	*	5.72
F-test	*	*	*	*	
CV (%)	7.05	6.99	10.89	11.86	

[/1] Means followed by the capital and small letters in the same column and row are not significantly difference at 95% ($P \leq 0.05$)

6.2.3 Virulence test of Entomopathogenic nematode against chilli fruit fly pupa

Significantly different results were found when applied three different species of EPNs on pupa of chili fruit fly at the different concentrations at 0, 2,000, 2,500, 3,000, 3,500 and 4,000 IJs/pupa. LD_{50} and LD_{90} of *S. siamkayai* gave the lowest value of 2,293 and 4,224 IJs/pupa, respectively (Table 6). Followed by *S. carpocapsae* with 3,054 and 4,801 and *H. bacteriophora* with 3,602 and 5,552 IJs/pupa, respectively. Result from Gehan and Mona (2014) also gave the same trends. *S. carpocapsae* gave lower value of LC_{50} than *H. bacteriophora* on pupa of *B. zonata* at 25 C° with the value of 2,260 and 2,889 IJs/pupa, respectively. Fetoh et al. (2011) performed even lower LC_{50} and LD_{90} at 540.2, 235 and 1,785, 1,167 IJs/pupa after treated with *S. carpocapsae* and *H. bacteriophora*, respectively. These differences can be explained by the different of age of pupa or the fitness of EPNs. Abbas and Basma (2009) also tested their *S. riobravis* on pupal stage of *B. zonata* at the concentration of 25, 50, 100, 200 and 400 IJs/cm^2. Their result indicated that *S. riobravis* at 200 and 400 IJs/cm^2 can kill pupa only 8%. Barbercheck and Kaya (1991) suggested that high mortality of host depended on EPNs species, number of EPNs penetrate to host and size of host. Another possibility is that *S. siamkayai* is superior than *S. carpocapsae* and *H. bacteriophora* might come from their body size (length 446 and width 21 *μm*) which is relatively smaller than *S. carpocapsae* and *H. bacteriophora* with 558, 25 and 570, 24 *μm,* respectively (Stock et al., 1998). The small size of body can help them easily penetrate through natural opening holes of insect host. Moreover, species of insect host can be one of the reasons that could help EPNs to be success to locate host. Different insect morphology such as size of natural opening holes, thickness of cuticle, scale or hair on insect body can give a significant on host mortality (Bedding and Molyneux, 1982). From their suggestion, our result found that the small size EPNS *S. siamkayai* can enter to the pupal stage of *B. latifrons* better than *S. carpocapsae* and *H. bacteriophora* with the LD_{50} and LD_{90} at 2,293 and 4,224 IJs/pupa, respectively.

Table 6 Median lethal dose of EPNs on pupa of fruit fly

EPNs species	LD_{50} [1/]	LD_{90} [1/]
Steinernema siamkayai	2,293 A	4,224 A
Steinernema carpocapsae	3,054 B	4,801 B
Heterorhabditis bacteriophora	3,602 C	5,552 C

[/1] Means followed by the same letter are not significantly difference at 95% (P≤ 0.05)

Mortality rate of pupa after applied with *S. siamkayai* was higher than *S. carpocapsae* and *H. bacteriophora* in all concentrations. All species of EPNs can kill pupa better than control though *S. siamkayai* at the highest concentration can cause the highest mortality rate at 86.66% followed by *S. carpocapsae* and *H. bacteriophora* at 76.66 and 59.16%, respectively (Table 7)

Table 7 Mortality rate of last instar larva after treated with EPNs

Concentration (IJs/pupa)	Mortality rate of pupa of fruit fly (%) ±SD [1/]			F-test	CV(%)
	Steinernema siamkayai	***Steinernema carpocapsae***	***Heterorhabditis bacteriophora***		
0 (Control)	4.16±1.66 Da	1.66±1.92 Ea	0.83±1.66 Ea	ns	7.18
2,000	48.33±4.30 Ca	25.83±3.19 Db	15.83±5.69 Dc	*	16.81
2,500	56.66±4.71 Ca	35.83±5.00 CDb	24.16±4.19 Cc	*	12.61
3,000	65.83±3.19 Ba	43.33±5.44 Cb	29.16±3.19 Cc	*	9.44
3,500	79.16±5.00 Aa	64.16±6.30 Bb	50.83±4.19 Bc	*	7.37
4,000	86.66±2.72 Aa	76.66±6.08 Ab	59.16±6.87 Ac	*	7.42
F-test	*	*	*		
CV(%)	6.50	10.98	15.15		

[/1] Means followed by the capital and small letters in the same column and row are not significantly difference at 95% (P≤ 0.05)

When compared the LD_{50} of *S. siamkayai S. carpocapsae* and *H. bacteriophora*, all EPNs species can kill last instar larva better than pupa. Mahmoud and Osman (2007) showed similar mortality rate of *S. feltiae* on final instar larva (4-56%) and pupa (20-32%) of *B. zonata.* Eliza et al. (2014) tested on *B. tryoni* and found that last instar larva is more susceptible to EPNs than pupal stage.

6.2.4 Host finding ability

EPNs can move at the distance between 0 and 2.5 cm. as shown in percentage of pupa mortality of 96.25 and 97.5% respectively. Followed by the distance of 5, 7.5, 10, 12.5 and 15 cm. with the mortality rate of 72.5, 42.5, 20, 5 and 2.5%, respectively (Table 8). James and Gaugler (1993) inspected the movement of 5 spp. of *H. bacteriophora, S. feltiae, S. glaseri, S. carpocapsae* and *S. scapterisci.* Their result showed that *S. carpocapsae* and *S. scapterisci* can move in a small area of 2.5-3.0 mm^2 and move in horizontal distance for 4.7 mm. whereas *H. bacteriophora, S. feltiae* and *S. glaseri* can move further to 13.7-26.6 mm^2 and move in longer horizontal distance for 18.6-24.3 mm. From our result, *S. siamkayai* can actively move at 0-2.5 cm. and tended to decrease their movement when distance increases. Therefore, we can assume that *S. siamkayai* can have mix movement behaviors between cruises and ambushes. Burman and Pye (1980) and Pye and Burman (1981) proposed that soil texture, soil moisture, CO_2 from insect host, host movement, host temperature and host cues might help EPNs to detect and lead their way to the host.

Table 8 Host searching ability of *Steinernema siamkayai*

Distance of pupa from EPNs inoculation point (cm.)	Mortality rate of fruit fly (%)±SD[1/]
0	96.25±1.73 A
2.5	97.50±5.02 A
5	72.50±8.45 B
7.5	42.50±5.10 C
10	17.50±8.20 D
12.5	5.00±5.80 E
15	2.50±5.00 E
F-test	*
CV (%)	16.03

[/1] Means followed by the same letter are not significantly difference at 95% (P≤ 0.05)

6.2.5 Effect of various insecticides on the survival of EPNs

The survival of *Steinernema siamkayai* after 24 hours of being treated with insecticides showed that the EPNs which were mixed with imidacloprid gave the highest survival rate and no statistically significant difference (P≤0.05) with control (distilled water). The survival rate was 78.5%, followed by EPNs mixed with thiamethoxam, cypermethrin and carbosulfan with the survival rates of 56.5, 15.5 and 0.75%, respectively. After 48-72 hours, survival rate in all treatments that were treated with insecticides tended to decrease except control that still survive 95%, followed by the EPNs mixed with imidacloprid, thiamethoxam, cypermethrin and carbosulfan with the survival rates of 60.5, 41.5, 9.5 and 0%, respectively (Table 9). Similar result was reported from Nimkingrat and Anupap (2017) after being treated EPNs with imidacloprid for 72 hours. The survival rate was 99.1. Grewal (2000) suggested that EPNs can only tolerate to some group of insecticides. This experiment showed that carbosulfan which belongs to the carbamate group is highly toxic to EPNs. Hara and Kaya (1982) reported that carbamate and organophosphate groups were found to be toxic to *Steinernema* spp. and *Heterorhbditis* spp. Although thiamethoxam and cypermethrin are less toxic to EPNs than carbosulfan but the survival rate was still very low compared to imidacloprid.

Table 9 Survival rate of *Steinernema siamkayai* after treated with various insecticides.

Treatments	Survival rate (%±SD)[/1]		
	24 h	**48 h**	**72 h**
EPN + water	96.50±2.12 A	95.50±0.71 A	95.00±1.40 A
EPN + imidacloprid	78.50±3.54 A	68.00±5.66 B	60.50±3.52 B
EPN + thiamethoxam	56.50±6.36 B	48.50±3.53 C	41.50±2.11 C
EPN + cypermethrin	15.50±4.95 C	14.00±2.83 D	9.50±0.71 D
EPN + carbusulfan	0.75±0.45 D	0.06±0.12 E	0 E
F-test	*	*	*
CV (%)	8.06	4.77	3.01

[/1] Means followed by the same letter are not significantly difference at 95% (P≤ 0.05)

6.2.6 Effect of insecticides on the infectivity of EPNs

Steinernema siamkayai survived more than 50% after mixing with imidacloprid and thiamethoxam was then washed and inoculated to fruit fly pupa. EPN without any treatment (control) was the most virulent to pupa which gave 87.5% mortality and significantly different (P≤0.05) with the other treatments, followed by thiamethoxam and imidacloprid with the mortality rate of 52.5 and 45%, respectively (Table 10). Radová (2010) investigated that EPN which was mixed with imidacloprid will lose their infectivity to 35% after sprayed on wireworm. Similar result was tested by Nimkingrat and Anupap (2017). Their result indicated that the application of EPN only gave higher mortality of wireworm at 96% than EPN mixed with thiamethoxam with the mortality at 30%. Although imidacloprid and thiamethoxam did not cause mortality on EPN but it did effect on their infectivity.

Table 10 Infectivity of EPN on chili fruit fly pupa after treated with insecticides.

Treatments	Mortality rate (%±SD)[/1]
EPN + water	87.50±2.90 A
EPN after treated with thiamethoxam	52.50±8.70 B
EPN after treated with imidacloprid	45.00±5.80 C
F-test	*
CV (%)	9.48

[/1] Means followed by the same letter are not significantly difference at 95% (P≤ 0.05)

6.2.7 Effect of EPNs against chili fruit fly under semi-field condition

The net house result revealed that numbers of fruit fly which were collected from sticky trap from EPNs treated net house were lower than untreated net house with the number of fruit fly in week 2, 3 and 4 at 6, 22, 25.50 and 62.50, 9.50 and 32.60 adult/plant, respectively (Table 11). The control efficacy from EPN treated net house gave a significantly different compared to untreated net house at 70.86% at week 4 (Fig. 8). According to result from Saenbudda (2017) which gave similar result after sprayed EPNs at the control rate of 74.59%.

Table 11 The number of adult chili fruit fly collected from yellow sticky trap

Treatments	**Number chili fruit fly per week**[1/]			**F-test**	**CV (%)**
	2	**3**	**4**		
EPN	6 Bb	25.50 Ba	9.50 Bb	**	36.34
Control	22 Ab	62.50 Aa	32.60 Ab	**	11.44
F-test	*	**	**		
CV (%)	30.72	15.58	5.63		

[1/] Means followed by the capital and small letters in the same column and row are not significantly different by Tukey's HSD test at 95% (P<0.05)

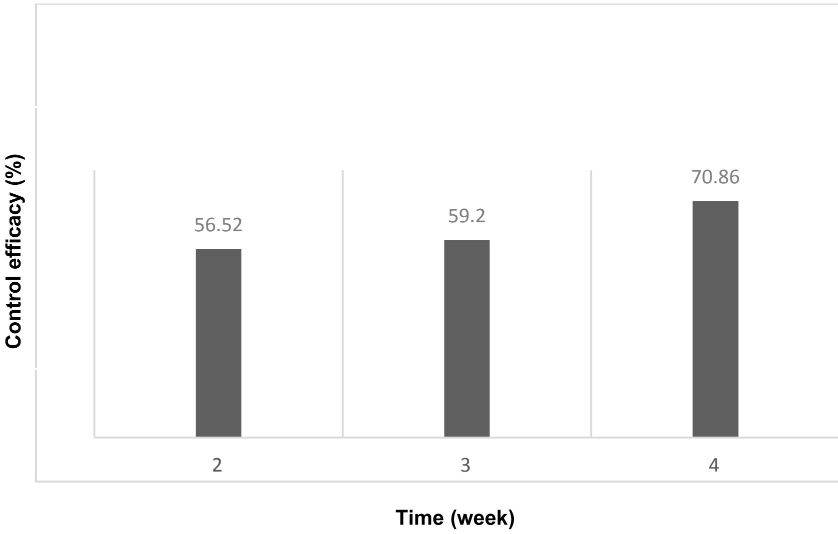

Figure 8 Control efficacy of chili fruit fly after treated with EPNs

6.2.8 Effect of EPNs against chili fruit fly population under field condition

Result from field condition was comparable to net house condition where EPNs treated plot was better than untreated plot. Four weeks after EPNs application, number of adult fruit fly was lower in EPNs treated plot at 13.60 compared to untreated plot at 38.90 adult/plant. The control efficacy of EPNs under field condition reached up to 65.04% (Table 12). Similar trend was found in Saenbudda (2017). His result showed that EPNs could reach up to 72.44% of control efficacy when applied EPNs under filed condition compared to untreated plot.

Table 12 Mean number of chili fruit fly and control efficacy of EPNs

Treatments	Number of fruit fly (individual/plant)[1/]	S.D.	t	Sig.	Control efficacy (%)
EPN	13.60 B	6.57	9.65	0.00**	65.04
Control	38.90 A	23.57			

[1/] Means followed by different letter are significantly different by Tukey's HSD test at 95% ($P<0.05$)

7. Conclusion

S. siamkayai can kill the last instar larva and pupa of chili fruit fly up to 94.44 and 86.66% when applied 3,000 IJs/larva and 4,000 IJs/pupa, respectively. The LD_{50} and LD_{90} of *S. siamkayai* on last instar larva and pupa were 1,246, 2,327 IJs/larva and 2,292, 4,224 IJs/pupa which is much better than *S. carpocapsae* and *H. bacteriophora.* Host searching ability of *S. siamkayai* varied from 0-2.5 cm. with the mortality rate of pupa at 98%. The use of *S. siamkayai* in combination with imidacloprid has no effect on survival compared to control (distilled water) whereas carbosulfan and cypermethrin can cause more than 50% mortality. However, when mixed EPNs with imidacloprid and thiamethoxam It seemed to lose its infectivity compared to EPNs without any insecticides treatment (88%). The control efficacy from EPNs treated net house gave a better result compared to untreated net house at 70.86% and similar result was found when applied EPNs under field condition. The control efficacy of EPNs under field condition can reach up to 65.04%.

8. Recommendation

Result from this study can be used as a guideline to encourage farmer to reduce the use of insecticides and replace with EPNs. The correct dose of EPNs application, EPNs species, and suitable insecticides used with EPNs can give direct impact on control efficacy.

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10. Output

10.1 Publication/Presentation

ประกายจันทร์ นิ่มกิ่งรัตน์ นุชรีย์ ศิริ วนศักดิ์ เกื้อหนุน และภูพิงค์ ต๊ะอุ่น. 2562. การศึกษาผลของเนื้อดินต่อการเข้าดักแด้ของแมลงวันผลไมศัตรูพริกและประสิทธิภาพการเข้าทำลายแมลงวันผลไมศัตรูพริกของไส้เดือนฝอยสายพันธุ์ไทย. วารสารแก่นเกษตร. 47(1); 68.

Nimkingrat, P., Guaenoon, W., Siri, N., Ehlers, R-U. and Burana, K. 2018. Effect of insecticides on the survival and control rates of entomopathogenic nematode against chilli fruit fly pupae (*Bactrocera latifrons* Hendel). Communications in agricultural and applied biological sciences. ISSN 1379-1176. Vol 83(2): 202.

วนศักดิ์ เกื้อหนุน ภานุพงศ์ แสนบุดดา นุชรีย์ ศิริ และประกายจันทร์ นิ่มกิ่งรัตน์. 2560. ผลของสารฆ่าแมลงที่มีต่อการรอดชีวิตและความสามารถในการเข้าทำลายเหยื่อของไส้เดือนฝอยศัตรูแมลงในดักแด้แมลงวันผลไมศัตรูพริก. ในการประชุมวิชาการอารักขาพืชแห่งชาติ ครั้งที่ 13. วันที่ 21-23 พฤศจิกายน 2560. โรงแรมเรือรัษฎา จังหวัดตรัง. หน้าที่ 95.

10.2 Speakers

- International seminar on Increase the capacity of about soil ecology and sustainable rubber management in South-East Asia at the faculty of Agriculture Nabong (Vientinae, Lao PDR) during 2 October to Wednesday 4 October 2017.

- International Floriculture Training Course for Participant from College of Natural Resources (CNR) Bhutan under the Project of Capacity Development of CNR-Follow up Phase Cooperate by TICA and Khon Kaen University (KKU) during 1 - 26 February 2018 at Khon Kaen University

10.3 academic services to community

- โครงการอบรม การใช้ศัตรูธรรมชาติป้องกันกำจัดศัตรูพริกโดยชีววิธี ในวันที่ 16 พฤษภาคม 2560 ณ อำเภอซำสูง จังหวัดขอนแก่น โดยได้รับทุนสนับสนุนจาก โครงการบริการวิชาการแก่สังคม ปีงบประมาณ 2560 มหาวิทยาลัยขอนแก่น

- โครงการอบรม การใช้ศัตรูธรรมชาติควบคุมแมลงวันผลไม้ศัตรูพริก เพื่อการผลิตพริกปลอดภัย ในวันที่ 30 มีนาคม 2561 ณ บ้านนาน้ำซำ อำเภอภูผาม่าน จังหวัดขอนแก่น โดยได้รับทุนสนับสนุนจาก โครงการบริการวิชาการแก่สังคม ปีงบประมาณ 2561 มหาวิทยาลัยขอนแก่น

11. Appendix

11.1 Annex 1: maunuscript (1st draft)

Interaction between insecticides and Entomopathogenic nematode against chili fruit fly pupae (*Bactrocera latifrons* Hendel)

Abstract

As part of Integrated Pest Management (IPM), Entomopathogenic nematode (EPN) has been found to be an effective bio-agent against chili fruit fly in many countryjtyies. Recently, EPN has begun to be used in Thailand. This study assessed the interaction of Thai isolate EPN, *Steinernema siamkayai*, and four common types of insecticides used in Thailand, namely imidacloprid, thiamethoxam, cypermethrin and carbosulfan with respect to EPN survival and infectivity for the control of chili fruit fly pupae. The results show that the survival rate of EPN mixed with imidacloprid was not *statistically significantly* different to insecticide-free EPN. The survival rate for other EPN and insecticide mixtures, were significantly different from the insecticide-free EPN [thiamethoxam (56.50%), cypermethrin (15.50%) and carbosulfan (0.75%)]. The surviving EPN were then tested against chili fruit fly pupae compared to insecticide-free EPN (control). The highest mortality rate was observed in the control (87.50%). Mortality was significantly low in EPN treated with thiamethoxam (52.50%) and imidacloprid (45%) ($P \leq 0.05$). Although imidacloprid did not affect EPN's survival rate, it does affected infectivity. Therefore, these results indicate that none of the tested insecticides were suitable for combination with EPN for the control of chili fruit fly pupae.

Introduction

Chili is one of the important economic and cultural herbs in Thailand. As a result, the growing area of chili for household consumption and international trade increased yearly. Information and Communication Technology Center, Department of Agricultural Extension (2017) reported area of large chili cultivation in the country in 2016 up to 20,633 ha. Two out of three of the major chili growing areas are located in the Northeast. The plantation area is approximately 13,465 ha and the yield is 112,587 tons, consistent with the increasing trend of export data. The export volume of dried chili between 2014 and 2016 was 9,000 to 12,000 tons, valued at 12 - 14 million U.S. dollars. Although growing areas and yields across the country are increasing each year. The problem of insect damage is a major obstacle that can result in lower production. Siri et al. (2010) reported the chili insect pests in Thailand which includes broad mite, whitefly, thrip, aphid, mealybug, common cutworm, beet armyworm, bollworm and fruit fly. The important and difficult to control insect pests that are severely damaged during the inflorescence period are *Bactrocera latifrons* (Diptera: Tephritidae) which can be found in all regions of the country. In addition, it can also destroy 17 other species, especially the family of Solanaceae, such as chilli, eggplant, Thai eggplant, turkey berry, brinjal and crape myrtle. In areas without protection, fruit fly can damage up to 100% (Nakprasert and Songsopha, 2015). The damage is caused by the fruit fly started from the gravid female chili fruit fly depositing her eggs in the chili fruit. Newly hatched larvae will chew the flesh inside the fruit, resulting in rotting and falling (Siri, 2007; Stonehouse et al., 2004).

In general, the farmers usually protect their crop with insecticides. The routine insecticide application starts from the beginning of flowering to stop adults from laying eggs. The insecticides recommended by the Department of Agriculture (DOA) in Thailand are malathion 83%EC, cypermethrin 35%EC, dimethoate 40%EC and dichlorvos 50%EC but insecticides which are frequently and widely used by farmers are carbosulfan, imidacloprid, cypermethrin and thiamethoxam. The use of insecticides to prevent fruit fly larvae is quite troublesome since the larvae live inside the fruit. It is hard for insecticides to make direct contact with the larvae and cause death. As a consequence, insecticide spraying cannot effectively control the population of fruit fly larvae and can also cause insecticide residues in the product (Kreeppha, 2014). The control of adult and pupa can be an alternate choice to cut down the occurrence of offspring. Therefore, the combined use of insecticides and natural enemies can be an interesting tool to help control a wide range of insect pests at the different developmental stages at the same time.

Entomopathogenic nematode (EPN) is a natural enemy that is gaining attention from farmers around the world (Nimkingrat et al., 2014). EPN can kill insects at varied developmental stages such as larva and pupa. Their mode of action started from Infective Juvenile (IJ) enters the insect's natural opening and make its way to haemocoel after that EPN will releases the symbiotic bacteria and breaks down the cells inside the insect, resulting in death which can be called septicemia within 24-48 hours (Simoes and Rosa, 1996). EPN will reproduce and develop inside the insect host until no food source is available then they will migrate from insect cadaver and search for new hosts (Poinar, 1990). The advantage of EPN is not only for ease of mass rearing but also high host search ability and can be used with some insecticides. This is an opportunity to control the number of pest populations in almost every developmental stage and to provide an alternative tool for farmers to apply the natural enemies together with the insecticides in a form of Integrated Pest Management (IPM).

Many reports indicated that the use of EPN together with insecticides is more effective than EPN or insecticides alone (Koppenhofer et al., 2002). Nimkingrat and Thipsukon (2017) suggested that some insecticides can be mixed with EPN and has no effect to EPN mortality and infectivity. Although there are a number of studies focused on the combination of EPN and chemical but none of them were focused on Thai strain, *Steinernema siamkayai* and insecticides which have been commonly used in Thailand. Therefore, the objective of this study was to investigate the effect of different insecticides on the survival and infectivity of EPN on chilli fruit fly pupa. The results of this study will be useful for farmers to select a proper insecticide that does not affect the mortality rate and promotes further nematode efficacy.

Materials and methods

1. Mass rearing

1.1 *Bactrocera latifrons* Hendel

The fruit fly infested chili fruits were collected from field (xxxxx) and placed into plastic rearing box 13 x 13 x 9 cm with the rice husk ash in the bottom. Maintain in the ventilated room with the temperature at 25 ± 2 ºC (%RH and light) for 1-2 weeks. Pupae were collected by sieving the rice husk ash and divided into two portions, one for the experiment and another one for mass rearing. For mass rearing, pupae were transferred into new net cage 17 x 23 x 16 cm. Distilled water and supplement food (1:3 ratio of honey and dry yeast (xxxx)) were provided for adult feeding. One week after, fresh chili fruits were supplied to the gravid females to lay their eggs. The mass rearing steps were then followed as above to obtain sufficient fruit flies for the experiment.

1.2 Entomopathogenic nematode

Thai EPN strain, *Steinernema siamkayai*, was reared in last instar of the wax moth, *Galleria mellonella* F. (Lepidoptera: Pyralidae) according to protocol of Kaya and Stock (1997) under laboratory room at 25 ºC. The freshly emerged infective juveniles (IJs) were harvested from a White trap (White, xxxx) and kept for up to one week in the storage solution (11.25 g NaCl, 0.525 g Cacl2.2H2O, 0.315 g MgSO4, 0.1 g ascorbic acid in 1 l distilled water) at 15 ºC before use.

2. Insecticides

Four commercial insecticides that are widely used in Thailand to control chili insect pests were tested as follows: (1) carbosulfan (100 CC per 20 liters; 20%EC xxx; xxxx, xxxx, xxx); (2) imidacloprid (3 g per 20 liters; 70% WG xxx; xxxx, xxxx, xxx); (3) cypermethrin (10 ml per 20 liters of water; 35% EC; xxx; xxxx, xxxx, xxx); (4) thiamethoxam (2 g per 20; 25% WG; xxx; xxxx, xxxx, xxx).

3. Effect of insecticides on the survival of EPN

The experimental statistical design was completely randomized design (CRD) of 5 treatments with 4 replications was used to assess the EPN survival after treated with four different commercialized insecticides. All insecticides above concentration were prepared. One hundred infective juveniles per replication were transferred into different treatments inside Petri dishes of 10 cm diameter according to the following treatments: 1. distilled water (control); 2. carbosulfan; 3.
imidacloprid; 4. cypermethrin and 5. thiamethoxam. All Petri dishes were then transferred to 24 h dark room at 25 ºC. The numbers of dead and alive infective juvenile in each treatment were recorded every 24 hours for 3 days by using stereo microscopy.

4. Effect of insecticides on the infectivity of EPN

The experimental statistical design was completely Randomized Design (CRD) with 3 treatments and 8 replications. Fruit fly pupae of one-three days old were placed inside the Petri dish 10 cm diameter filled with 10% moist sand. Alive EPN from previous experiment which a higher 50% survival rate (imidacloprid and thaimethoxam) were carefully washed with distilled water to remove any insecticide residue on EPN cuticle. Infective juveniles (4,000 IJs/pupa) (Saenbudda et al., 2015) were then inoculated into pupa of each replication. Insecticide-free infective juveniles were used as control. The numbers of dead and alive pupae were recorded after one week by using stereo microscopy.

5. Statistically analysis

Means value of dead infective juveniles and pupae were submitted to analysis of variance and differences between treatments were compared by using Tukey's HDS test at $P < 0.05$ probability (XLSTAT 2006; XLSTAT, New York, NY, USA).

<u>Result and discussion</u>

1. Effect of various insecticides on the survival of EPN

The survival of *Steinernema siamkayai* after 24 hours of being treated with insecticides showed that the EPN which was mixed with imidacloprid gave the highest survival rate and no statistically significant difference ($P \leq 0.05$) with control (distilled water). The survival rate was 78.5%, followed by EPN mixed with thiamethoxam, cypermethrin and carbosulfan with the survival rates of 56.5, 15.5 and 0.75%, respectively. After 48-72 hours, survival rate in all treatments that were treated with insecticides tended to decrease except control that still survive 95%, followed by the EPN mixed with imidacloprid, thiamethoxam, cypermethrin and carbosulfan with the survival rates of 60.5, 41.5, 9.5 and 0%, respectively (Table 1). Similar result was reported from Nimkingrat and Thipsukon (2017) after being treated EPN with imidacloprid for 72 hours. The survival rate was 99.1. Grewal (2000) suggested that EPN can only tolerate to some group of insecticides. This experiment showed that carbosulfan which belongs to the carbamate group is highly toxic to EPN. Hara and Kaya (1982) reported that carbamate and organophosphate groups were found to be toxic to *Steinernema* spp. and *Heterorhbditis* spp. Although thiamethoxam and cypermethrin are less toxic to EPN than carbosulfan but the survival rate was still very low compared to imidacloprid.

2. Effect of insecticides on the infectivity of EPN

Steinernema siamkayai survived more than 50% after mixing with imidacloprid and thiamethoxam was then washed and inoculated to fruit fly pupa. EPN without any treatment (control) was the most virulent to pupa which gave 87.5% mortality and significantly different ($P \leq 0.05$) with the other treatments, followed by thiamethoxam and imidacloprid with the mortality rate of 52.5 and 45%, respectively (Table 2). Radová (2010) investigated that EPN which was mixed with imidacloprid will lose their infectivity to 35% after sprayed on wireworm. Similar result was tested by Nimkingrat and Thipsukon (2017). Their result indicated that the application of EPN only gave higher mortality of wireworm at 96% than EPN mixed with thiamethoxam with the mortality at 30%. Although imidacloprid and thiamethoxam did not cause mortality on EPN but it did effect on their infectivity.

Conclusion

The use of Thai EPN (*Steinernema siamkayai*) in combination with imidacloprid has no effect on survival compared to control (distilled water) whereas carbosulfan and cypermethrin can cause more than 50% mortality. However, when mixed EPN with imidacloprid and thiamethoxam It seemed to lose its infectivity compared to EPN without any insecticides treatment. The information from this experiment can be used to advise farmers to choose a suitable insecticide when they want to combine.

Table 1 Survival rate of *Steinernema siamkayai* after treated with various insecticides.

Treatments	Survival rate (%±SD) [1]		
	24 h	48 h	72 h
EPN + water	96.50±2.12 A	95.50±0.71 A	95.00±1.40 A
EPN + imidacloprid	78.50±3.54 A	68.00±5.66 B	60.50±3.52 B
EPN + thiamethoxam	56.50±6.36 B	48.50±3.53 C	41.50±2.11 C
EPN + cypermethrin	15.50±4.95 C	14.00±2.83 D	9.50±0.71 D
EPN + carbusulfan	0.75±0.45 D	0.06±0.12 E	0 E
F-test	*	*	*
CV (%)	8.06	4.77	3.01

[1] Means followed by the same letter are not significantly difference at 95% ($P \leq 0.05$)

Table 2 Infectivity of EPN on chili fruit fly pupa after treated with insecticides.

Treatments	Mortality (%±SD) [1]
EPN + water	87.50±2.90 A
EPN after treated with thiamethoxam	52.50±8.70 B
EPN after treated with imidacloprid	45.00±5.80 C
F-test	*
CV (%)	9.48

[1] Means followed by the same letter are not significantly difference at 95% ($P \leq 0.05$)