A simple bioassay method for international comparison of insecticide susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith)

The fall armyworm (FAW), *Spodoptera frugiperda*, was a native pest in the Americas (Fig. 1). However, FAW has recently invaded Africa and Asia and is rapidly expanding its distribution. This insect is a polyphagous pest and prefers maize. Due to the frequent use of certain inexpensive and easily available insecticides, resistance development is a concern in Asia. Because of the long-distance migration ability of FAW, if a strain develops resistance to insecticides in one country, it is likely to spread rapidly to neighboring countries. Therefore, it is essential to conduct insecticide susceptibility monitoring using the same methods and share the results to manage insecticide resistance development. For this purpose, we developed a simple insecticide susceptibility testing method using relatively easily available materials to monitor the insecticide susceptibility of FAW in Southeast Asia, including developing regions.

This method can easily evaluate insecticide susceptibility, in contrast to existing methods such as molecular biology techniques and topical application. It consisted of the following procedures: collecting test insects, rearing them with an artificial diet made from relatively easily available materials, and susceptibility testing (Fig. 2). The artificial diet was composed of three fractions (Table 1). By feeding this artificial diet, 1st instar larvae could be raised to pupae (Fig. 3). We conducted diet-overlay bioassays using the 3rd instar larvae within three generations after collection to assess susceptibility. We applied 200 µl of insecticide serially diluted to any multiple using distilled water to 5 ml of artificial feed. After drying, ten 3rd instar larvae were introduced, and the number of dead individuals was counted 72 hours later. From the results obtained, the LC50 value was calculated. We evaluated the susceptibility of the insecticides that are applied in Southeast Asian countries to several FAW populations collected in Thailand using the developed method. The results suggested that the susceptibility of several insecticides decreased over time (Table 2).

The developed method showed enough accuracy and can be used for international comparison to develop resistance management measures. In Thailand, the susceptibility of FAW to several insecticides has decreased. Therefore, there is an urgent need to develop alternative control methods against FAW. The survival rate of 1st and 2nd instar larvae when fed an artificial diet was lower than when fed fresh maize leaves. Thus, fresh leaves are suitable for feeding the young larvae if it is necessary to examine a lot of insecticides in the same period.

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Fig. 1. Fall armyworm larva feeding on maize

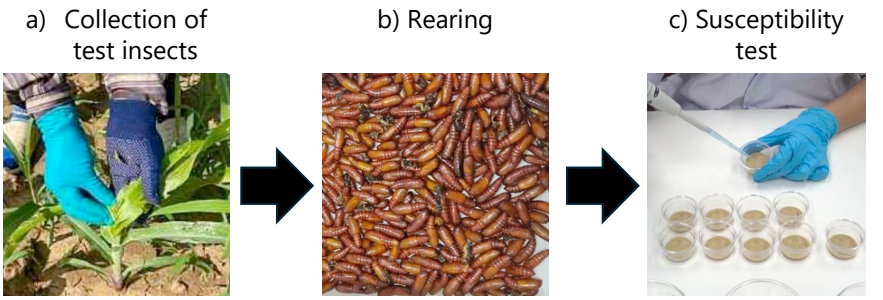

Fig. 2. Summary of the simple insecticide susceptibility test method

Table 1. Composition of the artificial diet

	Ingredients	Quantity
Fraction A	Agar powder	25g
	Reverse osmosis water	800ml
Fraction B	Formalin	4ml
	Yeast	20g
	Methyl paraben	5g
	Sorbic acid	3g
	Mungbean powder	240g
	Reverse osmosis water	800ml
Fraction C	Ascorbic acid (Vitamin C)	5g
	Vitamin stock[1)]	40ml

1) 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

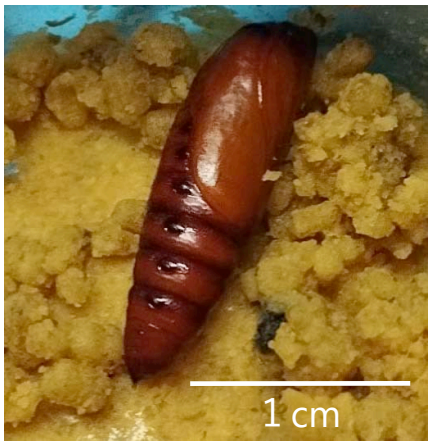

Fig. 3. FAW pupa reared by artificial diet

Table 2. Results of insecticide susceptibility test of several populations in Thailand

Insecticides	Collected year (Location)	LC50 (mg/L)[1)]
Emamectin benzoate 1.92% EC	2019 (Kanchanaburi)	0.014 (0.013–0.016)
	2019 (Tak)	0.015 (0.013–0.018)
	2021 (Suphan Buri)	0.017 (0.014–0.025)
	2021 (Lop Buri)	0.029 (0.023–0.039)
	2022 (Loei)	0.027 (0.021–0.036)
Indoxacarb 15% EC	2019 (Kanchanaburi)	1.526 (0.982–2.048)
	2019 (Tak)	1.877 (1.402–2.337)
	2021 (Lop Buri)	5.259 (3.554–9.019)
	2022 (Sa Kaeo)	7.530 (5.772–10.645)
	2022 (Loei)	10.466 (7.909–15.650)
Chlorfenapyr 10% SC	2019 (Kanchanaburi)	2.086 (1.268–3.450)
	2019 (Tak)	2.049 (1.243–3.360)
	2021 (Lop Buri)	7.056 (6.120–8.122)
	2022 (Sa Kaeo)	7.733 (6.714–8.915)
	2022 (Loei)	8.874 (7.669–10.284)

Reference: Thirawut et al. (2023) *CABI Agriculture and Bioscience* 4: 19.
https://doi.org/10.1186/s43170-023-00160-8
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