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Efficacy of Chemical and Botanical Insecticides Against the Potato Tuber Moth, *Phthorimaea operculella* (Zeller)

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Simple Summary

The efficacy of insecticides against the potato tuber moth (*Phthorimaea operculella*) depends on concentration and exposure time. Laboratory bioassays showed that greater toxicity at a fixed assessment time does not necessarily correspond to faster mortality. Abamectin had the lowest median lethal concentration (LC_{50}), whereas the organophosphates fenthion and diazinon acted most rapidly against newly hatched larvae. These organophosphates were included as comparative toxicity benchmarks rather than field recommendations. Cartap hydrochloride showed activity against eggs and neonate larvae, stages that may be accessible to treatment before larvae enter plant tissues. Methanolic extracts of sweet flag (*Acorus calamus*) rhizomes from South Korea and Nepal had similar LC_{50} values against neonate larvae, although their estimated lethal times differed. The extracts also differed in the relative abundance of *cis*- and *trans*-asarone. However, the contribution of individual asarone isomers to toxicity was not directly tested. Together, these laboratory results identify candidates for further evaluation in integrated potato tuber moth management; their field efficacy and safety require additional study.

Abstract

We evaluated ten insecticides and methanolic rhizome extracts of sweet flag (*Acorus calamus*) against the eggs and newly hatched larvae of the potato tuber moth, *Phthorimaea operculella*. Larval toxicity was assessed in glass scintillation vials coated with insecticides or plant extracts, and mortality was recorded at defined intervals. Ovicidal activity was evaluated using an egg-dipping assay. Median lethal concentrations (LC_{50}) at 24 h and median lethal times (LT_{50}) at the recommended field concentrations were estimated for the larval assays. Among the insecticides, fenthion had the shortest LT_{50} (0.37 h), whereas spinetoram elicited the longest (13.56 h). Based on median lethal concentration, abamectin showed the lowest LC_{50} (0.005 mg a.i./L), followed by emamectin benzoate (0.008 mg a.i./L). Cartap hydrochloride, emamectin benzoate, and diazinon caused 100% mortality of neonate larvae at a 100-fold dilution of the recommended field concentration after 24 h. Cartap hydrochloride caused egg mortality of 95.75%, 94.00%, 94.25%, and 89.75% in eggs aged one, two, three, and four days, respectively, indicating activity against eggs under laboratory conditions. HPLC profiles of sweet flag extracts differed by geographical origin, showing a relative peak-area percentage of 67.4% *cis*-asarone and 14.5% *trans*-asarone for the South Korean extract, compared with 49.8% and 28.7%, respectively, in the Nepali

Academic Editors: Maria C. Boukouvala and Nickolas G. Kavallieratos

Received: 12 October 2026
Revised: 19 November 2026
Accepted: 21 November 2026
Published: 26 November 2026

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extract. Despite these variations in relative peak-area profiles, the two extracts had similar larval LC_{50} values (36.40 and 36.82 ppm, for the South Korean and Nepali origin, respectively). These results provide a basis for further evaluation of insecticidal and botanical treatments against *P. operculella*.

Keywords: mortality; insecticides; recommended dose; emamectin benzoate; abamectin

1. Introduction

The potato tuber moth (PTM), *Phthorimaea operculella*, is a cosmopolitan pest [1] that has become established throughout South Korea following its introduction in 1968 [2–4]. Although it has a broad host range, it particularly damages solanaceous crops, including potato and tomato [4,5]. The larval stage causes injury by feeding on potato foliage in the field and on tubers before and after harvest [6,7]. In the absence of adequate postharvest management, reported losses caused by *P. operculella* range from 25% to 100% in stored potatoes [8–13]. Field yield losses may reach 70% when tubers are infested early [14]. Warm conditions favor the distribution and abundance of this pest. One climate-change projection [5] estimated that the area supporting four generations per year could increase from 30.1% to 42.4% of global potato-growing regions by 2050. The same study projected additional areas at risk in Asia, North America, and Europe. In South Korea, the pest reportedly expanded its range approximately 100 km northward over three decades [3], potentially aided by cold tolerance [15]. Integrated pest management (IPM) options include cultural practices, host-plant resistance, natural enemies, pheromone-based approaches, and botanical products. Chemical insecticides may be considered when pest pressure exceeds an appropriate management threshold [16].

Several insecticide groups have been evaluated for controlling *P. operculella*, including organophosphates, carbamates, pyrethroids, neonicotinoids, insect growth regulators, and botanical products. Earlier studies reported activity of azinphos-ethyl and endosulfan against leaf-mining larvae [6], as well as reduced damage following applications of quinalphos and diflubenzuron [17]. Other compounds were also investigated in historical control programs [18], although some are now obsolete or subject to regulatory restrictions. More recent field experimental studies have reported the efficacy of chlorantraniliprole, indoxacarb [19], and cartap hydrochloride against lepidopteran pests [20]. In South Korea, insecticides from several chemical groups have been compared across the egg, larval, pupal, and adult stages of *P. operculella* [21]. Additional studies have examined their effects on newly hatched larvae [22] and the field efficacy of emamectin benzoate, sulfoxaflor, and indoxacarb [23]. These findings highlight the importance of identifying compounds that act during the brief period when vulnerable pest stages are exposed.

The insecticidal activity of sweet flag (*Acorus calamus* L.) rhizome powder, solvent extracts, and essential oils has been investigated against several insect pests [24–29]. Asarone is among the principal constituents associated with this activity [30,31]. Previous studies have suggested that *cis*- and *trans*-asarone may differ in their toxic and antifeedant effects [25]. Sweet flag preparations have also been reported to suppress stored-product beetles and potato tuber moth infestation during storage [24,27]. However, information on the persistence of protection provided by a single application of *A. calamus* rhizome powder, obtained from different geographical origins, against successive PTM infestation during potato storage remains limited. Furthermore, the comparative toxicity of crude methanol rhizome extracts obtained from two country-specific origins against newly hatched *P. opercullela* larvae remains to be fully understood.

https://doi.org/10.3390/insects17121235

Eggs and newly hatched larvae are potentially accessible targets for insecticide treatment because neonates remain exposed only briefly before entering host tissues. Eggs may also be susceptible to compounds that penetrate or affect the chorion. Although several studies have investigated insecticide toxicity against *P. operculella*, comparative evaluations of multiple insecticides against eggs of different ages, together with their contact toxicity against newly hatched larvae, remain relatively limited. Therefore, this study aimed to (i) evaluate the contact toxicity of selected insecticides against neonate larvae of *P. operculella*, (ii) assess their effects on egg mortality across egg ages, (iii) investigate the toxicity of methanolic *A. calamus* rhizome extracts obtained from two geographical origins, and (iv) evaluate the residual efficacy of a single application of rhizome powder from two geographical origins against successive PTM infestations during potato storage bioassay. By integrating these assessments, this study provides comparative perspectives into the effects of synthetic insecticides and botanical preparations from different geographical origins for managing the most exposed developmental stages of *P. operculella,* thus contributing to potato protection under field and storage conditions.

2. Materials and Methods

2.1. Insect Rearing

PTM eggs were obtained from Highland Research Institute (HARI), Pyeongchang, Gangwon-do, South Korea, and maintained at the Insect Ecology Laboratory, Andong National University for two years. The potato tubers were placed in plexiglass container boxes (Ø40 × 40 × 40 cm) lined with fine sterilized sand at the bottom and incubated at 25 ± 1 °C and 65 ± 5% relative humidity (RH). The dry sand in the container served as the pupation medium. When the larvae had completed the larval stage, the pupae (with their cocoons) were harvested after sieving (2.0 mm mesh, LK Labkorea, Namyangju (Namyangju-si), Gyeonggi-do, Republic of Korea). Cocoons were removed, and pupae were surface-sterilized by washing them in a sodium hypochlorite (Samchun Pure Chemical Co., Ltd., Pyeongtaek, Gyeonggi-do, Republic of Korea) solution (0.3%) [32]. The collected pupae were air-dried and placed in a plastic container (Ø12 cm × 14 cm depth), which was covered with a mesh cloth. After adult emergence, a filter paper was placed on the mesh cloth as an oviposition medium. Adults were fed a 5% sugar solution, which was dropped on the edges of the filter paper. The filter papers (Hyundai Micro Co., Ltd., Seoul, Republic of Korea) containing the eggs were changed daily and collected. Eggs were allowed to hatch and used for experiments or for further rearing. The rearing cycle was continued to multiply the PTM population to provide sufficient eggs for the experiments [15,33].

2.2. Insecticides Used for Toxicity Evaluation

The insecticides were selected from products used against *P. operculella* or other lepidopteran pests and listed by the Korean Crop Protection Association, Seoul, Republic of Korea [34]. Commercial formulations, their chemical groups, modes of action, and application concentrations are summarized in Table 1. Ten insecticides were tested: spinetoram (Dongbu Farmhannong, Seoul, Republic of Korea), cartap hydrochloride (Kyeongnong, Seoul, Republic of Korea), emamectin benzoate (Arysta LifeScience, Chungju, Republic of Korea), abamectin (Taejun Agrotech, Seongnam, Republic of Korea), thiamethoxam (Syngenta, Seoul, Republic of Korea), dinotefuran (Namhae, Yeosu, Republic of Korea), etofenprox (Dongbu Farmhannong, Seoul, Republic of Korea), diazinon (Seongbo Chemicals, Ansan, Republic of Korea), fenthion (Dongbu Farmhannong, Seoul, Republic of Korea), and indoxacarb (Taejun Agrotech, Seongnam,

Republic of Korea). The compounds representing several modes of action include spinosyns, avermectins, nereistoxin analogues, neonicotinoids, pyrethroid-like compounds, organophosphates, and oxadiazines. The insecticide concentrations (mg a.i./L) were calculated based on the amount of active ingredients (a.i.) per liter in each commercial formulation.

Table 1. Insecticides evaluated against *P. operculella* eggs and neonate larvae, including chemical group, mode of action, formulation, and recommended field application rate.

Insecticide	Chemical Subgroup	Mode of Action Classification *	Formulation	Field Dose/L of Water	Conc (mg a.i. \L)
Spinetoram	Spinosyns	Nicotinic acetylcholine receptor (nAChR) allosteric modulators—Group 5	5% (SG)	0.5 g	25
Cartap hydrochloride	Nereistoxin	Nicotinic acetylcholine receptor (nAChR) channel blockers—Group 14	50% (SP)	1 g	500
Abamectin	Avermectins	Glutamate-gated chloride channel (GluCl) allosteric modulators—Group 6	1.8% (EC)	0.335 mL	6.03
Emamectin Benzoate	Avermectins	Glutamate-gated chloride channel (GluCl) allosteric modulators—Group 6	2.15% (EC)	0.5 mL	10.75
Thiamethoxam	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators—Group 4A	10% (SG)	0.5 g	50
Dinotefuran	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators—Group 4A	20% (SG)	1 g	200
Etofenprox	Pyrethroids	Sodium channel modulators—Group 3A	20% (EC)	1 mL	200
Diazinon	Organophosphates	Acetylcholinesterase (AChE) inhibitors—Group 1B	34% (EC)	1.25 mL	425
Fenthion	Organophosphates	Acetylcholinesterase (AChE) inhibitors—Group 1B	50% (EC)	1 mL	500
Indoxacarb	Oxadiazines	Voltage-dependent sodium channel blockers—Group 22A	30% (WG)	0.17 g	51

* Source: IRAC, 2026 [35].

2.3. Effects of Insecticides on P. operculella Eggs

One-to-four-day-old eggs were treated with the insecticide solutions prepared at recommended field concentrations (Table 1). Because PTM eggs remained attached to the filter paper used as an oviposition medium, the filter paper with the required number of eggs attached to it was cut into small pieces. Each solution was prepared in 1 L of water, and the filter paper pieces with attached eggs were immersed for 30 s before being transferred to small Petri dishes (50 × 15 mm; SPL Life Sciences, Pocheon, Republic of Korea) for incubation under laboratory conditions [16]. Four replicates of 100 eggs were used for each egg age and treatment.

Egg hatchability was assessed by observing larval emergence. Eggs were considered hatched when larvae emerged, leaving an empty chorion, and unhatched when no larval emergence occurred. Observations continued for an additional 10 days after the normal hatching period to confirm that no further hatching occurred. Egg mortality was calculated based on the number of unhatched eggs.

2.4. Glass-Vial Bioassay of Insecticide Toxicity to Neonate Larvae

Each insecticide was prepared in tap water at its recommended field concentration and at four successive 10-fold dilutions (Table S1). Tap water was used to represent the water sources commonly available to farmers for insecticide preparation. The same water

source was used for the control treatments. Glass scintillation vials (20 mL) were used for the bioassays. The inner surface of each vial was coated with 100 µL of the test solution by rotating the vial to distribute the liquid evenly [36–39]. The coated vials were allowed to dry completely before the introduction of larvae. Ten neonate larvae that had hatched within a 12-h period were then introduced into each vial [40]. Four replicate vials were used for each insecticide–concentration combination (40 larvae in total), and water-coated vials served as controls, exhibiting a 2.5% background mortality rate (one dead larva out of 40). No food was provided. Mortality was recorded at 15, 30, and 60 min hourly thereafter up to 10 h; after an additional 3 h; and subsequently at 6-h intervals until the control larvae died of starvation. Larvae were gently stimulated with a camel-hair brush and classified as dead if they showed no movement. Thiamethoxam was excluded from the final lethal-concentration analyses because its mortality effect was negligible and comparable to that of the control.

2.5. Collection and Methanolic Extraction of Sweet Flag Rhizomes

Acorus calamus rhizomes were harvested from the grounds of Andong National University, Republic of Korea, in June 2018. They were washed, cut into pieces of ca. 1 cm^3, air-dried in the shade (Figure S1), ground, sieved to obtain a fine powder, and stored at 4 °C until use. Powdered *A. calamus* rhizomes from Nepal were obtained from the National Entomology Research Center, Nepal Agricultural Research Council. Additionally, commercially available dried *A. calamus* rhizomes were obtained from Jecheon Medical Herb, South Korea, and used in the dusting bioassays.

The Nepali rhizome powder and the powder prepared from the rhizomes harvested at Andong National University were separately extracted with methanol (Kanto Chemical Co., Inc. Tokya, Japan) by shaking with a digital orbital shaker (Daihan Scientific Co., Ltd., Wonju, SHO-2D, Republic of Korea) during a 72-h maceration period. The extracts were concentrated using a rotary evaporator (Eyela N-1110, Rikakikai Co., Ltd., Tokyo, Japan). The extraction of 100 g of Nepali *A. calamus* powder yielded 18.89 g of crude extract, whereas 25 g of Andong-harvested Korean *A. calamus* powder yielded 3.9 g of crude extract.

2.6. Bioassays of Sweet Flag Rhizome Preparations Against P. operculella Larvae

2.6.1. Effects of Sweet Flag Extracts on Neonate Larvae

A crude sweet flag extract stock solution was prepared in methanol at a weight-to-volume (w/v) concentration of 4 mg/10 mL, followed by two-fold serial dilution with methanol to obtain 400, 200, 100, 50, 25, and 12.5 mg/L. Pure methanol served as the solvent control. The inner surfaces of glass scintillation vials were coated with 100 µL of each solution by rotating the vials until the solvent evaporated. Each vial containing ten neonate larvae was treated as an experimental unit, with ten replicate vials per concentration (100 larvae per treatment). Larvae were monitored frequently during the initial exposure period to record early effects; observation intervals were subsequently increased up to 16 h and then continued at 8-h intervals until control larvae died. No food was supplied. Larvae were gently touched with a camel-hair brush and considered dead if no movement was observed.

2.6.2. Potato-Tuber Storage Bioassay with Rhizome Powder

Potato tubers (cv. Superior) were treated with dried sweet flag rhizome powder at rates of 0, 0.5, 1, 2, 3, and 4 g/kg of tubers. Powder was distributed over the tubers, which were placed in plastic containers. Each container (replicate) contained one or two potato tubers with a total weight of 100 g [41]. Four replicates were established for each treatment. Fifty neonate larvae were introduced into each container at monthly intervals over a three-

month period to assess residual efficacy. The number of emerging adults was recorded each month. The containers (12 cm in diameter × 14 cm in depth) were maintained at 25 ± 1 °C and 65 ± 5% relative humidity (RH).

2.7. High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was performed to examine the chromatographic profile of *Acorus calamus* methanol extract from two geographical origins and compare it with the previously reported profile of *cis*- and *trans*-asarone isomers. The analysis was performed using a Shimadzu Prominence UFLC system equipped with a DGU-20A3 degasser (Shimadzu Corporation, Kyoto Japan) , dual LC-20AD pumps, SIL-20A autosampler, CTO-20A column oven, and SPD-20A UV detector. Chromatographic separation was achieved using a Shiseido Capcell Pak C18 MG column (250 × 4.6 mm, 5 μm). The mobile phase consisted of solvent A (0.1% phosphoric acid in water) and solvent B (80% acetonitrile). Gradient elution was performed at a flow rate of 0.8 mL/min according to the following program: 0–5 min, 80% A; 5–10 min, 80–70% A; 10–20 min, 70–55% A; 20–30 min, 55–30% A; 30–35 min, 30% A; 35–40 min, 30–20% A; 40–60 min, 20% A; 60–62 min, 20–80% B; and 62–70 min, 80% B. The column temperature was maintained at 30 °C, the injection volume was 20 μL, and detection was performed at 250 nm.

2.8. Statistical Analysis

Median lethal concentrations (LC_{50}) and median lethal times (LT_{50}) were estimated by Probit analysis for both synthetic-insecticide and botanical-extract vial bioassays [42]. Bioassays followed a completely randomized design. Observed mortality was adjusted using the following Schneider–Orelli correction equation [43].

$$Corrected\ mortality\ \% = \left(\frac{Mortality\ \%\ in\ treatement - Mortality\ \%\ in\ control}{100 - Mortality\ \%\ in\ Control}\right) \times 100$$

Concentration effects were assessed using Wald chi-square tests, and model goodness of fit was examined using Pearson chi-square statistics. When overdispersion was detected, a heterogeneity correction was applied to the covariance matrix. Differences in lethal-concentration or lethal-time estimates for *A. calamus* extracts were evaluated using the lethal-dose ratio test [44].

Egg mortality was analyzed by analysis of variance (ANOVA), followed by Tukey's post hoc comparisons. The residual effects of sweet flag powder in the potato-tuber assay were analyzed by repeated-measures ANOVA after square-root transformation [√ (x + 0.5)]. Analyses were performed using SAS 9.4 [45]. Survival curves and residual graphs were generated using ggplot2 in R 4.4.3 through RStudio (version 2026.08.2) [46].

3. Results

3.1. Effects of Insecticides on P. operculella Eggs

Among the ten insecticides tested, cartap hydrochloride showed the highest mortality across all four egg ages (Table 2), with mean activity rates of 95.75%, 94.00%, 94.25%, and 89.75% in one-, two-, three-, and four-day-old eggs, respectively. Treatment effects were highly significant at the four egg ages (one-day-old: $F_{10,33} = 686.23$, $p < 0.0001$; two-day-old: $F_{10,33} = 440.32$, $p < 0.0001$; three-day-old: $F_{10,33} = 245.15$, $p < 0.001$; four-day-old: $F_{10,33} = 405.57$, $p < 0.0001$.

Table 2. Effects of insecticides on the mortality of *P. operculella* eggs of different ages.

Insecticide	Mean Percentage of Egg Mortality (±SE)			
	1d Old	2d Old	3d Old	4d Old
Spinetoram	14.25 ± 1.49 b	9.75 ± 2.39 b	6.75 ± 1.80 b	7.7 ± 1.97 b
Cartap hydrochloride	95.75 ± 1.11 a	94.00 ± 1.68 a	94.25 ± 3.47 a	89.75 ± 2.56 a
Abamectin	3.75 ± 1.54 cd	5.75 ± 1.75 bc	9.75 ± 2.09 b	2.00 ± 1.41 bc
Emamectin Benzoate	1.75 ± 0.85 cd	1.25 ± 0.47 c	4.75 ± 1.88 b	1.00 ± 0.57 c
Thiamethoxam	3.25 ± 1.11 cd	1.25 ± 0.48 c	5.25 ± 1.10 b	2.75 ± 0.85 bc
Dinotefuran	4.25 ± 0.48 cd	3.50 ± 0.87 bc	4.75 ± 1.10 b	6.00 ± 1.10 bc
Etofenprox	4.25 ± 0.63 cd	7.00 ± 1.08 b	3.50 ± 0.95 b	3.05 ± 1.25 bc
Diazinon	6.75 ± 0.85 c	4.75 ± 0.94 bc	6.50 ± 1.25 b	5.50 ± 0.87 bc
Fenthion	5.25 ± 1.31 cd	4.50 bc ± 1.19	6.00 ± 1.35 b	5.75 ± 0.95 bc
Indoxacarb	2.25 ± 1.01 cd	2.50 ± 1.25 c	7.25 ± 1.11 b	1.50 ± 0.5 bc
control	1.00 ± 0.57 d	2.25 ± 0.75 c	3.00 ± 0.91 b	1.25 ± 0.48 c

Within a column, means followed by the same letter do not differ significantly according to Tukey's significant difference test ($p \leq 0.05$).

3.2. Toxicity of Insecticides to Neonate Larvae in Glass-Vial Bioassays

Based on 24-h LC_{50} estimates, larval toxicity decreased in the following order: abamectin > emamectin benzoate > spinetoram > indoxacarb > diazinon > cartap hydrochloride > etofenprox > fenthion > dinotefuran (Table 3). Estimates ranged from approximately 0.005 mg a.i./L for abamectin to 2.34 mg a.i./L for dinotefuran, indicating that abamectin elicited the highest toxicity, whereas dinotefuran showed the lowest toxicity in this assay. Comparisons of relative toxicity normalized to recommended concentrations are presented in Table 3. The insecticides were also grouped according to their relative toxicity, with avermectin showing the highest toxicity (Table S2).

Table 3. Median lethal concentrations (LC_{50}: mg a.i./L) of insecticides against neonate *P. operculella* larvae after 24 h of exposure in glass-vial bioassays.

Insecticides	LC_{50} (mg a.i./L) at 24 h (95% CL)	Wald χ^2 (Dose Effect)	Slope	Intercept	*p*	RT	Pearson $\chi 2$ (Goodness of Fit)	Heterogeneity Factor (h)
Spinetoram	0.04 (0.01–0.07)	49.67	0.58	0.84	<0.0001	0.001	5.12 (df = 3, $p = 0.163$)	-
Cartap hydrochloride	0.56 (0.15–2.80)	20.03	1.76	0.44	<0.0001	0.0011	7.40 (df = 3, $p = 0.060$)	2.47
Emamectin Benzoate	0.008 (0.005–0.01)	45.40	1.82	3.78	<0.0001	0.0008	2.10 (df = 3, $p = 0.552$)	-
Abamectin	0.005 (0.003–0.009)	55.98	1.06	2.41	<0.0001	0.0009	2.51 (df = 3, $p = 0.473$)	-
Dinotefuran	2.34 (0.49–12.17)	35.04	0.89	−0.33	<0.0001	0.0117	6.38 (df = 3, $p = 0.095$)	2.13
Etofenprox	0.89 (0.46–1.90)	48.54	0.85	0.03	<0.0001	0.0045	3.71 (df = 2, $p = 0.156$)	-
Diazinon	0.21 (0.13–0.32)	43.35	1.75	1.18	<0.0001	0.0005	0.81 (df = 3, $p = 0.847$)	-
Fenthion	2.14 (0.39–12.19)	24.35	1.18	−0.38	<0.0001	0.0043	8.16 (df = 3, $p = 0.043$)	2.72
Indoxacarb	0.13 (0.002–1.60)	17.33	0.76	0.68	<0.0001	0.0026	11.46 (df = 3, $p = 0.010$)	3.82

Lower relative toxicity ratio (RT) indicates a lower LC_{50} relative to the recommended concentration. RT = LC_{50}/recommended field concentration [47]. Control mortality at 24 h was 2.5% (one death among 40 larvae).

Median lethal times (LT_{50}) were estimated at the recommended field concentrations. Fenthion acted most rapidly (LT_{50} = 0.37 h), whereas spinetoram had the longest estimated LT_{50} (13.56 h; Table 4).

Table 4. Median lethal times (LT_{50}; h) of insecticides at the recommended field concentrations against neonate *P. operculella* larvae in glass-vial bioassays.

Insecticide	LT_{50} (h) (CL 95%)	Wald χ^2	Slope	Intercept	*p*	Pearson χ2 (Goodness of Fit)	Heterogeneity Factor (h)
Spinetoram	13.56 (12.72–14.51)	77.99	8.15	−9.24	<0.001	4.98 (3, $p = 0.173$)	—
Cartap hydrochloride	2.03 (0.78–3.90)	14.20	4.63	−1.43	0.0002	26.33 (2, $p < 0.001$)	13.17
Emamectin Benzoate	5.28 (4.92–5.61)	68.40	7.37	−5.34	<0.001	10.36 (4, $p = 0.035$)	2.59
Abamectin	2.33 (2.06–2.56)	42.20	6.49	−2.39	<0.001	8.25 (2, $p = 0.016$)	4.13
Dinotefuran	9.09 (8.70–9.51)	63.17	10.70	−10.26	<0.001	2.00 (4, $p = 0.735$)	—
Etofenprox	4.67 (4.42–4.91)	84.99	10.45	−7.00	<0.001	1.51 (4, $p = 0.824$)	—
Diazinon	0.54 (0.46–0.64)	57.50	3.67	0.96	<0.001	1.67 (2, $p = 0.435$)	—
Fenthion	0.37 (0.31–0.42)	38.50	4.57	1.96	<0.001	3.28 (1, $p = 0.070$)	3.28
Indoxacarb	8.23 (7.86–8.63)	90.50	9.22	−8.45	<0.001	2.29 (6, $p = 0.891$)	—

Fenthion induced the shortest estimated lethal time against neonate larvae, followed by diazinon, cartap hydrochloride, and abamectin (Table 4). Cartap hydrochloride, emamectin benzoate, and diazinon caused 100% mortality after 24 h even at a 100-fold dilution of their recommended field concentrations (Figure 1).

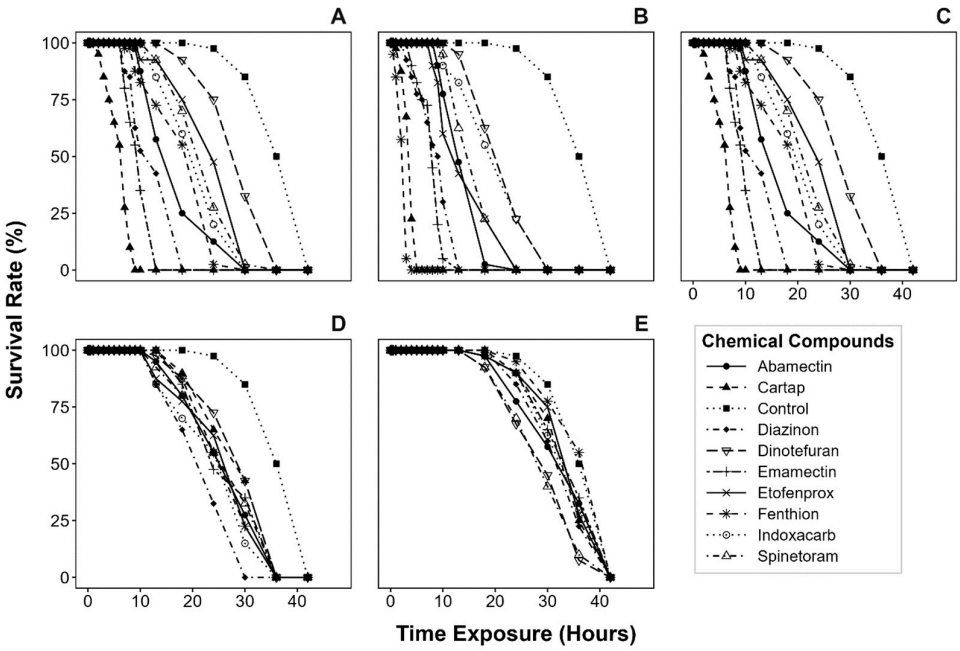


Figure 1. Survival curves of neonate *P. operculella* larvae exposed to insecticides at different concentrations: (**A**) recommended concentration, (**B–E**) 10-, 100-, 1000-, and 10,000-fold dilutions, respectively. The corresponding active ingredient concentration (mg a.i./L) for each insecticide is provided in Table S1. Plots were generated using ggplot2 in R.

3.3. Effects of Sweet Flag Extracts on Neonate Larvae

The methanolic sweet flag extracts from Nepal and South Korea produced similar 24-h LC_{50} estimates against neonate larvae: 36.8 and 36.4 mg/L, respectively (Table 5). The estimated LT_{50} values at 100 mg/L were 3.06 h for the Nepali extract and 2.11 h for the South Korean extract. The reported lethal-concentration ratio of 1.01 (95% confidence interval: 0.44–2.31) did not indicate a statistically significant difference in LC_{50} between

origins. Differences in estimated lethal times should be interpreted separately from this concentration-ratio comparison.

Table 5. LC_{50} (mg/L) and LT_{50} (h) estimates for sweet flag extracts from Nepal and South Korea against neonate *P. operculella* larvae in glass-vial bioassays.

	Sweet Flag Origin	Value (CL 95%)	χ^2	Slope	Intercept	*p*	Pearson χ^2	Heterogeneity Factor (H)
LC_{50}	Nepal	36.8 (17.26–75.22)	16.35	2.9	−4.6	<0.0001	46.90 ($p < 0.0001$)	11.71
	Korea	36.4 (18.89–68.07)	18.66	3.0	−4.7	<0.0001	40.65 ($p < 0.0001$)	10.16
LT_{50}	Nepal	3.06 (1.99–5.27)	26.28	3.1	−1.5	<0.0001	27.53 ($p < 0.0001$)	6.88
	Korea	2.11 (1.92–2.33)	191.8	3.6	−1.17	<0.0001	5.56 ($p = 0.235$)	-

- Lethal-concentration ratios were calculated following Robertson et al. (2017) [44].
- A 95% confidence interval for the LC_{50} ratio that includes 1 indicates no statistically significant difference at the 5% level.
- LT_{50} values were estimated at 100 mg/L. No control mortality was observed during the first 8 h; therefore, no mortality correction was applied for that interval.

At concentrations of 400, 200, and 100 mg/L, the Nepali extract reportedly caused 100% larval mortality within 1, 2, and 8 h, respectively. Corresponding values for the South Korean extract were 2, 4, and 8 h (Figure 2). At lower concentrations, survival patterns approached those observed in the control. The observed time courses should be considered when interpreting differences between the fitted LT_{50} estimates.

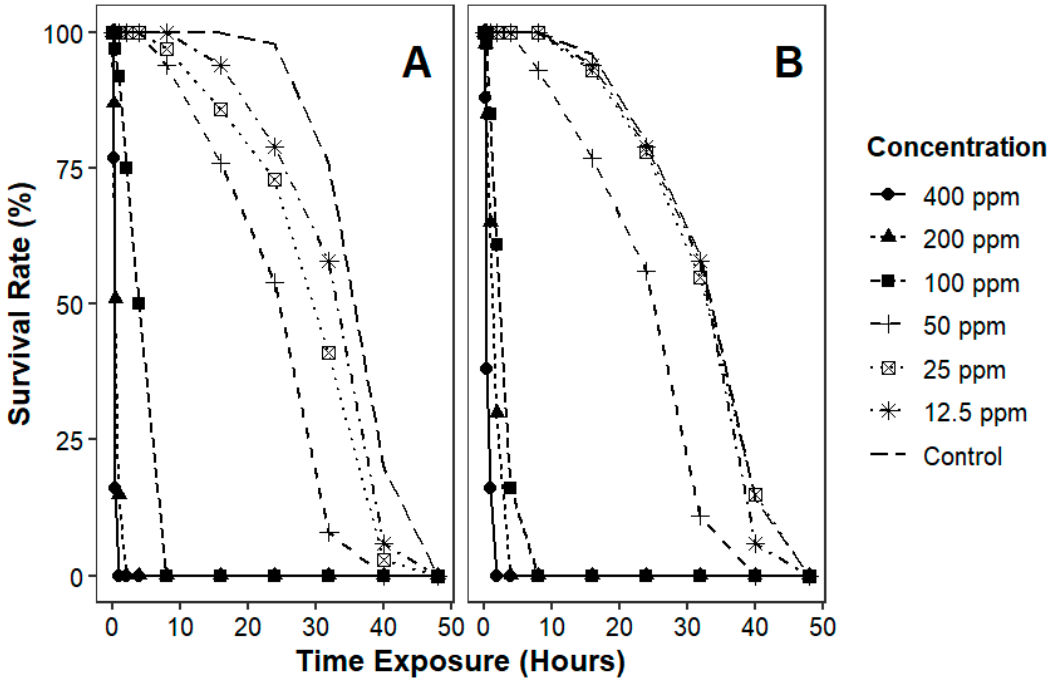


Figure 2. Survival of neonate *P. operculella* larvae exposed to methanolic *A. calamus* extracts from (**A**) Nepal and (**B**) South Korea at six concentrations in a glass-vial bioassay. A nominal 400 mg/L stock was serially diluted two-fold.

3.4. Chromatographic Profiles of the Major Components of Sweet Flag Methanol Extract

HPLC profiles of methanolic extracts from sweet flag rhizomes collected in Andong, South Korea, and Nepal are shown in Figures 3A and 3B, respectively. In the South Korean extract, *cis*-asarone and *trans*-asarone accounted for 67.4% and 14.5% of the reported

chromatographic peak composition. The corresponding proportions in the Nepali extract were 49.8% and 28.7%, respectively. Thus, the South Korean sample showed a higher relative proportion of *cis*-asarone and a lower proportion of *trans*-asarone than the Nepali sample. Despite the differences in the relative proportions of *cis*- and *trans*-asarone between the two extracts, LC_{50} values did not differ significantly (Table 5).

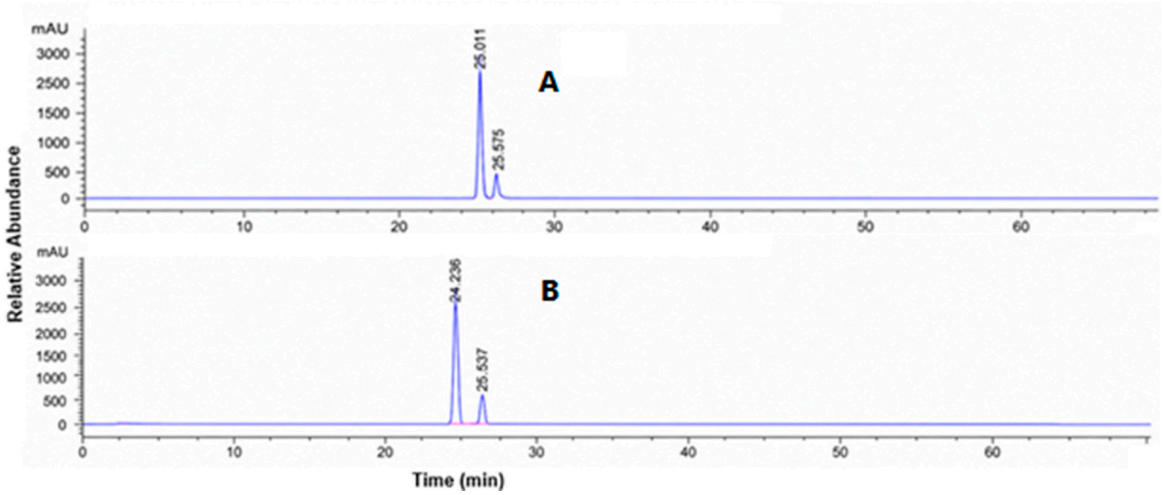


Figure 3. HPLC chromatogram of crude methanolic extract from (**A**) *A. calamus* rhizomes collected in Andong, South Korea. Peaks were assigned to *cis*-asarone (retention time, 25.011 min; 67.4%) and *trans*-asarone (25.575 min; 14.5%); (**B**) *A. calamus* rhizomes (Nepal Origin), showing peaks of *cis*-asarone (RT = 24.236; 49.8%) and *trans*-asarone (RT = 25.537; 28.7%).

3.5. Residual Effects of Sweet Flag Rhizome Powder

In the storage assay, the number of surviving insects varied significantly over time for the Nepali powder ($F_{2,36}$ = 563.04, $p < 0.0001$), with a significant treatment-by-time interaction ($F_{10,36}$ = 118.80, $p < 0.0001$). Similar patterns were found for the South Korean powder (time: $F_{2,36}$ = 184.85, $p < 0.0001$; treatment × time: $F_{10,36}$ = 40.33, $p < 0.0001$; Figure 4 and Table S3). At the end of the three-month trial, no visible tuber damage was observed in the 3- and 4-g/kg treatments (Figure S2). The commercial South Korean powder did not differ significantly from the control at the first assessment and was therefore excluded from subsequent inoculations (Figure 4A).

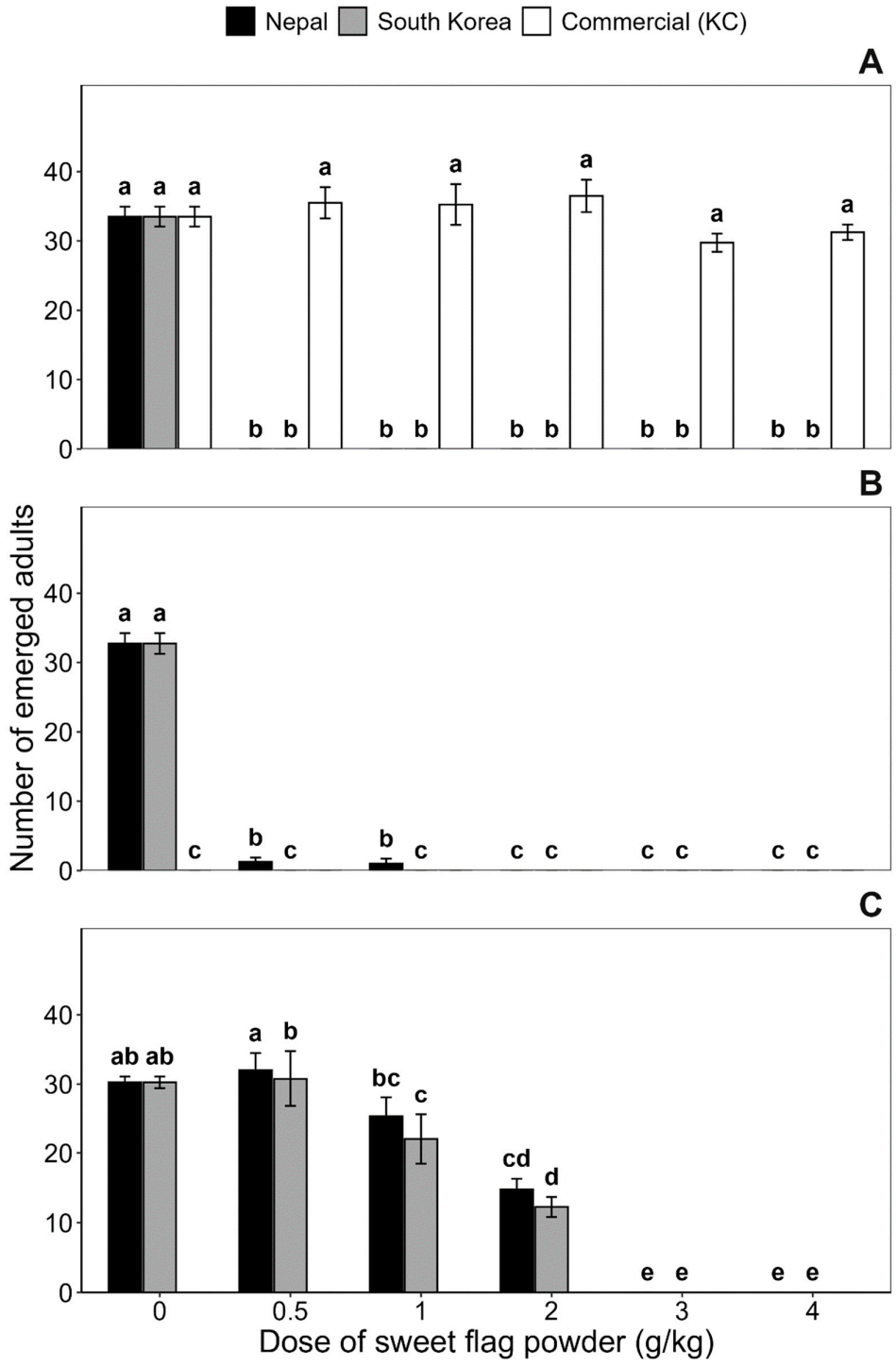


Figure 4. Residual effects of sweet flag rhizome powder from Nepal, South Korea, and a commercial South Korean source (KC) against *P. operculella* over three monthly larval inoculations: (**A**) first, (**B**) second, and (**C**) third inoculation. The commercial preparation was excluded after the first assessment because its effect was not significantly different from the control. Error bars indicate ±SE. Different lowercase letters above the bars within each panel indicate significant differences among treatments (Tukey's HSD test, $p \leq 0.05$).

4. Discussion

As the geographical distribution of *P. operculella* expands, baseline information on insecticide toxicity may help identify candidate treatments for further evaluation. This study compared chemical insecticides in egg and neonate-larval bioassays and assessed the activity of *A. calamus* rhizome extracts and powders under laboratory and storage conditions. The results distinguish concentration-dependent toxicity from speed of action and indicate differences among pest life stages and botanical preparations.

4.1. Effects of Insecticides on Eggs and Neonate Larvae

We observed that cartap hydrochloride significantly inhibited hatchability of 1-, 2-, 3-, and 4-day-old eggs (95.75%, 94.00%, 94.25%, and 89.75%, respectively). Similarly, Silva et al. [48] reported more than 80% mortality of *Neoleucinodes elegantalis* eggs with cartap hydrochloride treatment, accompanied by alterations to the egg chorion and disruption of larval hatching. In the present study, cartap hydrochloride showed substantial ovicidal activity across all tested egg ages, whereas the other insecticides generally showed limited egg mortality. However, Gavcheva and Dimitrov [19] reported PTM egg mortality up to 49.6% and 38.72% upon treatment with neonicotinoids and indoxacarb, respectively. In their study, the eggs deposited on filter paper were sprayed twice with insecticides, which may explain the higher egg mortality observed for these insecticide groups compared with the corresponding treatments in our study. Cartap hydrochloride demonstrated 100% ovicidal efficacy against the eggs of the red-necked longhorn beetle, *Aromia bungii* (Faldermann) (Coleoptera: Cerambycidae) [49]. These studies provide supporting evidence of the ovicidal properties of cartap hydrochloride, which may be associated with its effect on the egg chorion. Its relatively low molecular weight (237.3 g/mol) may potentially allow it to better penetrate into the chorion [50,51]. Silva et al. [48] reported that treatment with cartap hydrochloride disrupted the innermost layer of the endochorion, potentially allowing the insecticide to penetrate the eggshell and reach the serosal embryonic membrane. Cartap hydrochloride belongs to the nereistoxin analogue group (IRAC Group 14), which blocks insect nicotinic acetylcholine receptor (nAChR) ion channels and disrupts normal nerve transmission [35]. Recent research has identified α6-containing nAChRs as an important molecular target of cartap, providing further insight into its channel-blocking mechanism [52]

The insecticides evaluated in this study represent several modes of action targeting the insect nervous system [53]. Abamectin and emamectin benzoate are avermectins (IRAC group 6) that act on glutamate-gated chloride channels, increasing chloride ion influx and disrupting nerve and muscle function, ultimately causing paralysis and death [35,54]. Spinetoram acts as an allosteric modulator of nicotinic acetylcholine receptors, disrupting normal neuronal activity. Thiamethoxam and dinotefuran are neonicotinoids (IRAC Group 4A) that act as competitive modulators of nicotinic acetylcholine receptors, resulting in abnormal nerve signal transmission, whereas etofenprox acts on voltage-gated sodium channels, causing abnormal nerve excitation and paralysis. Diazinon and fenthion are organophosphates (IRAC Group 1B) that inhibit acetylcholinesterase, resulting in acetylcholine accumulation at synapses and continuous nerve stimulation [35]. These different physiological effects may contribute to the variation in toxicity observed among the tested insecticides. Newly hatched *P. operculella* larvae were selected for evaluation because they remain exposed on the plant surface for a short period before entering into plant tissues.

The conventional insecticides remain in use and provide useful comparative toxicity information. Symington and Horne [55] evaluated several conventional insecticides, including organophosphates, synthetic pyrethroids, and neonicotinoids against first

instar *P. operculella*. Their study provides a comparison of insecticide activity against PTM, although their application method differed from our glass-vial contact bioassay: insecticides were applied using a Potter spray tower in Petri dishes. In the present study, abamectin demonstrated the highest toxicity with an LC_{50} value of 0.005 mg a.i./L. Similarly, Kay [56] reported that abamectin (8.1 g a.i./ha) was effective against both exposed neonate larvae and larvae within leaf mines of *P. operculella* in a tomato plant bioassay. This finding supports the efficacy of abamectin against PTM. An et al. [21] found an LC_{50} of 0.05 ppm for the 3rd instar larvae of PTM in a feeding bioassay with emamectin benzoate in 48 h, while our result showed an LC_{50} of 0.008 ppm at 24 h for a day-old neonate larva. This difference may reflect the different exposure routes and experimental conditions, particularly the use of an insecticide-treated diet in the feeding assay compared with insecticide-coated glass vials without food in our study. Although nutritional status and detoxification enzymes, such as P450s and esterases, may influence insecticide toxicity [57,58], these factors were not investigated in the present study.

Fenthion and diazinon at their recommended field dose caused 100% mortality within 1 and 2 h, respectively. The estimated LT_{50} values were 0.37 h and 2.33 h for fenthion and abamectin, respectively. A similar pattern was reported by Reynolds et al. [59], who estimated LT_{50} values of 50 and 275 min in insects fed insecticide-treated fruits. In topical application assays against adults, they reported LT_{50} values of 16, 53 and 97 min for fenthion, abamectin and emamectin benzoate, respectively. Although fenthion and diazinon caused rapid mortality, these organophosphates raise important environmental and human-health concerns [60,61]. They were included as comparative toxicity benchmarks rather than recommended field treatments.

Insecticidal performance depends on both concentration and exposure duration; a lower LC_{50} does not necessarily imply a shorter LT_{50} [62]. Abamectin had the lowest 24-h LC_{50} in this study, whereas fenthion had the shortest estimated LT_{50} at the tested field concentration. This distinction is consistent with the importance of exposure time emphasized by Reynolds et al. [59].

Our vial bioassay does not establish the field concentrations required for effective control. Toxicity ratios relative to the recommended concentrations were calculated as described by AI-Antary et al. [47]. The ratios were 0.0005 for diazinon, 0.0008 for emamectin benzoate, and 0.0009 for abamectin. Lower values indicate a lower LC_{50} relative to the listed recommended concentration. These ratios should be interpreted in the context of the tested formulations and laboratory exposure conditions, rather than as a direct measure of field efficacy or speed of action.

Overall, cartap hydrochloride showed substantial activity against both eggs and neonate larvae in our laboratory assays. This activity may be useful because newly hatched larvae remain exposed on the plant surface for only a short period before entering leaf tissue [63,64]. However, its effectiveness under field conditions requires further evaluation.

4.2. Effects of Sweet Flag Extracts and Powders on Neonate Larvae

Koul et al. [25] isolated the *cis* and *trans* isomers of asarone from *Acorus calamus* L. oil using HPLC. In the present study, HPLC analysis of *A. calamus* methanol extract revealed a similar chromatographic profile to that reported by Koul et al. [25]. However, authentic reference standards and analytical validation were not used to confirm the identities or determine the absolute concentrations of the major *cis*- and *trans*-asarone isomers. Although Koul et al. [25] reported isolating these isomers using HPLC, the individual isomers were not isolated or tested separately in our study.

The insecticidal properties of sweet flag (*A. calamus*) rhizome dust and extracts have been investigated by several researchers [24,25,27,28]. For instance, Park et al. [28]

reported that asarones isolated from methanol extracts of *Acorus gramineus* were effective against three coleopteran stored-product pests. The extracts, when applied to filter paper, caused 100% mortality of *Sitophilus oryzae* and *Callosobruchus chinensis* at a dose of 0.51 mg/cm^2 after seven days. Also, an *A. calamus* extract caused 100% mortality of diamondback moth 3rd instar larvae within 24 h [65]. In the present study, LC_{50} values of the *A. calamus* extracts from Nepal (36.82 mg/L) and South Korea (36.40 mg/L) were statistically comparable, as their 95% confidence intervals overlapped. Furthermore, the lethal-dose ratio test also revealed no significant difference between the extracts. These low LC_{50} values (36.82 and 36.40 mg/L) indicate high susceptibility of neonate *P. operculella* to sweet flag extracts when evaluated under a glass-vial bioassay. Similar results were observed by Jiyavorranant et al. [65], who demonstrated that active chromatographic fractions of an *A. calamus* extract caused 100% mortality of lepidopteran larvae within 24 h. These findings indicate that *A. calamus* extracts can exhibit insecticidal activity against various insect pests, although their efficacy may vary with the insect species, extract composition, and exposure conditions.

The South Korean *A. calamus* methanol extract had a lower estimated LT_{50} than the Nepali extract at the concentration used for the time-response assay. However, the Nepali *A. calamus* methanol extract exhibited a more rapid initial knockdown effect at the highest concentrations (200 and 400 mg/L). *Cis*- and *trans*-asarone might contribute to this difference, although the cause cannot be inferred. Previous studies have reported insecticidal, antifeedant, and repellent effects [25,30,66] associated with asarone isomers. Furthermore, the *A. calamus* essential oil has also been shown to exhibit insecticidal activity against *Sitophilus zeamais* [29].

Since commercially available Korean sweet flag did not cause significant mortality relative to the control during the initial inoculation, it was excluded from subsequent larval inoculations. This might be due to the differences in processing methods, which may have resulted in the loss of active compounds. In this study, the residual effect of the sweet flag rhizome powder varied significantly with exposure time and dose. Among the tested doses, 0.5 to 4 g/kg of potato tuber caused almost 100% mortality of the PTM larvae for up to two months. Paneru et al. [27] reported that when sweet flag rhizome powder was admixed with 2% w/w wheat, it caused mortality of *Sitophilus granarius* and *Sitophilus oryzae* within 7 days after treatment. Similarly, Paneru and Shivakoti [67] found that lentils admixed with sweet flag rhizome powder at 1 and 2% (w/w) showed 100% mortality of *Callosobruchus maculatus* after 4–6 days. Application of 5 g/kg of *A. calamus* powder to potato tubers in farmer rustic storage showed high efficacy in protecting the tubers from damage against PTM up to four months [24]. In another study, Giri et al. [68] reported that application of 0.67 g/kg to 5 g/kg of *A. calamus* powder to potato tubers caused 90 to 100% mortality of neonate larvae. *A. calamus*-treated potato tubers had less infestation during all observation periods when compared to farmers' practice [69]; however, some damage was observed after nine weeks at an application rate of 150 g/100 kg of potato. These studies indicate the potential of sweet flag rhizome powder as a locally available protectant for stored potato. Although these storage assays demonstrate promising potential, large-scale application, proper formulation, and food safety parameters require further investigation.

Rhizome powders and extracts of *A. calamus* have previously been tested against several stored-product pests, and the present study extends these assays to neonate *P. operculella*. Further work should standardize extraction and formulation procedures and evaluate efficacy under operational storage conditions. In addition, because asarone constituents may present toxicological concerns [70], botanical treatments also require careful assessment of residue levels, exposure, and food safety before practical application.

5. Conclusions

Overall, selected synthetic insecticides and *A. calamus* preparations showed activity against *P. operculella* under the laboratory conditions tested. Cartap hydrochloride showed both ovicidal and larvicidal activity, making it a candidate for further evaluation against exposed early life stages. However, laboratory vial and egg-dipping assays cannot establish field efficacy, and the performance of these treatments under realistic crop and storage conditions remains to be tested. Limiting infestations in the field could potentially reduce subsequent tuber infestation in storage. Nevertheless, from an integrated pest management (IPM) perspective, *A. calamus* may have potential as a botanical pesticide because it is locally available and has demonstrated insecticidal and residual activity in laboratory and storage assays. Future research should examine the stability, efficacy, and safety of standardized *A. calamus* formulations. Separate bioassays with purified *cis*- and *trans*-asarone are needed to determine whether their effects differ in toxicity, repellency, and feeding deterrence. Such experiments would help distinguish associations between extract composition and biological activity from causal effects of individual constituents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects17121235/s1, Figure S1: Process of the *A. calamus* rhizome to make coarse dust and application in potato tubers; Figure S2: After completion of third inoculation of neonate PTM and they emerged out, the potatoes were chopped and damage was observed. From the left (0, 0.5, 1, 2, 3, and, 4 g of rhizome dust/ kg of potato); Table S1: Active ingredient concentrations (mg a.i./L) of the 9 insecticides used in Figure 1 at the recommended field concentrations and their serial dilutions; Table S2: Relative toxicity among the insecticide groups tested against PTM based on 24-h LC_{50} values;; Table S3. Repeated Measures ANOVA statistics for the residual efficacy of *Acorus calamus* powder treatments against potato tuber moth.

Author Contributions: Conceptualization, S.A., C.J., and A.D.; Writing—original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. S.A. and A.D.; Validation, Methodology, Investigation, Formal analysis, Data curation. C.J. Writing—review and editing, Validation, Supervision, Conceptualization, C.J. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1A6A1A03024862).

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments: Sunil Aryal thanks the Nepal Agricultural Research Council for its support.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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