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Evaluation of Susceptibility of *Spoladea recurvalis* (Lepidoptera: Crambidae) to Five Insecticides in Spinach

Young Su Lee*, Hee-A Lee, So-Hee Kim, Jong Yoon Choi, Hyun Ju Lee, Sang-Woo Lee and Jung-Soo Park
Gyeonggi-do Agricultural Research and Extension Services, Hwaseong 18388, Korea

시금치 해충 흰띠명나방(*Spoladea recurvalis*)의 살충제 감수성

이영수* · 이희아 · 김소희 · 최종윤 · 이현주 · 이상우 · 박중수
경기도농업기술원 환경농업연구과

ABSTRACT: The susceptibilities of five insecticides to the larvae of *Spoladea recurvalis*, a major pest of spinach, were evaluated. Lufenuron EC, chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW showed 90% or more insecticidal activity at dilution concentrations of 2 (12.5 ppm), 4 (12.5 ppm), 8 (2.5 ppm), 4 (20.0 ppm), and 8 (12.5 ppm), respectively. At their recommended concentrations, Chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW showed very high insecticidal activity of 98.3%, 100%, 95.0%, and 100%, respectively, after 72 h on *S. recurvalis* larvae exposed after 7 days of treatment. Field tests at two spinach greenhouses in Hwaseong and Yeoncheon showed a high control effect of over 90% after 7 days of treatment in both areas. In addition, the insecticides can be used as exclusive agents for *S. recurvalis* that occurs in spinach, as there is no phytotoxicity even at double doses.

Key words: Spinach, *Spoladea recurvalis*, Insecticide, Susceptibility

초 록: 시금치의 주요 해충인 흰띠명나방(*Spoladea recurvalis*) 유충의 살충제 5종에 대한 감수성을 검정하였다. Lufenuron EC, chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, pyridalyl EW는 각각 2(12.5 ppm), 4(12.5 ppm), 8(2.5 ppm), 4(20.0 ppm), 8(12.5 ppm)배의 희석농도에서 90% 이상의 높은 살충활성을 보였다. 추천농도로 경엽처리 후 7일이 경과된 시금치 잎에 흰띠명나방 유충이 72시간 동안 노출되었을 경우 chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, pyridalyl EW의 살충률은 각각 98.3%, 100%, 95.0%, 100%로 나타나 높은 잔효성을 보였다. 흰띠명나방에 대한 방제효과를 2개소(화성, 연천)에서 포장검정 결과, 5종의 약제 모두 2개소에서 약제처리 7일 경과 후 90% 이상의 방제효과를 보였으며 2배량에서도 약해가 없어 향후 흰띠명나방 방제약제로 시금치에 활용이 가능할 것으로 판단된다.

검색어: 시금치, 흰띠명나방, 살충제, 감수성

The Hawaiian beet webworm (*Spoladea recurvalis*) is widely distributed worldwide, including countries in Africa and Asia, such as Korea (Bae and Paek, 2006; Kim et al., 2014; Jeyasankar and Gokilamani, 2016; Ehsine and Aoun, 2020; Husain et al., 2020; Kwon et al., 2021). Although *S. recurvalis* is considered to belong to the *Spoladea* genus in most countries, including Korea (Bae and Paek, 2006), it is considered to belong to the *Hymenia* genus in some countries. The larvae attack Chenopodiaceae, Amaranthaceae, and Liliaceae, and they fold leaves using thread and feed the interior soft tissue of the stem(Jeyasankar and Gokilamani, 2016; Kim et al., 2014). Depending on the environment, *S. recurvalis* passes either through the 4th-instar larval stage (Jeyasankar and Gokilamani, 2016) or 5th-instar (Lee et al., 2013). Egg hatching is possible at 15°C, but development into the 2nd-instar does not occur, and the total developmental period is 14.6-51.0 days at 17.5-35.0°C

***Corresponding author:** yslee75@gg.go.kr
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(Lee et al., 2013). In South Korea, representative host crops, such as spinach, beet, beetroot, and amaranth, that are damaged by *S. recurvalis* show a decrease in the quantity (Bae and Paek, 2006; Kim et al., 2014; Kwon et al., 2021).

The major insect pests of spinach that have been reported are aphids, mites, spinach leaf miner (*Pegomya Hyoscyami*), tomato leaf miner (*Liriomyza bryoniae*), and tortoise beetle (*Cassida nebulosa*). However, studies on the occurrence and optimal control time of pests through precise diagnosis are still lacking in South Korea (RDA, 2011). When *S. recurvalis* occurs on spinach, young larvae start to attack the tender leaves first, followed by the rear side of leaves. As mature leaves are folded, the commercial value and quantity are significantly damaged when left without control (Fig. 1-A, B).

Several studies have been conducted to establish control strategies against *S. recurvalis* in South Korea, including a study on the occurrence patterns in several vegetable greenhouses (Lim et al., 2012; Kwon et al., 2021), temperature-dependent development models (Lee et al., 2013), and determination of the economic injury level of *S. recurvalis* on beet (Kim et al., 2014). For controlling *S. recurvalis*, several studies have been conducted globally using insecticides, microorganisms, and plant extracts (Jeyasankar and Gokilamani, 2016; Muralikrishna et al., 2019). However, in South Korea, as of 2019, 13 active ingredients for the chemical control of *S. recurvalis* are currently registered for three minor crops, such as leaf beet and pigweed, under the government pesticide registration project in accordance with the positive list system (PLS) (RDA, 2022). As no insecticide is registered for spinach in South Korea, there is an urgent need of an insecticide to control *S. recurvalis* occurrence.

Therefore, this study was conducted to establish an effective control strategy against *S. recurvalis* and to register five insecticides for spinach by inspecting insecticidal susceptibility, residual activity, and field control tests.

Fig. 1. Damage to spinach by Spoladea recurvalis and the spinach greenhouses for the field test of the five insecticides.

Materials and methods

Insect rearing

The *S. recurvalis* used in the insecticidal activity testing was mass-reared using a population that occurred on a spinach field in the Hwaseong region of Gyeonggi Province was collected in 2019. After sowing in a square acrylic container (30 × 30 × 30 cm), 30-day-old spinach seedlings were supplied as food to breed at least three generations in the laboratory. The breeding conditions were 25±1°C, 60%–70% relative humidity, and 16L:8D as the photoperiod.

Test Chemical Agent

A total of five chemical agents were used in the insecticidal susceptibility testing of *S. recurvalis* larvae (Table 1). At the

Table 1. List of the five tested insecticides

Insecticide	AI[†] (%)	Formulation[‡]	RC[§] (ppm)	Group
Lufenuron	5	EC	25	Benzoylureas
Chromafenozide	5	EC	50	Diacylhydrazines
Chlorantraniliprole	4	WP	20	Diamides
Tebufenozide	8	WP	80	Diacylhydrazines
Pyridalyl	10	EW	100	Not known

[†]Active ingredient, [‡]EC; emulsifiable concentration, WP; wettable powder, EW; emulsion in water, [§]RC; Recommended concentration.

time of conducting these experiments, these insecticides were not registered for the control of *S. recurvalis* on spinach in South Korea.

Insecticidal activity in laboratory

The insecticidal effect on 3rd-instar *S. recurvalis* larvae was investigated. Each insecticide was diluted to seven concentrations below the recommended concentration. Spinach leaves were added as a food source to the insect breeding dish (Φ9.0 × 4.0 cm, SPL, Korea) with filter paper on top of moistened cotton wool. After inoculating the *S. recurvalis* larvae, the diluted insecticide was sprayed on the larvae body 5 times (approximately 10 ml) using a small sprayer (4 × 14 cm, 100 ml). Mortality was evaluated after 24, 48, and 72 h after spraying, and dead individuals were enumerated in the untreated group. The corrected mortality (%) was calculated using the Abbott formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Treated mortality} - \text{Untreated mortality}}{100 - \text{Untreated mortality}} \times 100$$

Dead individuals were determined when they did not stand upright or move at least the length of their body when nudged with a brush. The test was conducted under laboratory conditions (25±1°C, 50%–60%, 16L:8D) in three repetitions of 20 larvae per repetition.

Residual effect

The residual effects of the five insecticides on the 3rd-instar *S. recurvalis* larvae were tested. The recommended diluted concentration of each insecticide was sprayed sufficiently on the leaves of spinach seedlings placed in a greenhouse exposed to natural light. After 3, 5, and 7 days from chemical spraying, they were moved to the laboratory and used for the test. The leaves sprayed in advance were fed to the larvae, and the mortality was evaluated after 24, 48, and 72 h under laboratory conditions (25±2°C, 50%–60%, 16L:8D). The test was repeated thrice, with 20 larvae per repetition.

Control Effect in Field

The field control effect was conducted for the five insecticides at two spinach (cultivar Ultra) greenhouses in Hwaseong and Yeoncheon in Gyeonggi Province. The spinach was sown directly in the greenhouse, and a drip irrigation system was used to provide water. To unify the density and larval developmental stage of *S. recurvalis* before treatment, 50 larvae were selected and inoculated per replicate at Yeoncheon (September 15) and Hwaseong (September 24). The plot was arranged in repetitions of three randomized block designs, and a net cage (BugDorm-3 rearing cage, Seoul, Korea) covered the inoculation point to minimize loss due to external factors such as birds and larval self-defeating (Fig. 1-C). Each insecticide was diluted to the recommended concentration, and the number of living larvae was determined after 3, 5, and 7 days from insecticide spraying. The control value (%) was calculated as [(percent living in untreated–percent living in treated)/percent living in untreated] × 100. In addition, these five insecticides were not registered for spinach, and the presence of external phytotoxicity was examined in the shoots and leaves after 3, 5, and 7 days from spraying at the recommended and double concentrations.

Statistical Analysis

The indoor insecticidal activity and field control effect for each insecticide were compared using the SAS statistics program (SAS Institute, 2011) by testing the difference between means using the Duncan's multiple range test (DMRT).

Results

Insecticidal activity in laboratory

Table 2 shows the insecticidal activity of the five insecticides that have not been registered for spinach against *S. recurvalis*. Pyridalyl EW, with a yet unreported mode of action, showed 100% insecticidal activity after 24 h of spraying at the recommended concentration. Chlorantraniliprole WP, a compound of the diamides group, showed a 98.3% insecticidal activity after 48 h of spraying. Lufenuron EC, chromafenozide EC, and

Table 2. Comparison of susceptibility of *Spoladea recurvalis* 3rd-instar larva to five insecticides at different concentrations under laboratory conditions

Insecticide	Conc. (ppm)	% Corrected mortality (mean±SE[†])		
		24h	48h	72h
Lufenuron	25.0	45.0±18.03 a[‡]	75.0±10.00 a	96.7±2.89 a
	12.5	43.3±7.64 a	73.3±5.77 ab	90.0±0.00 a
	6.3	38.3±7.64 a	71.7±7.64 ab	86.7±7.64 a
	3.1	33.3±7.64 a	70.0±8.66 ab	83.3±7.64 a
	1.6	25.0±18.03 a	55.0±15.00 b	75.0±13.23 a
	0.8	3.3±2.89 b	6.7±5.77 c	8.3±7.64 b
	0.4	0.0±0.00 b	3.3±5.77 c	16.7±24.66 b
Chromafenozide	50.0	26.7±12.58 a	78.3±12.58 a	96.7±2.89 a
	25.0	25.0±8.66 a	73.3±7.64 a	96.7±2.89 a
	12.5	21.7±10.41 a	73.3±2.89 a	96.7±2.89 a
	6.3	20.0±10.00 a	71.7±10.41 a	85.0±5.00 b
	3.1	10.0±5.00 ab	53.3±7.64 b	83.3±7.64 b
	1.6	2.1±3.61 b	3.8±3.31 c	39.6±8.32 c
	0.8	1.6±2.75 b	6.5±2.61 c	23.0±6.07 d
Chlorantraniliprole	20.0	86.7±5.77 a	98.3±2.89 a	100±0.00 a
	10.0	81.7±2.89 a	83.3±5.77 b	96.7±5.77 a
	5.0	76.7±2.89 a	81.7±2.89 b	95.0±5.00 a
	2.5	61.7±7.64 b	80.0±5.00 b	90.0±5.00 a
	1.3	31.7±12.58 c	55.0±13.23 c	65.0±10.00 b
	0.6	1.9±3.21 d	3.6±3.13 d	22.7±7.83 c
	0.3	0.0±0.00 d	1.4±2.51 d	7.6±8.92 d
Tebufenozide	80.0	50.0±18.03 a	81.7±10.41 a	100±0.00 a
	40.0	45.0±5.00 a	81.7±5.77 a	100±0.00 a
	20.0	36.7±10.41 a	80.0±21.79 a	100±0.00 a
	10.0	20.0±5.00 b	61.7±7.64 a	81.7±2.89 b
	5.0	11.7±5.77 bc	36.7±7.64 b	43.3±2.89 c
	2.5	1.8±3.04 c	7.8±2.26 c	13.3±15.57 d
	1.3	1.7±2.89 c	1.7±2.89 c	0.0±0.00 e
Pyridalyl	100.0	100±0.00 a	100±0.00 a	100±0.00 a
	50.0	100±0.00 a	100±0.00 a	100±0.00 a
	25.0	96.7±5.77 a	100±0.00 a	100±0.00 a
	12.5	93.3±7.64 a	100±0.00 a	100±0.00 a
	6.3	43.3±7.64 b	68.3±10.41 b	80.0±13.23 b
	3.1	5.0±8.66 c	11.7±5.77 c	23.3±2.89 c
	1.6	6.7±5.77 c	6.7±5.77 c	8.3±2.89 d
	df	6	6	6
	F-value	7.87	33.07	26.79

[†]Standard Error.

[‡]Means followed by the same letter within a column are not significantly different at $p < 0.05$ by Duncan's multiple range Test (SAS Institute, 2011).

tebufenozide WP, which belong to the insect growth regulator group, exhibited low insecticidal activities of 45.0%, 26.7%, and 50.0% after 24 h of spraying, which then increased to 96.7%, 96.7%, and 100% after 72 h, respectively. After 72 h of insecticide spraying, lufenuron EC, chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW showed insecticidal activities of 90% or more, even at 2 (12.5 ppm), 4 (12.5 ppm), 8 (2.5 ppm), 4 (20.0 ppm), and 8 (12.5 ppm) times diluted concentrations.

Residual effects testing

Fig. 2 shows the residual effect of the five insecticides on 3rd-instar *S. recurvalis* larvae. When the larvae were inoculated on the spinach leaves after 3, 5, and 7 days from insecticide spraying, high insecticidal activities were observed, similar to the insecticidal activity observed in the laboratory (day 3: $df = 5$; $F = 239.43$; $p < 0.01$; day 5: $df = 5$; $F = 89.95$; $p < 0.01$; and day 7: $df = 5$; $F = 216.64$; $p < 0.01$). Chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW showed 98.3%, 100%, 95.0%, and 100% mortality when the *S. recurvalis* larvae were exposed 7 days after spraying, respectively. In particular, pyridalyl EW showed a high residual effect and showed the most rapid insecticidal activity (86.7%) after 24 h of exposure to *S. recurvalis* larvae 7 days after treatment. In contrast, lufenuron EC showed 96.8% insecticidal activity after 72 h of exposure to *S. recurvalis* larvae 3 days after treatment, but the insecticidal activity decreased to less than 75% 5 days after treatment.

Control effect field testing

Field control tests were conducted for the five insecticides at the two spinach greenhouses located in Hwaseong and Yeoncheon in the Gyeonggi Province. The control value showed an increasing trend as the number of days passed since insecticide spraying in both locations. Lufenuron EC, chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW showed high control effects of 98.5%, 100%, 97.1%, 100%, and 100% after 7 days of treatment at Hwaseong region, respectively (Tables 3, 4), and the control values were 99.3%, 100%, 97.1%, 100%, and 100% at Yeoncheon region, respectively (Tables 5, 6). In addition, these insecticides did not cause any phytotoxicity to the leaves and stems of spinach, even at

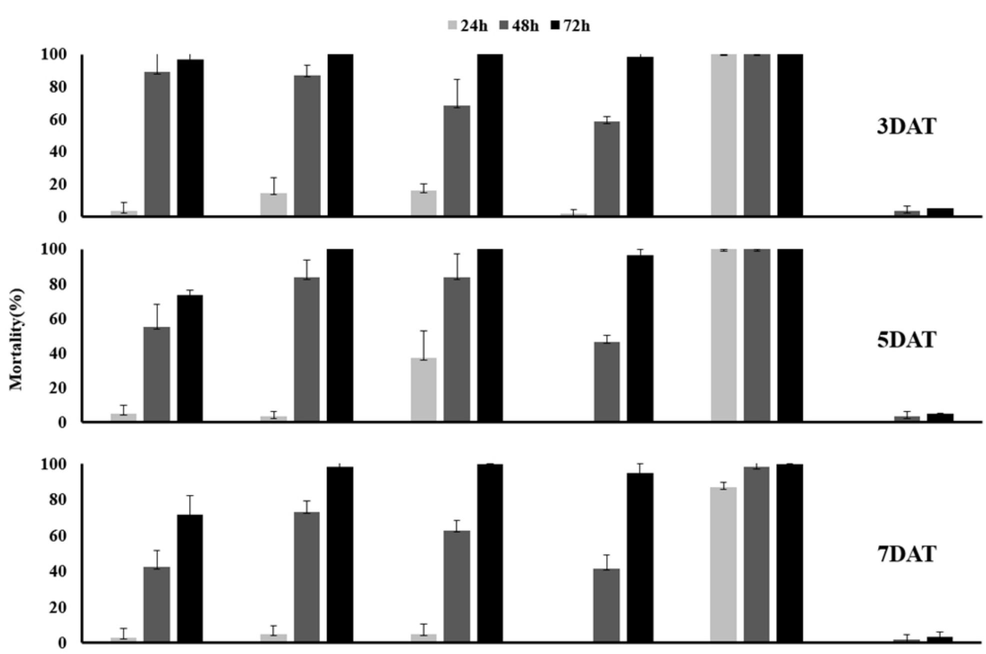

Fig. 2. Residual effect of five insecticides on *Spoladea recurvalis* 3rd instar larva at recommended concentrations (the upper, middle, and lower parts of the graph indicate that larvae were inoculated 3, 5, and 7 days after treatment, respectively).

Table 3. Control effect of *Spoladea recurvalis* larvae to five insecticides in spinach greenhouse in Hwaseong regions after 3 days of treatment (n=50)

Insecticide	Survival rate (%)				Control value (%)	DMRT‡
	I	II	III	Mean†		
Lufenuron	10.0	4.0	8.0	7.3	92.3	b
Chromafenozide	0.0	0.0	0.0	0.0	100	c
Chlorantraniliprole	4.0	4.0	10.0	6.0	93.7	b
Tebufenozide	0.0	0.0	0.0	0.0	100	c
Pyridalyl	0.0	0.0	0.0	0.0	100	c
Control	98.0	96.0	92.0	95.3	-	a

†Coefficient of variation: 12.5

‡Means followed by the same letters are not significantly different at $p < 0.05$ by Duncan's multiple range test (SAS Institute, 2011).

Table 4. Control effect of *Spoladea recurvalis* larvae to five insecticides in spinach greenhouse in Hwaseong regions after 7 days of treatment (n=50)

Insecticide	Survival rate (%)				Control value (%)	DMRT‡
	I	II	III	Mean†		
Lufenuron	2.0	0.0	2.0	1.3	98.5	b
Chromafenozide	0.0	0.0	0.0	0.0	100	b
Chlorantraniliprole	0.0	2.0	6.0	2.7	97.1	b
Tebufenozide	0.0	0.0	0.0	0.0	100	b
Pyridalyl	0.0	0.0	0.0	0.0	100	b
Control	96.0	92.0	86.0	91.3	-	a

†Coefficient of variation: 15.4

‡Means followed by the same letters are not significantly different at $p < 0.05$ by Duncan's multiple range test (SAS Institute, 2011).

Table 5. Control effect of *Spoladea recurvalis* larvae to five insecticides in spinach greenhouse in Yeoncheon regions after 3 days of treatment (n=50)

Insecticide	Survival rate (%)				Control value (%)	DMRT‡
	I	II	III	Mean†		
Lufenuron	2.0	4.0	2.0	2.7	97.3	c
Chromafenozide	0.0	0.0	0.0	0.0	100	c
Chlorantraniliprole	6.0	2.0	2.0	3.3	96.6	b
Tebufenozide	0.0	2.0	0.0	0.7	99.3	c
Pyridalyl	0.0	0.0	0.0	0.0	100	c
Control	100	96.0	98.0	98.0	-	a

†Coefficient of variation: 8.1

‡Means followed by the same letters are not significantly different at $p < 0.05$ by Duncan's multiple range test (SAS Institute, 2011).

Table 6. Control effect of *Spoladea recurvalis* larvae to five insecticides in spinach greenhouse in Yeoncheon regions after 7 days of treatment (n=50)

Insecticide	Survival rate (%)				Control value (%)	DMRT‡
	I	II	III	Mean†		
Lufenuron	0.0	2.0	0.0	0.7	99.3	b
Chromafenozide	0.0	0.0	0.0	0.0	100	b
Chlorantraniliprole	4.0	0.0	2.0	2.0	97.1	b
Tebufenozide	0.0	0.0	0.0	0.0	100	b
Pyridalyl	0.0	0.0	0.0	0.0	100	b
Control	96.0	92.0	94.0	94.0	-	a

†Coefficient of variation: 12.5

‡Means followed by the same letters are not significantly different at $p < 0.05$ by Duncan's multiple range test (SAS Institute, 2011).

Table 7. Phytotoxicity of five insecticides to in spinach in two regions after 3, 5, and 7 days of treatment

Insecticide	Crop (Cultivar)	Phyto-toxicity(0-5)	
		Recommended concentration	Double concentration
Lufenuron	Spinach (Ultra)	0	0
Chromafenozide		0	0
Chlorantraniliprole		0	0
Tebufenozide		0	0
Pyridalyl		0	0

double concentrations (Table 7).

Discussion

This study provides effective five insecticides against *S. recurvalis* through insecticidal susceptibility, residual activity, and field control tests. In this study, the five insecticides used also showed similarly high insecticidal activity at the recommended concentrations, and it was observed that *S. recurvalis* showed very high susceptibility even when exposed to leaves 7 days after insecticide spraying, even at concentrations below the recommended values. Therefore, it was determined that these insecticides will result in an effective control at the early stage of *S. recurvalis* occurrence. Five insecticides showed a time difference in insecticidal activity depending on the mode of action, but all insecticides showed high insecticidal activity against the *S. recurvalis* larvae (3rd-instar). Studies on the insecticidal activity of insecticides against *S. recurvalis* are insufficient. Moreover, there have been no reports on insecticide resistance. In the bioassay of 11 insecticides of 10 modes of action against the *S. recurvalis* larvae (2nd-instar), Muralikrishna et al. (2019) reported that all 11 insecticides, including chlorantraniliprole, showed 100% insecticidal activity after 36 h of spraying. This result was similar to our results that chlorantraniliprole, a diamide insecticide, showed a 97.1% control on *S. recurvalis* at the recommended concentration even in the field conditions. However, the resistance of diamide insecticides to moth pests, including *Spodoptera exigua*, is rapidly progressing in Korea and China (Nauen and Steinbach, 2016; Cho et al., 2018; Wang et al., 2021). Therefore, to prevent the emergence of insecticide-resistant populations in the future, an alternating insecticide spraying program with different modes of action should be developed.

In the residual effect test of five insecticides, it can be deduced that lufenuron EC should be sprayed at least every 3 days, and chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW sprayed at 7 day intervals to control *S. recurvalis* larvae on spinach. However, in this experiment, spinach, which was pre-treated with the insecticides, was exposed to sunlight but was not in contact with water through the drip irrigation system. Therefore, the efficacy duration may decrease depending on various irrigation methods, such as sprinklers.

In the field test in spinach greenhouse at two regions, lufenuron EC, chromafenozide EC, chlorantraniliprole WP, tebufenozide WP, and pyridalyl EW showed high control effect of 97.1% or more and did not cause any phytotoxicity to the leaves and stems of spinach, even at double concentrations. In the absence of pesticides registered in spinach for the control of *S. recurvalis,* the results of this study will be basic data for establishing an integrated control strategy for *S. recurvalis*. Besides, all tests were conducted according to the pesticide registration test method of South Korea, so this result means these five insecticides are very likely to be registered in spinach.

Although most of the spinach from Gyeonggi area is cultivated in greenhouses, it is sometimes grown in open fields. This difference can result in different insecticidal activities due to photosynthesis from natural light and rain (Hulbert et al., 2011; Hossain et al., 2013). However, when the control of the open field was conducted by selecting minimal rainfall periods, it was expected that control effects would be similar to those under greenhouse conditions.

These results provide basic data for establishing chemical control strategies for *S. recurvalis* in various crops including spinach. Furthermore, in order to satisfy consumers who want eco-friendly agricultural products and minimize adverse effects on environment, biological pesticides such as neem extracts (Muralikrishna et al., 2019), *B. bassiana* (Opisa et al., 2018), natural enemies, and even resistant cultivars need to be considered (Othim et al., 2018). In the future, we will do further study to make integrated pest management (IPM) program by mixing the five pesticides selected in this study with various insecticidal plant extracts to minimize the use of pesticides.

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Statements for Authorship Position & Contribution

Lee, Y.S.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Designed the research, conducted experiments, analyzed the results, and wrote the manuscript.

Lee, H-A.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Validated insecticide efficiency.

Kim, S-H.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Validated insecticide efficiency.

Choi, J.Y.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Validated statistics analysis.

Lee, H.J.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Validated insecticide efficiency.

Lee, S-W.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Edited the manuscript.

Park, J-S.: Gyeonggi-do Agricultural Research and Extension Services, Researcher; Edited the manuscript.

All authors read and approved the manuscript.

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