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Test of Larvicidal Effect of Some Commercial Natural Products on Lepidopteran *Plutella xylostella* and *Spodoptera litura* Larvae

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A diverse kind of environment-friendly agricultural materials (EFAM) for the control of insect pests is on the market. These EFAMs are a part of essential sources for the accomplishment of successful, sustainable, and environment-friendly agriculture. Thus, accurate information of these EFAMs is one that required for the success of environment-friendly agriculture, but, in reality, still appropriate information is absolutely in shortage. In this study, we, therefore, tested the efficacy of commercial EFAMs against two lepidopteran insect larvae, the diamondback moth *Plutella xylostella* (Lepidoptera: plutellidae) and the tobacco cutworm *Spodoptera litura* (Lepidoptera: Noctuidae). After the two insect pests were successfully stabilized in indoor environment the larvicidal activity was tested at $24 \pm 1^\circ\text{C}$, relative humidity (RH) of $60 \pm 5\%$, and a photoperiod of 16L : 8D, and mortality was determined 48 hrs after EFAMs are treated. The EFAMs that showed more than 90% of larvicidal activity were each six among 16 against both *P. xylostella* and *S. litura* and only three of them showed consistent larvicidal activity against both species, signifying species specificity of EFAMs and importance of selection of proper EFAMs depending on target insect pest.

Key words: Larvicidal Effect, Plant extracts, *Plutella xylostella*, *Spodoptera litura* Laboratory test

Introduction

With the increasing concern on the environment and health many researches are underway in the field of environment-friendly biological control. In fact, an excessive number of environment-friendly agricultural materials (EFAM), some of which are derived from microbial organisms (e.g., Kennedy and Thilagam, 2005) and plant extracts (i.e., Rao, 1997; Rao *et al.*, 2005; Isman, 2006) are manufactured and on the market. Nevertheless, still more EFAMs may be needed, because these EFAMs are a part of essential sources for the accomplishment of successful, sustainable, and environment-friendly agriculture.

With the shortage of the EFAM products, another problem of EFAMs is uncertainty of efficacy and applicable range. For example, many on-the-market products control possibly limited number of taxonomic group or species, but, in reality, the efficacy of their products is overstated in that they appear to control most, if not all, of species belong in a certain insect order, although no such experimental data are specifically provided. Furthermore, on the market, local dealers describe applicable range of EFAMs obscurely, rendering farmers to believe as said by them. Therefore, it is essential to provide the farmers with the accurate information of the on-the-market EFAMs, by thoroughly testing their efficacy and applicable range.

Therefore, in this study, we tested several on-the-market EFAMs that state larvicidal efficacy against lepidopteran insect larvae. For the purpose of experiment, a total of 16 EFAMs that state larvicidal efficacy was purchased from local markets in Jeollanamdo Province and tested with two lepidopteran species, the tobacco cutworm, *Spodoptera litura*, and the diamondback moth, *Plutella xylostella*, that were reared for over several generations in laboratory condition. As well known, the diamondback moth is a serious insect pest of cruciferous plants, distributed

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Table 1. List of environment-friendly agricultural materials

Product	Company	Insecticidal Range	Source	Recommended dilution*
A	B	ML	Plant extracts	20 mL
B	NB	AS, SE	"	13 ~ 20 mL
C	FM	Insect pests	"	20 mL
D	F	ML	"	20 mL
E	K	M, other insects	"	16 ~ 20 mL
F	K	Insect pests	"	16 ~ 20 mL
G	PD	ML, A	"	20 mL
H	B	ML, M	"	20 mL
I	G	ML	<i>B. thuringiensis</i>	50 mL
J	NB	ML	Plant extracts	13 ~ 20 mL
K	K	A, other pests	"	16 ~ 20 mL
L	B	A, F other pests	"	20 mL
M	A	T, SE, Be etc.	"	40 mL
N	A	A, Be, SE etc.	"	40 mL
O	D	ML	<i>B. thuringiensis</i>	10 g
P	F	A, other pests	"	20 mL

*per 20 liter of water.

Abbreviations in insecticide range represent A for aphid, AS for *Agrotis segetum*, Be for *Bemisia tabaci*, F for flies, M for mite, ML for moth larvae, SE for *Spodoptera exigua*, and T for thrips.

world-widely, and the damage caused by the larvae of this insect amounts to at least one-billion dollars annually. In Korea, it overwinters at least in southern region in all stages (Kim and Lee, 1991), and seriously damages the cabbage. The tobacco cutworm is also a worldwide, extremely damaging pest, the larvae of which can defoliate many economically important crops. In Korea, the species is polyphagous, damaging more than 100 species belonging in ~40 plant families. In particular it seriously damages leguminous plants (Bae, 1999).

Materials and Methods

EFAMs

For the test of larvicidal efficacy 16 environment-friendly agricultural materials (EFAM) were purchased from local markets located in Jellanamdo Province in 2007 (Table 1). To avoid unnecessary advertisement of the products used in this study, product names and companies were initial-ized in Table 1. The applicable range of EFAMs and source of the products are listed in Table 1. Briefly, sources of most products were plant extract, but two products, T and S were originated from *Bacillus thuringiensis*. Detailed components were not obtainable. According to the product description, they were all targeted at least for the control of the larvae of lepidopteran insects. These

products were diluted as recommended for the test of larvicidal efficacy (Table 1).

Test insects

Larvae of the healthy diamondback moth, *Plutella xylostella* (Lepidoptera: plutellidae), and the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), were selected for the study. The pests were reared and maintained in the laboratory at $25 \pm 1^\circ\text{C}$, relative humidity (RH) of $60 \pm 5\%$, and a photoperiod of 16L:8D. To remove any possible abnormality, the two insect pests were reared in the laboratory for several generations after collected from wild and their life cycles were roughly checked with at least 20 individuals. For the larvicidal activity, 3rd and 4th instar larvae were used in the case of *P. xylostella*, because these ages are at the beginning of greatest appetite. On the other hand, *S. litura* larvae were subjected to experiment at 2nd instar, because preliminary experiment at each individual larval stage showed that most EFAMs did not show much efficacy at the stage older than 3rd instar, preventing test of available EFAMs at all.

Diets

The diamondback moths were provided with the pesticide-free cabbages purchased from a market. To collect eggs, on the other hand, the seeds of Elgari Chinese cabbage were sowed in a plastic cage (6 cm × 6 cm × 10 cm)

Table 2. Developmental periods of two lepidopteran insect pest reared at 25°C(a) *Plutella xylostella*

Diet	Duration (days)						Accumulative duration (days)
	Egg	1 st instar	2 nd instar	3 rd instar	4 th instar	Pupa	
Chinese cabbage	3.5	3.5	1.5	2.2	2.3	4.5	17.5

(b) *Sodoptera litura*

Diet	Instar duration (days)						Accumulative duration (days)
	1 st instar	2 nd instar	3 rd instar	4 th instar	5 th instar	6 th instar	
Gangnankong	3.5	2.0	2.0	2.0	2.5	3.5	15.5

and provided to adult diamondback moths 25 days after growing in a growth cage (165 cm × 83 cm × 124 cm) with a photoperiod of 18L : 6D. In the case of the tobacco cutworm, the purchased seeds of kidney bean were germinated at 28°C for two days with wet towel covered and provided 20 days after growing in a growth cage (165 cm × 83 cm × 124 cm) with a photoperiod of 18L : 6D.

Screening bioassay

A spraying method was used to evaluate the activity of the test samples. In the case of diamondback moth, a plain cabbage leaf was cut into Φ10 cm in size, inoculated with 20 healthy 3rd and 4th instar larvae, and sprayed with recommended concentration of EFAMs in a round plastic cage (Φ10 cm × 6 cm). In the case of tobacco cutworm, a similar sized leaf of kidney bean was selected from whole kidney bean plants, end of petiole was placed in a micro-centrifuge tube, filled with wet cotton to prevent shrivel, inoculated with 20 healthy 2nd instar larvae, and sprayed with recommended concentration of EFAMs in a round plastic cage (Φ10 cm × 6 cm). Every six hrs, the larvae were checked, dead larvae were removed, and eventually mortality was determined 48 hrs after EFAMs are treated. Only distilled water was used for control. Each set of experiment was carried out in triplicate. Using SAS program, Duncan's multiple range test ($p < 0.05$) was performed to test if any significant difference in the larvicidal effect exists among EFAMs.

Results and Discussion**Life cycle of test insects**

Before larvicidal efficacy of EFAMs are tested against two lepidopteran larvae, the insects were indoor reared for several generations after brought into laboratory, their life cycles were roughly compared with the published date, and finally utilized for the test of larvicidal efficacy of EFAMs. The developmental period of the diamondback

moth reared at 25°C are presented in Table 2. The egg period was 3.5 days, larval period was 9.5 days, and pupa period was 4.5 days. Similar experiment with more robust sampling at the same temperature scheme showed 3 days for egg, 8.5 days for larvae, and 4.7 days for pupa (see Kim and Lee, 1991). Thus, egg period (0.5 day longer) and larval period (1 day) are longer, but pupa period is 0.2 day shorter. Nevertheless, overall developmental period is similar, and the difference may have been caused by less through data collection in this study and difference in minor environmental factors. In the case of tobacco cutworm, larval duration was 15.5 days and pupal duration was 17.5 (Table 2). Similar experiment with the same condition (temperature scheme and diet) showed that larval duration was 13.9 days and pupal duration was 13.5 days (Bae, 1999), showing somewhat longer developmental period in this study. The exact reason for the difference is unknown, but overall the insect pests utilized in this study seem normal enough to test efficacy of the EFAMs.

Larvicidal efficacy

Among 16 EFAMs, six showed more than 90% of larvicidal activity against *P. xylostella* (Table 3). These are O, F, D, I, A, and M. Along with these, H, E, and K showed ~80% larvicidal efficacy. Thus, it seems that many commercialized EFAMs truly have larvicidal efficacy. In the case of larvicidal activity against *S. litura*, also six EFAMs showed more than 90% of larvicidal efficacy. These include A, C, E, F, J, and D (Table 4). These six most effective EFAMs are not identical to those of *P. xylostella*. Only three of them showed enough efficacy in both species of larvae (F, D, and A). Considering the EFAMs tested in this study state that they have efficacy to at least to the tested insects, it seems that applicable range of the on-the-market EFAMs are over-described in their range of application. Clearly, only a few of them work both species of larvae. Thus, the description of most products should be revised accurately.

Some of the EFAMs showed only ~50% of larvicidal

Table 3. Larvicidal effect of on-the-market EFAMs on the diamondback moth larvae

Products	% mortality (\pm SD) after			
	12 hrs	24 hrs	36 hrs	48 hrs
O	11.7 \pm 2.9hij	93.3 \pm 2.9a	100.0 \pm 0.0a	100.0 \pm 0.0a
F	40.0 \pm 5.0def	66.7 \pm 14.4bcd	91.7 \pm 5.8abc	100.0 \pm 0.0a
D	48.3 \pm 7.6cde	65.0 \pm 15.0	93.3 \pm 2.9ab	98.3 \pm 2.9ab
I	1.7 \pm 2.9ij	50.0 \pm 17.3def	91.7 \pm 2.9abc	98.3 \pm 2.9ab
A	68.3 \pm 15.3abc	80.0 \pm 8.7abc	88.3 \pm 2.9abc	91.7 \pm 5.8abc
M	86.7 \pm 2.9a	88.3 \pm 2.9ab	90.0 \pm 5.0abc	90.0 \pm 5.0abcd
H	75.0 \pm 8.7ab	85.0 \pm 5.0ab	86.7 \pm 2.9abc	88.3 \pm 5.8abcde
E	13.3 \pm 2.9ghij	23.3 \pm 7.6gh	56.7 \pm 5.8defg	80.0 \pm 5.0bcdef
K	25.0 \pm 5.0fgh	28.3 \pm 2.9fgh	48.3 \pm 12.6efg	80.0 \pm 5.0bcdef
G	25.0 \pm 8.7fgh	45.0 \pm 8.7defg	63.3 \pm 15.3def	78.3 \pm 2.9cdef
P	61.7 \pm 7.6bcd	76.7 \pm 10.4abc	76.7 \pm 10.4bcd	76.7 \pm 10.4cdef
J	23.3 \pm 12.6fghi	28.3 \pm 5.8fgh	53.3 \pm 12.6efg	75.0 \pm 10.0cdef
C	11.7 \pm 20.2hij	15.0 \pm 8.7hi	58.3 \pm 20.2defg	71.7 \pm 10.4def
L	58.3 \pm 25.7bcd	65.0 \pm 30.4bcd	65.0 \pm 30.4de	65.0 \pm 30.4fgh
B	23.3 \pm 18.9fghi	28.3 \pm 23.1fgh	38.3 \pm 12.6g	53.3 \pm 10.4gh
N	35.0 \pm 15.0efg	40.0 \pm 13.2efg	41.7 \pm 11.5fg	48.3 \pm 12.6h
R	0.0 \pm 0.0j	0.0 \pm 0.0i	0.0 \pm 0.0h	0.0 \pm 0.0i

Table 4. Larvicidal effect of on-the-market EFAMs on the tobacco cutworm

Products	% mortality (\pm SD) after			
	12 hrs	24 hrs	36 hrs	48 hrs
A	78.3 \pm 12.6bc	95.0 \pm 5.0a	100.0 \pm 0.0a	100.0 \pm 0.0a
C	93.3 \pm 5.8a	100.0 \pm 0.0a	100.0 \pm 0.0a	100.0 \pm 0.0a
E	95.0 \pm 5.0a	100.0 \pm 0.0a	100.0 \pm 0.0a	100.0 \pm 0.0a
F	80.0 \pm 10.0b	100.0 \pm 0.0a	100.0 \pm 0.0a	100.0 \pm 0.0a
J	83.3 \pm 5.8ab	98.3 \pm 2.9a	100.0 \pm 0.0a	100.0 \pm 0.0a
D	95.0 \pm 5.0a	95.0 \pm 8.7a	98.3 \pm 2.9a	98.3 \pm 2.9a
M	66.7 \pm 11.5c	68.3 \pm 10.4c	68.3 \pm 10.4b	68.3 \pm 10.4b
N	28.3 \pm 7.6d	50.0 \pm 5.0c	51.7 \pm 2.9c	51.7 \pm 2.9c
I	8.3 \pm 7.6ef	16.7 \pm 2.9def	31.7 \pm 12.6d	31.7 \pm 12.6d
L	25.0 \pm 5.0d	26.7 \pm 5.8d	26.7 \pm 5.8d	26.7 \pm 5.8d
O	15.0 \pm 13.2de	18.3 \pm 10.4de	23.3 \pm 15.3de	23.3 \pm 15.3de
P	16.7 \pm 7.6de	18.3 \pm 7.6de	20.0 \pm 10.0de	21.7 \pm 7.6de
B	6.7 \pm 7.6ef	10.0 \pm 5.0efg	11.7 \pm 5.8ef	13.3 \pm 2.9ef
K	6.7 \pm 2.9ef	6.7 \pm 2.9fg	6.7 \pm 2.9f	8.3 \pm 5.8fg
H	3.3 \pm 2.9ef	6.7 \pm 7.6fg	6.7 \pm 7.6f	6.7 \pm 7.6fg
G	3.3 \pm 2.9ef	3.3 \pm 2.9g	3.3 \pm 2.9f	5.0 \pm 5.0fg
R	0.0 \pm 0.0f	0.0 \pm 0.0g	0.0 \pm 0.0f	0.0 \pm 0.0fg

effects against *P. xylostella* larvae (Table 3). These products are questionable for the field efficacy for larvicidal effect and maybe not effective at all in the field, because the field situation might be much coarse for the EFAMs to

exert efficacy. Thus, much caution is required when these EFAMs are actually applied in the field. Furthermore, several products tested against *S. litura* larvae showed substantially low activity (>40% larvicidal efficacy) (Table

4). Considering that we used only 2nd instar larvae of *S. litura*, because several EFAMs showed very weak or no efficacy against the *S. litura* larvae older than 2nd instar in the preliminary experiment, these EFAMs must not be recommended at least for tobacco cutworm until proper experimental data are provided. In regard to EFAMs, Yu *et al.* (2006) and Kang *et al.* (2007) tested abundant number of on-the-market products, which were originated from a diverse source, but these were all about the effect of the products on the natural insect parasitoids and predators, but not about the efficacy of the EFAMs themselves. Thus, this study is a relatively recent, urgent issues that are required for the farmers who are willing to accomplish successful, sustainable, and environment-friendly agriculture.

We tested the on-the-market EFAMs only with two lepidopteran larvae. Thus, it is far less to complete the whole larvicidal efficacy of those EFAMs to other lepidopteran insects. Nevertheless, it is important to find out that some EFAMs tested in this study do not have larvicidal efficacy to the tobacco cutworm, considering that the pest is one of the most damaging lepidopteran insect. Furthermore, it is important to know that many EFAM products that describes their products to have larvicidal efficacy to many, if not all, lepidopteran insects do not have as stated, and may need more accurate explanation on their products.

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References

- Bae, S. D. (1999) Leaf characteristics of leguminous plants and the biology of tobacco cutworm, *Spodoptera litura* Fabricius: I. The larval development and leaf feeding amount. *Korean J. Appl. Entomol.* **38**, 217-224.
- Isman, M. B. (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **51**, 45-66.
- Kang, M.-K., E.-J. Kang, H.-J. Lee, D.-H. Lee, H.-B. Seok, D.-A Kim, M.-L. Gil, M.-J. Seo, Y.-M. Yu and Y.-N. Youn (2007) Effects of environment friendly agricultural materials to *Phytoseiulus persimilis* (Acari: Phytoseiidae) in the laboratory. *Korean J. Entomol.* **46**, 87-95.
- Kennedy, J. S. and P. Thilagam (2005) Qualitative and quantitative assessment of synergism between *Bacillus thuringiensis* var. *kurstaki* and insecticides; in *Green pesticides for insect pest management*. Ignacimuthu, S. and S. Jayaraj (eds.), pp. 190-196, Narosa Publishing House, New Delhi.
- Kim, M.-H. and S. C. Lee (1991) Bionomics of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) in southern region of Korea. *Korean J. Entomol.* **30**, 169-173.
- Rao, A. P. J. (1997) Botanical Pesticides in Agriculture. Lewis Publishers, Boca Raton.
- Rao, N. Venugopala, T. Uma Maheswari and K. Manjula (2005) Review of botanical pesticides as tools of pest management; in *Green pesticides for insect pest management*. Ignacimuthu, S. and S. Jayaraj (eds.), pp. 1-16, Narosa Publishing House, New Delhi.
- Yu, Y.-M., E.-J. Jin, M.-J. Seo, M.-G. Kang, H.-J. Lee, D.-A Kim, M.-L. Gil and Y. N. Youn (2006) Effects of environment friendly agricultural materials to insect parasitoids in the laboratory. *Korean J. Entomol.* **45**, 227-234.