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Status of phosphine resistance of *Tribolium castaneum* in Indonesia

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Abstract

Storage period is the most vulnerable stage in post-harvest systems of all agricultural products. Fumigation using phosphine has long been an option in managing stored-product insect pests during the storage period. The long-term use of phosphine can lead to the development of resistant strains of stored-product insects. This study aimed to determine the distribution and resistance status of *Tribolium castaneum* in Indonesia. Samples of *T. castaneum* were obtained from various food and feed warehouses in 33 cities from 13 provinces in Indonesia which routinely conduct fumigation. The study was conducted using the FAO method for resistance test of stored-product insect pests. It was found that resistance cases had been spread out in various cities in Indonesia, and almost all *T. castaneum* samples collected had shown their resistance against phosphine. As many as 24 samples of *T. castaneum* collected from 24 cities in 11 different provinces (75% from total samples) had shown their resistance to phosphine with resistance factors (RF) ranging from 1.2 – 350.7 times. There were 8 cities from 6 provinces that had not experienced resistance to phosphine. The province with the lowest RF value was East Kalimantan at 0.6.

Keywords: Distribution, Phosphine, Resistance, *Tribolium castaneum*

Introduction

Storage period is the most vulnerable stage in post-harvest systems of all agricultural products. Insect pest attacks during the storage period can cause losses in quantity and quality of commodities. The magnitude of stored-product losses depends on the insect species involved, storage duration, storage facilities, and the pest control method implemented. Management of stored-product insects is most commonly carried out by fumigation.

Methyl bromide and phosphine are two fumigants that are commonly applied. Phosphine has been widely used as a fumigant for the control of stored-product insects for almost half a century (Price and Mills, 1988; Chaudhry, 2000). Since 1935, when phosphine gas was used for the first time as a fumigant, this gas has been used widely as a standard fumigant to manage insect pests in food storage facilities all over the world (Reichmuth, 1994; Chaudhry, 2000; Nath et al., 2011). Currently, phosphine is the main fumigant because methyl bromide has been banned due to its ozone-depleting capacity (Waterford et al., 1994). The use of phosphine to fumigate cereal grain in Australia has reached 80% (Cao and Wang, 2001). Meanwhile in China, it has reached up to 90% (Ling et al., 2004).

In Indonesia, phosphine has also been used intensively. Phosphine has been chosen as the main alternative to methyl bromide because it has ideal characteristics as a fumigant, such as ease of application, no residues left in commodities, inexpensive, high penetration into any commodities and packaging materials, quick distribution throughout fumigation enclosures, and no impact on seed viability (Taylor, 1989; Liang, 1989, 1994; Afbh and Aciar, 1991; Chaudhry, 2000; Cao and Wang, 2001; Nath et al., 2011, Prijono, 2006; Nayak, 2012). These characteristics have allowed phosphine to play a critical role in stored-product pest management. Using phosphine as the main fumigant for warehouse treatment was supported by the Montreal Protocol which decided to phase out methyl bromide in 2015.

Repeated applications of phosphine in poorly sealed warehouses have been cited as the cause of the resistance development (Chaudry, 2000). Control failures by phosphine treatment have been reported in some countries (Taylor, 1989; Collins et al., 2002). Based on its survey in 1972-73, the Food and Agricultural Organization (FAO) reported the resistance of stored-product insects to phosphine in 33 out of 82 countries sampled (Champ and Dyte, 1976). In Indonesia, although studies on insect pests of stored products resistant to phosphine have been conducted since 2011 by Entomology Laboratory, SEAMEO BIOTROP, information about phosphine resistance of stored-product insect pests is still very limited. The insect samples for this study were collected from several provinces. This research aimed to monitor the resistance level of stored-product insects in Indonesia to phosphine, so that resistance management can be developed to minimize the problems.

Materials and methods

Insects

Between 2011 and 2018, a total of 33 samples containing *Tribolium castaneum* from 32 cities in 12 different provinces in Indonesia were collected by visiting food and feed storages in survey locations. In each visit, insect pests found in these facilities were collected manually using small paintbrushes and aspirators. Then, the insect samples were taken to the Entomology Laboratory in Bogor and used for resistance tests.

Resistance testing method

The test insects used were the first progeny (F1) of the collected insects, and the phosphine gas used was extracted from phosphine tablets (AlP) using H_2SO_4 10%. Extraction of phosphine gas was conducted using the apparatus for generating phosphine based on the FAO method (FAO 1975). Test insects of 50 adult beetles were introduced into a PVC insect cage (diameter and height of 2.5 cm) covered at both sides by a thin muslin cloth. Then, 2 such insect cages were placed on a wire mesh hung in the middle of a glass jar. Thus, each experimental unit was composed of one glass jar containing two insect cages. The lid of each glass jar had a hole for injecting phosphine gas, and this hole was plugged with a rubber stopper. The rubber stopper was then sealed with plasticine after phosphine gas was injected into the jar in order to prevent phosphine gas leakage.

Phosphine gas extracted from AlP (FAO 1975) was injected into the jars using a syringe at respective concentrations of 0.000, 0.005, 0.014, 0.023, 0.031, and 0.040 mg/L. A magnetic stirrer was used to stir the gas in the jars. Fumigation was conducted for 20 h exposure. After fumigation was completed, the test insects were taken out from the jars and put into new jars containing their appropriate feed. The test insects were kept in these respective jars for 14 d before their mortality was observed. If the test insects were still alive, there was indication of a resistance factor of more than 1. After that, a confirmation test was conducted by re-fumigation of those strains for 48 h. This test was intended to confirm that the insects were resistant to phosphine.

Data analysis

Mortality of the tested insects was observed 14 d after fumigation, and the data were analyzed using Probit Analysis (Polo PC) to obtain LC_{50} and $LC_{99.9}$ values from each tested insect sample. Those LC_{50} and $LC_{99.9}$ were then compared with Discriminating Concentration from FAO Manual No. 23 (FAO 1975) to obtain the resistance level. Resistance Factor (RF) was calculated using the formula:

$$RF = LC_{99.9} \text{ of test insect/Discriminating Concentration}$$

If the value of $LC_{99.9}$ was more than the discriminating concentration, the insects tested were considered resistant and were confirmed with a 48 h test. After the confirmation test, if RF > 1, then the insects tested were confirmed as resistant strains to phosphine. However, if RF ≤1 after the confirmation test, then the resistance of these tested insects could not be determined and another round of tests was conducted.

Results and discussion

A total of 32 samples containing T. castaneum was collected from 12 provinces in Indonesia. The 12 provinces were: Bali, Banten, Central Java, East Borneo, East Java, Lampung, North Sulawesi, South Sulawesi, South Sumatera, West Java, West Nusa Tenggara, and West Sumatera. These provinces represent the big islands in Indonesia which include Java, Sulawesi, Sumatera, and Borneo, as well as the small islands including Bali and Nusa Tenggara. The test results from Borneo Island showed that T. castaneum samples from the East Borneo Province did not yet have resistance with an RF value of only 0.6-fold (Table 1). Overall, insect resistance had been distributed amongst almost all of the big islands in Indonesia including Java, Sulawesi, and Sumatera (Fig. 1).

More than 75% of the *T. castaneum* studied in this research showed their resistance against phosphine. The resistance levels varied with an RF value of 1.2 - 350.7 folds. A significant difference in resistance level from one city to another within the same province occurred in Banten Province with an RF value of 1 - 350.7 folds. This significant difference in resistance levels was thought to have occurred due to ineffective fumigation practices. Poor fumigation techniques (such as maintaining the fumigation chamber at a low air-tightness) could be one of the reasons resulting in fumigation failure and triggering the development of insect-resistant strains. According to Benhalima et al. (2004) and Collins et al. (2005), an insect population that has been under high selection pressure for many years may result in low mortality at the discriminating concentration.

Table 1. Probit analysis of 32 *Tribolium castaneum* assays using the FAO recommended method.

No.	City	Province	DC[a] (mg/L)	LC_{50}[a] (mg/L)	$LC_{99.9}$[a] (mg/L)	RF[b]	Confirmation
1	Balikpapan	East Borneo	0.040	0.010	0.020	0.600	Susceptible
2	Pare-pare	South Sulawesi	0.040	0.007	0.029	0.730	Susceptible
3	Wajo	South Sulawesi	0.040	0.008	0.032	0.800	Susceptible
4	East Lombok	West Nusa Tenggara	0.040	0.002	0.037	0.930	Susceptible
5	Lapadde	South Sulawesi	0.040	0.010	0.039	0.980	Susceptible
6	Surabaya	East Java	0.040	0.010	0.040	1.000	Susceptible
7	Jatake	Banten	0.040	0.020	0.040	1.000	Susceptible
8	Padang	West Sumatera	0.040	0.005	0.040	1.000	Susceptible
9	Tanette	South Sulawesi	0.040	0.006	0.048	1.200	Resistant
10	Bitung	North Sulawesi	0.040	0.004	0.049	1.220	Resistant
11	Makassar	South Sulawesi	0.040	0.013	0.050	1.250	Resistant
12	Mataram	West Nusa Tenggara	0.040	0.001	0.051	1.280	Resistant
13	East Lombok	West Nusa Tenggara	0.040	0.006	0.054	1.350	Resistant
14	South Padang	West Sumatera	0.040	0.009	0.062	1.550	Resistant
15	Sidrap	South Sulawesi	0.040	0.006	0.095	2.360	Resistant
16	Palembang	South Sumatera	0.040	0.006	0.106	2.650	Resistant
17	Painan	West Sumatera	0.040	0.011	0.112	2.800	Resistant
18	Tegal	Central Java	0.040	0.013	0.113	2.825	Resistant
19	Badung	Bali	0.040	0.009	0.113	2.830	Resistant
20	Central Lombok	West Nusa Tenggara	0.040	0.013	0.127	3.180	Resistant
21	Semarang	Central Java	0.040	0.004	0.131	3.275	Resistant
22	Panakkukang	South Sulawesi	0.040	0.010	0.142	3.550	Resistant
23	Lampung	Lampung	0.040	0.018	0.160	4.000	Resistant
24	Medan	North Sumatera	0.040	0.020	0.160	4.000	Resistant
25	Probolinggo	East Java	0.040	0.004	0.219	5.475	Resistant
26	Kotamobagu	North Sulawesi	0.040	0.013	0.302	7.560	Resistant
27	Indramayu	West Java	0.040	0.024	0.340	8.500	Resistant
28	Tabanan	Bali	0.040	0.030	0.566	14.150	Resistant
29	Serang 1	Banten	0.040	0.010	0.580	14.550	Resistant
30	Manado	North Sulawesi	0.040	0.014	0.932	23.300	Resistant
31	Bogor	West Java	0.040	0.070	1.020	25.450	Resistant
32	Serang 2	Banten	0.040	0.030	14.030	350.700	Resistant

DC[a] = Discriminating Concentration; LC[a] = Lethal Concentration; RF[b] = Resistance Factor (FAO 1980).

Resistant and non-resistant strains found within the same province also occurred in East Java, South Sulawesi, West Nusa Tenggara, and West Sumatera Province with an average RF value below 5 folds. Meanwhile, several areas had an RF value of more than 20 folds, namely Manado in North Sulawesi (23.3 folds), and Bogor in West Java (25.45 folds). All samples obtained from cities in these two provinces having resistance, had also shown to spread evenly. Apart from being triggered by inappropriate fumigation practices, the spread of insect-resistant strains could also be triggered by inter-city and provincial commodity shipping activities. The movement of resistant strains of insects carried in shipping commodities between cities and provinces is possible. The movement of insects due to the commodity trade is one of the factors that can influence the evolution of phosphine resistance (Benhalima et al., 2004).

Fig. 1. Distribution of insect strains resistant to phosphine in Indonesia: resistant strain (red); susceptible strain (blue).

Most of the insect samples used in this study were collected from rice, which is the main food commodity in Indonesia. This commodity has a high distribution potential between provinces as not all regions can independently meet their rice needs. Therefore, the development of *T. castaneum* resistance to phosphine in Indonesia might be caused by local selection and/or broad dispersal of the resistant population by the distribution of rice or the grain trade. Commodity distribution and poor fumigation application are most likely the major forces driving the development of resistant strains and phosphine resistance spread. Based on these results, it is necessary to evaluate commodity distribution activities, improve fumigation techniques, and look for alternative techniques or other fumigants to control resistant strains.

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