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RESEARCH

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# Pesticide susceptibility monitoring of fall armyworms (*Spodoptera frugiperda* (J.E. Smith)): a simple methodology for information-sharing among Southeast Asian countries

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## Abstract

**Background** The fall armyworm, *Spodoptera frugiperda*, is a destructive moth pest. It is highly migratory and was first detected in Southeast Asia in 2018, rapidly becoming a major pest of corn production in this region. Monitoring the susceptibility of *S. frugiperda* populations is important for efficient insecticide resistance management. Because of the high mobility of this pest, information-sharing of susceptibility levels among neighboring countries is required for insecticide resistance management. To this end, we developed simple standard methods for pesticide susceptibility monitoring of *S. frugiperda* to contribute to information-sharing among Southeast Asian countries.

**Methods** The developed methods included mass rearing of larvae using an artificial diet and bioassay by diet overlay. The lethal concentrations for 50% and 95% mortality ($LC_{50}$ and $LC_{95}$) and resistance coefficient values were calculated. We tested the susceptibilities of samples of *S. frugiperda* collected from the six major corn planting areas in Thailand to emamectin benzoate, spinetoram, chlorantraniliprole, indoxacarb, chlorfenapyr, and lufenuron using the developed methods.

**Results** The mortality of artificial diet-fed larvae was higher than those fed corn leaves, especially in the early instars. However, more than half of the specimens reared on the artificial diet became pupae. In the case of three of the six pesticides, emamectin benzoate, indoxacarb, and chlorfenapyr, the $LC_{50}$ values of the samples collected in 2021 and 2022 were significantly higher than those collected in 2019, indicating increasing resistance to those three pesticides. According to the resistance coefficient values, only samples from one area exhibited low resistance to lufenuron.

**Conclusions** We developed a simple standardized methodology for Southeast Asian countries to compare insecticide susceptibility. The calculated $LC_{50}$ and resistance coefficient values can be used as a baseline for monitoring the development of pesticide resistance in the region. The $LC_{50}$ values of several pesticides have increased significantly over the years. However, the resistance coefficient values indicated that *S. frugiperda* developed low resistance to only one pesticide (lufenuron). This study offers helpful information for insecticide selection and improved resistance management of fall armyworms in Thailand.

**Keywords** Corn, Integrated pest management, Pesticide resistance, Resistance management, Thailand

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## Background

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous migratory pest native to the tropical and subtropical areas of the Americas. However, it has recently been reported as an invasive species in Africa and Asia (Goergen et al. 2016; Ganiger et al. 2018). Since 2018, it has rapidly invaded many countries in Southeast Asia and has become a significant pest of corn (*Zea mays* L.) in the region (Nagoshi et al. 2020). The first outbreak of FAW in Thailand was reported at the end of 2018, with results of a survey in Thailand from December 2018 to December 2019 showing that these outbreaks occurred in all corn cultivation areas (Chaireunkaew et al. 2022).

In Africa, the region where FAW was recently detected, there are several reports that estimated the impacts of FAW on maize production (e.g., Day et al. 2017; Early et al. 2018; Kumela et al. 2018; Akeme et al. 2021). These reports indicated that the damage caused by FAW in Africa has a critical impact on production. In Thailand, Punyawattoe (2021) reported that maize yield reduction caused by FAW might reach 25–40%, and that the overall cost to production in the country will rise to 26–52 million US$ per annum.

The long-distance migration ability of FAW is a crucial reason this insect has expanded rapidly throughout its newly invaded region and become large-scale outbreaks. The FAW adults can move hundreds of kilometers over several nights with high-altitude winds (Rose et al. 1975; Westbrook et al. 2016). Its ability to feed on a broad range of host plants has further contributed to its development as a major pest. Montezano et al. (2018) reported 353 host plants belonging to 76 plant families, principally representatives of Poaceae, Asteraceae, and Fabaceae.

Early infestation signs and symptoms of FAW include small pinholes and window pane-like damage resulting from feeding by early-stage larvae (Abrahams et al. 2017). Due to the feeding on folded leaves from the inside, holes in the maize leaves are formed. Old-stage larvae stay inside the funnel. This behavior protects from pesticide spray applications. In the case of fully grown plants, the middle- and old-stage larvae can bore into the maize cobs. This behavior further reduces yield quantity and quality (Abrahams et al. 2017).

The FAW life cycle is completed in approximately 30 days during the warm summer months but may extend to 60–90 days in cool temperatures, as FAW does not have the ability to undergo diapause (Prasanna et al. 2018). However, several studies have revealed that temperature is essential to FAW biology. Du et al. (2020) found that the duration of the FAW larval stage was $11.38 \pm 0.25$ d, and the development time from egg to adult was $22.38 \pm 0.27$ d when reared under controlled conditions at $30 \pm 1$ °C, $65 \pm 5$% RH, and a 14L:10D photoperiod. In Thailand, Hong et al. (2022) reported on the duration of the FAW life cycle when fed with maize, sweet corn, and waxy corn, finding significant differences in the duration of the larval stage but not in the overall life cycle duration.

Currently, chemical control is a critical strategic component for controlling this pest in Southeast Asia, including Thailand. Reliance on chemical control strategies in many countries has led to the development of resistance to 45 active insecticidal ingredients (Wu et al. 2019; Mota-Sanchez and Wise 2021). FAW is currently among the top 15 most insecticide-resistant species, with cases reported for different chemical classes of insecticides, such as organophosphates, pyrethroids, spinosyns, diamides, benzoylureas, and *Bacillus thuringiensis* Berliner (Bt) insecticidal Cry proteins expressed in transgenic crops, such as Cry1F, Cry1A.105, and Cry2Ab (Kulye et al. 2021). In Thailand, the Department of Agriculture recommended several chemicals for FAW control, including emamectin benzoate, spinetoram, chlorantraniliprole, indoxacarb, chlorfenapyr, and lufenuron (Punyawattoe 2021).

Considering the current situation of pesticide resistance in FAW and its dispersal ability, information exchange between countries in the same geographical region is essential for effective insecticide resistance management for this pest. In this study, we developed a simple method for Southeast Asian countries to compare insecticide susceptibility test results using the same method.

## Methods

We established standard methods of pesticide susceptibility monitoring by a combination of FAW rearing using an artificial diet (modified from the Department of Agriculture (2001)), bioassay by diet overlay using an artificial diet (similar to Cook et al. (2005) and Muraro et al. (2021)), and evaluation of the results using the lethal concentrations for 50% and 95% mortality ($LC_{50}$ and $LC_{95}$) values and the resistance coefficient (Roy et al. 2009; Węgorek et al. 2009).

### Insects

More than 300 FAW larvae were collected during 2019–2022 from each of the six provinces that are major corn production areas in Thailand (Table 1). The larvae were collected by hand from corn plants on a field. The insects were maintained under controlled conditions ($26 \pm 2$ °C, $60 \pm 10$% R.H., and photoperiod of 12 h: 12 h, light: dark) during all of the developmental stages. Larvae of all samples were maintained on an artificial diet (details provided below). In each culture, the adults that emerged

**Table 1** Sample information of *Spodoptera frugiperda* from Thailand between 2019 and 2022

| Sample | Location | Site | Collection time | Host plant | Number of larvae |
|---|---|---|---|---|---|
| TM2019 | Tha muang, Kanchanaburi | 13°58′54.3"N 99°38′52.6"E | February, 2019 | Sweet corn | 537 |
| SN2019 | Sam Ngao, Tak | 17°11′42.6"N 99°04′51.7"E | March, 2019 | Feeding corn | 742 |
| SP2021 | Si Prachan, Suphan Buri | 14°36′20.1"N 100°06′08.5"E | June, 2021 | Feeding corn | 368 |
| TL2021 | Tha Luang, Lop Buri | 15°02′47.9"N 101°13′52.5"E | May, 2021 | Feeding corn | 487 |
| KC2022 | Khao Chakan, Sa Kaeo | 13°37′10.9"N 102°04′40.0"E | July, 2022 | Feeding corn | 653 |
| WS2022 | Wang Saphung, Loei | 17°16′43.1"N 101°47′06.4"E | July, 2022 | Feeding corn | 325 |

were placed in egg-laying containers and supplied with a 5% honey solution. The egg-laying container contained a bundle of corn seedlings (cultivar: Nakhon Sawan 3) or Para grass (*Brachiaria mutica* Forsskål), and was covered with a paper towel on top for oviposition. Eggs laid on the raw material and paper towels were collected every 2 d. As described below, third instar larvae of F1–F3 progeny from all samples were tested during the bioassays (Torres-Vila et al. 2002; Avilla and Gonzalez 2010).

## Insecticides

Formulated insecticides were used in all bioassays. Emamectin benzoate (1.92% EC, Syngenta Crop Protection Co. Ltd.), spinetoram (12% SC, Dow AgroSciences, Thailand Co. Ltd.), chlorantraniliprole (5.17% SC, DuPont Agricultural Chemicals Co. Ltd.), indoxacarb (15% EC, DuPont Agricultural Chemicals Co. Ltd.), chlorfenapyr (10% SC, BASF (Thai) Co. Ltd.), and lufenuron (5% EC, Syngenta Crop Protection Co. Ltd.) were tested in this study.

## Artificial diet

An artificial diet for FAW was developed according to the Department of Agriculture (2001) protocols for beet armyworm, *Spodoptera exigua* (Hübner). The ingredients of the artificial diet are listed in Table 2. The 'Fraction A' ingredients (agar) were boiled while stirring periodically, and then left at room temperature to cool down. The 'Fraction B' ingredients were added to the blend and mixed for 1 min. The 'Fraction C' ingredients were then added, and the mixture was blended thoroughly using a blender for approximately 1 min at high speed. While hot, 5 ml of the mixture was poured into plastic cups (5 cm in diameter) and allowed to cool. After solidification at room temperature, the feeding medium was refrigerated until later use. The feeding medium was removed from the refrigerator and kept at room temperature for 2–3 h before use.

**Table 2** Composition of the artificial diet used for rearing *Spodoptera frugiperda* larvae

| Groups | Ingredients | Quantity |
|---|---|---|
| Fraction A | Agar powder | 25 g |
| | Reverse osmosis water | 800 ml |
| Fraction B | Formalin | 4 ml |
| | Yeast | 20 g |
| | Methyl paraben | 5 g |
| | Sorbic acid | 3 g |
| | Mungbean powder | 240 g |
| | Reverse osmosis water | 800 ml |
| Fraction C | Ascorbic acid (Vitamin C) | 5 g |
| | Vitamin stock* | 40 ml |

*Vitamin stock contains 5 mg biotin, 2.5 g thiamine (vitamin B1), 1.5 g pyridoxine (vitamin B6), 3 g riboflavin (vitamin B2), 20 mg cyanocobalamin (vitamin B12), 3 g D-Pantothenic acid hemicalcium salt, 10 g choline chloride, 2.5 g folic acid, 5 g inositol, 6 g nicotinic acid, distilled water 1,000 ml

## Evaluation of the performance of the artificial diet

Mortality was compared between artificial diet-fed and corn leaf-fed larvae. First instar larvae of the TM2019, KC2022, and WS2022 samples were reared individually on the artificial growth medium or corn leaves. Artificial growth medium was cut to 2 $cm^3$ and put in plastic cups with a diameter of 6 cm. In the experiment on corn leaf-fed larvae, prior to placing the corn leaves in plastic cups, a slightly moistened filter paper was placed inside, covering the bottom of each plastic cup. A piece of 5 $cm^2$ corn leaf (cultivar: Nakhon Sawan 3) was put inside each plastic cup. A neonate larva was added to each cup before capping it with a perforated plastic lid. The food was replaced every 1–3 days. Subsequently, the insects were cultured under laboratory conditions of 25 ± 2 °C and 60–80% R.H., until pupation. The experiments were replicated four times, with each replicate comprising 10 individuals. The growth stage and survival were monitored daily.

### Diet overlay bioassays

Diet overlay bioassay methods were established based on the previous studies by Cook et al. (2005) and Muraro et al. (2021). Third-instar FAW larvae were used as test specimens (similar to Kulye et al. (2021), Zhang et al. (2021), and Bird et al. (2022)). The main reasons for using third instar larvae as test specimens are that the early instars are sensitive to biotic and abiotic stresses and more susceptible to insecticides compared to the later instars (Ghidiu and Andaloro 1993; Adamczyk et al. 1999). In addition, the third instar larvae were easy to rear for the experimental preparations. Moreover, in actual cultivation conditions the third instar larvae are easy and cost-effective to control. Four replications per concentration were conducted, with 10 insects per replicate, primarily using the progeny from the F1 to the F3 generation reared in the laboratory. In all assays, 5 ml of the feeding medium was transferred to 60 ml plastic cups. The surface area per well was 12.56 $cm^2$. The formulated insecticides were serially diluted seven to nine times with distilled water, which was expected to induce 0 to 100% mortality from the preliminary bioassay. Thereafter, 200 µl of each concentration was applied to the surface of the feeding medium in each cup. The control treatment consisted of growth medium supplemented with distilled water. The cups were rotated to evenly distribute the solution over the surface of the growth medium. The treated medium was allowed to dry (by evaporation of the distilled water carrier) for approximately 1 h.

After drying, a third instar larva was added to each cup and capped with a perforated plastic lid. Mortality was assessed 72 h after insecticide exposure. The larvae were considered dead if they did not respond after being touched with a small brush or when they showed severe intoxication symptoms (slow movement, twitching, feeding cessation, and interrupted molting). Growth-retarded larvae approximately 1/3 of the size of the control larvae were considered strongly affected, and therefore scored as dead (Avilla and Gonzalez 2010; Hardke et al. 2011; Kulye et al. 2021).

### Data analysis

#### *Data on the evaluation of the performance of the artificial diet*

Mortality data are presented as the mean values and standard errors.

#### *The data on diet overlay bioassays*

Mortality data of diet overlay bioassays were corrected for using the control mortality by applying Abbott's formula (Abbott 1925) and analyzed by probit analysis (Finney 1971) using Polo PC (LeOra Software, Berkeley, California) to obtain $LC_{50}$ and $LC_{95}$ values. Data were considered significantly different based on non-overlap of the 95% confidence intervals. To assess the development of insecticide resistance in *S. frugiperda* from 2019 to 2022 in Thailand, the resistance coefficient (RC) values were calculated as follows: RC = $LC_{95}$ value/recommended field dose of each insecticide (Roy et al. 2009). The following criteria for resistance assessment were assumed (Węgorek et al. 2009): RC ≤ 1: None; RC = 1.1–2: low resistance; RC = 2.1–5: medium resistance; RC = 5.1–10: high resistance; and RC > 10: very high resistance.

## Results

### Evaluation of the artificial diet

Even though the mortality of the artificial diet-fed young larvae was higher than that of the corn leaf-fed larvae, more than half of the specimens reared on the artificial diet became pupae (Table 3).

### Susceptibility of FAW to six insecticides

The susceptibility of FAW to six insecticides was determined using samples collected from six provinces in Thailand between 2019 and 2022 using the methods, and the relevant $LC_{50}$ values are presented in Table 4. Regarding emamectin benzoate, the $LC_{50}$ values of the FAW samples collected in 2019 (TM2019 and SN2019) were significantly lower than those of the samples collected in 2021 and 2022 (TL2021 and WS2022, respectively). Similar trends were detected for indoxacarb (comparing TM2019 and SN2019 to TL2021, KC2022, and WS2022) and chlorfenapyr (comparing TM2019 and SN2019 to TL2021, KC2022, and WS2022). Overall, $LC_{50}$ values increased over time for all of the insecticides, except lufenuron. However, RC values indicated that only one sample, SP2021, developed resistance against lufenuron from 2019 to 2022 in Thailand (Table 4).

**Table 3** Mortality of artificial diet- and corn leaf-fed *Spodoptera frugiperda* larvae. Forty larvae were used to start each treatment

| Larval development stage | Mortality (%) (Means ± SE) | | | | | |
|---|---|---|---|---|---|---|
| | Artificial diet | | | Corn leaves | | |
| | TM2019 | KC2022 | WS2022 | TM2019 | KC2022 | WS2022 |
| 1st instar | 32.50 ± 0.25 | 30.00 ± 0.41 | 27.50 ± 0.25 | 10.00 ± 0.41 | 12.50 ± 0.25 | 15.00 ± 0.29 |
| 2nd instar | 10.00 ± 0.00 | 7.50 ± 0.25 | 5.00 ± 0.29 | 5.00 ± 0.29 | 7.50 ± 0.48 | 5.00 ± 0.29 |
| 3rd instar | 2.50 ± 0.25 | 0 | 2.50 ± 0.25 | 0 | 2.50 ± 0.25 | 0 |
| 4th instar | 0 | 0 | 0 | 0 | 0 | 0 |
| 5th instar | 0 | 0 | 0 | 0 | 0 | 0 |
| 6th instar | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4** Susceptibility and resistance level of *Spodoptera frugiperda* samples to six insecticides recommended for FAW control in Thailand

| Insecticides | Recommended dose (mg/L) | Sample | N | Slope ± SE | $LC_{50}$ (95% CL*) (mg/L) | $LC_{95}$ (95% CL*) (mg/L) | RC** | Resistance level |
|---|---|---|---|---|---|---|---|---|
| Emamectin benzoate 1.92% EC | 19.20 | TM2019 | 320 | 5.925 ± 0.785 | 0.014 (0.013 – 0.016) | 0.027 (0.022–0.036) | 0.001 | None |
| | | SN2019 | 320 | 4.929 ± 0.610 | 0.015 (0.013–0.018) | 0.032 (0.023–0.063) | 0.002 | None |
| | | SP2021 | 320 | 5.393 ± 0.592 | 0.017 (0.014–0.025) | 0.035 (0.025–0.092) | 0.002 | None |
| | | TL2021 | 320 | 3.249 ± 0.305 | 0.029 (0.023–0.039) | 0.093 (0.062–0.192) | 0.005 | None |
| | | WS2022 | 320 | 3.806 ± 0.360 | 0.027 (0.021–0.036) | 0.073 (0.050–0.142) | 0.004 | None |
| Spinetoram 12%SC | 120.00 | TM2019 | 320 | 1.846 ± 0.391 | 0.005 (0.002–0.008) | 0.040 (0.028–0.084) | 0.000 | None |
| | | SN2019 | 320 | 1.947 ± 0.306 | 0.009 (0.001–0.015) | 0.060 (0.031–1.260) | 0.001 | None |
| | | SP2021 | 320 | 1.231 ± 0.149 | 0.012 (0.006–0.020) | 0.270 (0.118–1.733) | 0.002 | None |
| Chlorantraniliprole 5.17% SC | 77.55 | TM2019 | 320 | 2.898 ± 0.304 | 0.433 (0.364–0.517) | 1.601 (1.211–2.384) | 0.021 | None |
| | | SN2019 | 320 | 2.610 ± 0.265 | 0.393 (0.328–0.475) | 1.680 (1.240–2.578) | 0.022 | None |
| | | SP2021 | 320 | 1.654 ± 0.170 | 0.747 (0.402–1.525) | 7.379 (2.909–82.452) | 0.095 | None |
| | | TL2021 | 320 | 1.962 ± 0.193 | 0.442 (0.306–0.629) | 3.046 (1.790–7.630) | 0.039 | None |
| | | KC2022 | 320 | 1.968 ± 0.208 | 0.270 (0.212–0.337) | 1.852 (1.307–3.042) | 0.024 | None |
| | | WS2022 | 320 | 1.698 ± 0.172 | 0.730 (0.376–1.569) | 6.793 (2.630–93.977) | 0.088 | None |
| Indoxacarb 15% EC | 225.00 | TM2019 | 320 | 1.906 ± 0.289 | 1.526 (0.982–2.048) | 11.129 (7.724–20.339) | 0.049 | None |
| | | SN2019 | 320 | 2.487 ± 0.354 | 1.877 (1.402–2.337) | 8.610 (6.347–14.054) | 0.038 | None |
| | | TL2021 | 320 | 1.448 ± 0.176 | 5.259 (3.554–9.019) | 71.888 (30.257–372.490) | 0.320 | None |
| | | KC2022 | 320 | 1.791 ± 0.227 | 7.530 (5.772–10.645) | 62.422 (34.671–161.009) | 0.277 | None |
| | | WS2022 | 320 | 1.938 ± 0.280 | 10.466 (7.909–15.650) | 73.854 (39.439–219.453) | 0.328 | None |
| Chlorfenapyr 10% SC | 150.00 | TM2019 | 320 | 1.963 ± 0.194 | 2.086 (1.268–3.450) | 14.369 (7.210–63.649) | 0.096 | None |
| | | SN2019 | 320 | 1.786 ± 0.181 | 2.049 (1.243–3.360) | 17.086 (8.343–77.231) | 0.114 | None |
| | | TL2021 | 320 | 4.492 ± 0.559 | 7.056 (6.120–8.122) | 16.397 (13.357–22.358) | 0.109 | None |
| | | KC2022 | 320 | 4.476 ± 0.558 | 7.733 (6.714–8.915) | 18.022 (14.621–24.761) | 0.120 | None |
| | | WS2022 | 320 | 4.174 ± 0.510 | 8.874 (7.669–10.284) | 21.987 (17.643–30.649) | 0.147 | None |
| Lufenuron 5% EC | 75.00 | TM2019 | 320 | 1.270 ± 0.151 | 2.359 (1.521–3.551) | 46.591 (21.594–189.192) | 0.621 | None |
| | | SN2019 | 320 | 1.271 ± 0.151 | 2.267 (1.373–3.573) | 44.631 (19.536–224.426) | 0.595 | None |
| | | SP2021 | 320 | 1.297 ± 0.154 | 4.558 (3.405–6.311) | 84.518 (43.602–236.717) | 1.127 | Low |
| | | TL2021 | 320 | 0.928 ± 0.125 | 1.034 (0.597–1.553) | 61.180 (29.292–203.736) | 0.816 | None |
| | | KC2022 | 320 | 1.097 ± 0.191 | 0.259 (0.095–0.451) | 8.180 (4.638–22.907) | 0.109 | None |
| | | WS2022 | 320 | 0.910 ± 0.172 | 0.209 (0.055–0.415) | 13.403 (6.691–52.303) | 0.179 | None |

* 95% fiducial limits

** RC = Resistance coefficient [$LC_{95}$ of each sample/recommended dose (mg/L)]. The following criteria for resistance assessment were assumed: RC≤: No resistance; RC = 1.1–2: Low resistance; RC = 2.1–5: Medium resistance; RC = 5.1–10: High resistance; RC >10: Very high resistance (Węgorek et al. 2009)

## Discussion

FAW has recently emerged as a severe corn pest in Southeast Asia. The development of broad-spectrum insecticide resistance has complicated its chemical control (Yu 1992; Yu et al. 2003; Yu and McCord 2007). However, the current FAW control relies mainly on the application of a variety of insecticides. Therefore, the selection of effective insecticides to control this pest is essential, and monitoring the insecticide susceptibility of FAW populations is required.

The principle of the bioassay test is to evaluate the toxicity of insecticides to the same species under the same test conditions. Bioassay methods commonly used for insecticide toxicity evaluation are topical application, the dipping method (leaf dip and larval dip), the dietary method, Potter's tower method, and the dry film method, among others (Paramasivam and Selvi 2017). For studies on FAW, different methods have been used. Kulye et al. (2021) adopted a diet incorporation assay in their research in India, while the topical application was used by Gutiérrez-Moreno et al. (2019) and Zhang et al. (2021) in Puerto Rico, Mexico and China, respectively. In addition, there were inconsistent approaches in each study. For example, second instar larvae were tested by Gutiérrez-Moreno et al. (2019), third instar larvae by Zhang et al. (2021), and fourth instar larvae by Yu (1992). Therefore, the results of those experiments were so different that they were not directly comparable. If we could follow the same approach for insecticide susceptibility testing, we would be able to compare the experimental results.

The method developed in this study is suitable as a standard approach for Southeast Asian countries, because it is simple and uncomplicated. In addition, the artificial diet used in this method has several advantages, such as the ease of purchasing the ingredients in Southeast Asia, quality control, and the low cost. Furthermore, the feeding medium can be stored in a refrigerator for at least three months. If all Southeast Asian countries use this method for insecticide susceptibility testing and resistance monitoring, it will standardize the data and help manage insecticide resistance in the region effectively.

The mortality of the larval stage of FAW on the developed artificial diet was higher than that of the larvae on corn leaves, but was less than 50%. Several previous studies have focused on artificial diets for FAW larvae recently (e.g., Jin et al. 2020; He et al. 2021). Particularly, Jin et al. (2020) compared the performance of FAW reared on various artificial diets. The mortality of the artificial diet developed in this study was not significantly different from the results of their study. The mortality rate of early-stage larvae reared on artificial diets was particularly higher than those reared on maize leaves. In other insects, Xu et al. (2012) studied the growth and reproduction of an artificial diet of rice leaf folder (*Cnaphalocrocis medinalis* Guenée). The results suggested that the larval mortality fed on the artificial diet was 77%. In the first 6–7 days after hatching, the larvae suffered high mortality. The survival ratio of the larvae tended to stabilize at approximately ten days after hatching. Similarly, Li et al. (2011) reported that the critical problem in *C. medinalis* rearing on an artificial diet was the high mortality of neonate larvae. Furuta et al. (1998) reported that the early-stage larvae of rice leaf folder should be reared on rice seedlings for the first week after hatching. And then, the larvae should be transferred onto the artificial diet. The artificial diet developed in this study showed a similar trend.

Because FAW is a new invasive pest in Southeast Asia, we did not have any susceptible populations for the resistance ratio calculation. Therefore, $LC_{50}$ and the RC values were calculated to identify the changing resistance levels of FAW in Thailand from 2019 to 2022. The $LC_{50}$ values increased as the years progressed for all of the insecticides tested, except for lufenuron, suggesting that FAW is developing resistance against several pesticides. In Thailand, emamectin benzoate is the currently the leading pesticide used for FAW management, while the other pesticides tested in the present study are infrequently used. However, the $LC_{50}$ and $LC_{95}$ values for indoxacarb and chlorfenapyr increased over time. This might have been caused by the invasion of resistant FAW populations from neighboring countries or vegetable fields in same location which were applied indoxacarb and chlorfenapyr. Information sharing among neighboring countries may help understand the reason for this trend. On the other hand, considering the RC values, all of the samples showed no resistance to emamectin benzoate, spinetoram, chlorantraniliprole, indoxacarb, and chlorfenapyr, while only the SP2021 showed low resistance to lufenuron. $LC_{50}$ and RC values can be used as a baseline for future pesticide susceptibility monitoring among Southeast Asian countries.

## Conclusions

In this study, a simple method for monitoring pesticide susceptibility was developed. This method could be the standard for comparing and monitoring pesticide susceptibility in Southeast Asian countries. The $LC_{50}$ and RC values calculated in this study can be used as a baseline for monitoring pesticide resistance in this region. Presently, the $LC_{50}$ value of several pesticides became significantly higher than the sample collected in 2019. The frequency of application of the insecticides should be reduced and rotated to the other recommended insecticides to improve the control of FAW in Thailand, and possibly elsewhere in Southeast Asia too. Continued monitoring in areas where it is applied can also provide clues to determine the causes and rates of increased resistance to insecticides by FAW.

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**Author contributions**
PP, UN, ST, and YK contributed substantially to the conceptualization of the study. AR contributed to the development of the artificial diet and data analysis. ST and WS contributed significantly to data analysis and interpretation of the bioassays. ST and YK contributed substantially to the manuscript drafting. All the authors critically reviewed and revised the manuscript draft and approved the final version for submission.

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**Availability of data and materials**
The datasets are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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