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Research Article

Resistance of *Ahasverus advena* and *Cryptolestes ferrugineus* to Phosphine on Imported Cocoa Beans from Cameroon, Ivory Coast, and Dominican Republic

Resistensi Ahasverus advena *dan* Cryptolestes ferrugineus *terhadap Fosfin pada Biji Kakao Impor dari Kamerun, Pantai Gading, dan Republik Dominica*

Franciskus Parasian[1)*], Y. Andi Trisyono[2)], & Edhi Martono[2)]

[1)]*Agriculture Quarantine Agency of Tanjung Priok*
Jln. Enggano No. 17 Tanjung Priok, Jakarta Utara 14310

[2)]*Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada*
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281

**Corresponding author. E-mail: parasian1986@gmail.com*

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ABSTRACT

Ahasverus advena and *Cryptolestes ferrugineus* were the most frequent pests found on cocoa beans in consignment. Their high infestation could contaminate cocoa beans and put the impact on its quality and economic value. Phosphine is one of the most commonly used fumigant in fumigation treatment to control the pest. The resistance status of *A. advena* and *C. ferrugineus* carried by cocoa beans from abroad to Indonesia against phosphine has not been reported. The purpose of this research was to determine the resistance of *A. advena* and *C. ferrugineus* to phosphine in the imported cocoa beans. The insects were collected from cocoa beans in consignment from Cameroon, Ivory Coast, Dominican Republic, and storing warehouse in Bandung, Indonesia. *C. ferrugineus* from Bogor (SEAMEO BIOTROP) and *A. advena* from Cianjur (PT IGE), Indonesia were utilized as the reference populations. Resistance assay was conducted based on Food Agriculture Organization's standard method. The resistance testing consisted of six phosphine concentrations: 0 (control), 0.005, 0.014, 0.023, 0.031, and 0.040 mg/l for 20 and 48 hours. The resistance classification testing was carried out with concentration 0.25 mg/l for 48 hours. *A. advena* originating from Cameroon, Ivory Coast, Dominican Republic and Bandung were susceptible to phosphine. *C. ferrugineus* coming from Cameroon, Ivory Coast (San Pedro and Abidjan) and Bandung were resistant to phosphine, while Dominican Republic's population remained susceptible to phosphine. *C. ferrugineus* from Cameroon, Ivory Coast (San Pedro and Abidjan) categorized into strong resistance, while the Bandung population was weakly resistant.

Keywords: *Ahasverus advena*, cocoa, *Cryptolestes ferrugineus*, phosphine, resistance

INTISARI

Ahasverus advena *dan* Cryptolestes ferrugineus *adalah hama yang sering ditemukan pada biji kakao di dalam petikemas. Infestasi hama ini dalam jumlah yang tinggi bisa mencemari biji kakao dan berdampak pada kualitas dan nilai ekonominya. Fosfin merupakan salah satu fumigan yang sering digunakan dalam perlakuan fumigasi untuk mengendalikan hama tersebut. Status resistensi* A. advena *dan* C. ferrugineus *yang terbawa biji kakao dari luar negeri ke Indonesia terhadap fosfin belum dilaporkan. Tujuan penelitian ini adalah untuk menentukan resistensi* A. advena *dan* C. ferrugineus *yang terbawa biji kakao impor terhadap fosfin. Serangga uji diambil dari biji kakao di dalam kontainer yang berasal dari Kamerun, Pantai Gading, Republik Dominica, dan gudang penyimpanan di Bandung, Indonesia.* C. ferrugineus *yang berasal dari Bogor (SEAMEO BIOTROP) dan* A. advena *dari Cianjur* (*PT IGE*), *Indonesia digunakan sebagai populasi referensi. Pengujian resistensi berdasarkan metode standar* Food Agriculture Organization. *Pengujian resistensi terdiri dari 6 (enam) konsentrasi fosfin yaitu 0 (kontrol); 0,005; 0,014; 0,023; 0,031; dan 0,040 mg/l selama 20 dan 48 jam. Pengujian klasifikasi resistensi dengan konsentrasi 0,25 mg/l selama 48 jam.* A. advena *yang berasal dari Kamerun, Pantai Gading, Republik Dominica dan Bandung rentan terhadap fosfin.* C. ferrugineus *yang berasal dari Kamerun, Pantai Gading (San Pedro dan Abidjan) dan Bandung resisten terhadap fosfin, sedangkan populasi asal Republik Dominica tetap rentan terhadap fosfin.* C. ferrugineus *yang berasal dari Kamerun, Pantai Gading (San Pedro dan Abidjan) tergolong resisten yang kuat* (strong resistant), *sedangkan populasi dari Bandung resisten yang lemah* (weak resistant).

Kata kunci: Ahasverus advena, Cryptolestes ferrugineus, *fosfin, kakao, resistensi*

INTRODUCTION

Indonesia is the third largest cocoa producing country in the world after Ivory Coast and Ghana. However, Indonesia still import cocoa beans to fulfill the need of industrial raw materials. Total of the imported cocoa beans in 2015 was about 51,620.4 tonnes which frequently originated from Cameroon, Ivory Coast, and Dominican Republic (Barantan, 2016). The imported cocoa beans have a potential to carry pests either which has not been found or already present in Indonesia. The carried pests in consignment may endanger Indonesia's agricultural products.

Ahasverus advena (Coleoptera: Silvanidae) and *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) were the most frequent pests found in cocoa beans (Cranham, 1960; Bateman, 2009), and it was confirmed that these pests were commonly discovered on cocoa beans in consignment coming to Indonesia (Barantan, 2016). In spite of they were considered as secondary pests, their high infestation may contaminate cocoa beans and leave their impact on its quality and economic value.

Pest infestation on agricultural commodities should be treated before they are exported to Indonesia. One of the treatments is by fumigation. Presently, phosphine (PH_3) is more frequently used as a fumigant rather than methyl bromide (CH_3Br) since it is more eco-friendly. Its application dose to treat cocoa beans in Asia is 1.5 g/m^3 or 2 g/ton (ACIAR, 1991). Until now, phosphine is still effective in controlling several insect pests. However, many reports stated that some species of insect pests had become resistant to phosphine. Klementz and Reichmuth (2007) in Jerman reported that *C. ferrugineus* collected from imported cocoa beans from South Africa could survive after fumigation with phosphine applied at 6 g/m^3 which was higher than the recommended dose. Resistance of *Araecerus fasciculatus* and *Tribolium castaneum* on cocoa beans to phosphine was reported occurring in storage warehouse of cocoa beans in South Sulawesi (Widayanti, 2016). Further development of *C. ferrugineus* resistance to phosphine was recognized as main problem of stored wheat in China (Xiaoping *et al*., 2007) and the highest resistance level to phosphine in *C. ferrugineus* in large bulk storages in Australia posed a serious threat to the biosecurity of Australian grain. The highest resistance level of *C. ferrugineus* detected in stored wheat in Australia was 875 times (Nayak *et al*., 2010).

High inter-country trade activity on cocoa beans increases the risk of getting insects resistant to phosphine. Introduction of resistant insects could affect the resistance level of the same species in Indonesia when they produce progeny. The crossing between susceptible and resistant insects could generate both the strong and weak resistant generations depending on the mode of inheritance (Collin *et al*., 2000). Currently *C. ferrugineus* and *A. advena* resistance carried by cocoa beans from abroad to phosphine has not been studied. The aim of this research was to determine the resistance status of *A. advena* and *C. ferrugineus* carried by imported cocoa beans to phosphine. A population from storing warehouse of cocoa beans in Bandung, Indonesia was included for comparison.

MATERIALS AND METHODS

Sampling of Tested Insects

A. advena and *C. ferrugineus* were collected from consignments containing imported cocoa beans in Tanjung Priok, Jakarta and Tanjung Perak, Surabaya, Indonesia seaports. Such consignments were selected based on the document of quarantine requirement stating that the commodity had been fumigated in the origin countries. Consignments arriving in Indonesia from each country vary. The time it takes the container to arrive in Indonesia varies from 38−50 days, but consignments contained in one document will arrive simultaneously in one ship. During the trip the container is not opened or unloaded, it is shown based on the same seal number and good seal condition. In addition, the tested insects were also sampled from private storing warehouse in Bandung which frequently imported cocoa beans. The length of storage of imported cocoa beans in storage warehouses ranged from 3−5 months. Adults were directly collected using smooth brush and aspirator from packaged or bulked cocoa beans. As many as 300 adults were sampled from the seaports, and brought to the laboratory for identification and rearing.

Preparation of Tested Insects

Three populations of *A. advena* were collected from cocoa beans coming from Ivory Coast (San Pedro), Dominican Republic, and Bandung. Five populations of *C. ferrugineus* came from Cameroon, Ivory Coast (Abidjan and San Pedro), Dominican Republic, and Bandung. The reference populations of *C. ferrugineus* was from Bogor (SEAMEO BIOTROP) and *A. advena* was orriginally collected from non-fumigated cocoa beans-storing warehouse in Cianjur (PT IGE), Indonesia and these two insects have been reared in the laboratory.

Mass Rearing of Tested Insects

Adults of *A. advena* were reared in plastic jars (in volume 1 L) containing 500 g artificial diet (40% of roaled oat, 40% of wheat grain and 20% of dry yeast); while *C. ferrugineus* were fed with other composition artificial media (95% of barley powder, 4.5% of wheat grain and 0.5% of dry yeast) under the temperature of 30 ± 2°C and relative humidity of 70 ± 5%. This first generation of each population was used for bioassays.

Resistance Assay

Resistance assay was carried out according to recommended method of FAO (Busvine, 1980). The method is based on exposure of adult insects to discrete atmospheres containing fumigant. Exposure periods are 20 and 48 hours for phosphine. Responses are determined 14 days following termination of the exposure. The experiments consisted of six concentrations of phosphine (0 as control, 0.005, 0.014, 0.023, 0.031 and 0.040 mg/L). Each concentration was repeated three times with 50 insects per each replication. Phosphine (SHENPHOS, aluminium phosphide 56%) was obtained from SEAMEO BIOTROP. Phosphine at given concentration was injected into a glass jar (2 L in volume) which had the tested insects using gastight syringe. For control, the tested insects were equally treated in a glass jar without any phosphine injection. The fumigation was performed for 20 and 48 h for the confirmation test. After fumigation, the tested insects were then transferred into plastic glass (100 mL in volume) and provided with similar artificial diet for rearing. The observation on mortality of the tested insect was conducted at the 14th day after treatment (Busvine, 1980; Lorini *et al*., 2007).

Classification of the Resistant Insects

This assay was carried out to categorize the resistant level: weak and strong resistant. The confirmed resistant tested insects on the previous assay was further treated with the recommended dose of FAO of 0.25 mg/L for 48 h. This concentration was applied on 150 tested insects with three repititions. The observation of mortality was performed at the 14th day after treatment (Collin *et al*., 2002; Lorini *et al*., 2007; Emery *et al*., 2011), using similar bio-assay procedure mentioned above.

Data Analysis

Probit analysis using Polo Plus version 1.0 program (Robertson *et al*., 2003) was conducted to obtain LC_{50} and LC_{99} values of tested insects from each original country, and the reference populations. Those values were then compared with such values of the reference insects to calculate the resistance factor (RF). The resistance factor was calculated by using the following formula:

$$RF = \frac{LC_{50}\ \text{values for tested insects}}{LC_{50}\ \text{values for reference insects}}$$

Probit analysis were performed only for the populations that showed dose-mortality dependent phosphine with mortality spreaded from below to above 50%.

The populations of *A. advena* were considered resistant if the RF values were greater than 1 and LC_{99} values were more than 0.03 mg/L; while populations of *C. ferrugineus* were resistant if the RF values were greater than 1 and LC_{99} values were more than 0.05 mg/L (Collin *et al*., 2002; Nayak *et al*., 2012; Kocak *et al*., 2015; Duong *et al*., 2016). The survival insects at concentration of 0.25 mg/L were considered to have strong resistance.

In addition, Analysis variance ($P<0.05$) (SAS Institute. 2002) was performed to see differences in the mortality at 48 h. Least Significant Differences at 5% level was conducted if significant difference based on ANOVA was present.

RESULTS AND DISCUSSIONS

***Resistance of* A. advena**

The LC_{50} and LC_{99} values of phosphine on *A. advena* originating from Cianjur, Bandung and Dominican Republic for 48 h could not be determined since the mortality of tested insects at the two low concentrations (0.005 and 0.014 mg/L) was more than 50%. The values of LC_{50} and LC_{99} for Ivory Coast were 0.014 and 0.029 mg/L (Table 1) and the RF value were not calculated since the LC_{50} and LC_{99} values of the reference population could not be calculated. All *A. advena* from Cianjur, Bandung and Dominican Republic died when those populations were exposed to 0.023 mg/L. However, the Ivory Coast population only reach mortality of 99.33% when they were exposed even up to 0.04 mg/L (Table 2). The values of LC_{50} and LC_{99} for Ivory Coast populations were slightly higher and the mortality did not reach 100% when they were treated at 0.04 mg/L. These findings may suggest that the migration might have occured after fumigation rather than fumigation failure in the origin countries. In addition, continous phosphine monitoring from the Ivory Coast is needed since early shift in resistant development might have occur. Based on the mortality and LC_{99} values, all populations of *A. advena* were susceptible to phosphine. These tested populations were more susceptible than the populations tested in the United Kingdom (UK). *A. advena* populations in UK treated at concentration of 0.05 mg/L for 23 hours died all (Clifton *et al*., 1995). Bell *et al.* (1996) also reported that other UK populations reached 100% mortality when they were treated with a concentration of 0.1 mg/L for 96 h.

Resistance of **C. ferrugineus**

The RF and LC_{99} value of phosphine on *C. ferrugineus* from Dominican Republic were 1 time compared to the reference population and 0.044 mg/L, respectively. These results indicated that this population was susceptible. In contrast, the populations of *C. ferrugineus* collected from Bandung were resistant, as indicated by the RF value of 1.75 and LC_{99} of 0.093 mg/L (>0.05 mg/L). The LC_{50}, LC_{99} and RF value from Cameroon, Ivory Coast (San Pedro and Abidjan) at 20 and 48 h were not calculated

Table 1. Resistance of *Ahasverus advena* collected from three countries after being fumigated with phosphine

Origin	df	Slope (± SE)	LC_{50} (mg/L) (95% CI)	LC_{99} (mg/L) (95% CI)	χ^2
Exposure for 20 h					
Indonesia (Cianjur [reference insects])	-	-	<0.014*	-	-
Indonesia (Bandung)	-	-	<0.014*	-	-
Dominican Republic	3	9.69 (± 0.77)	0.014 (0.013−0.014)	0.024 (0.022−0.027)	1.10
Ivory Coast (San Pedro)	3	5.87 (± 0.34)	0.015 (0.011−0.020)	0.039 (0.027−0.123)	27.64
Exposure for 48 h					
Indonesia (Cianjur (reference insects))	-	-	<0.014*	-	-
Indonesia (Bandung)	-	-	<0.014*	-	-
Dominican Republic	-	-	<0.005*	-	-
Ivory Coast (San Pedro)	3	6.93 (± 0.49)	0.014 (0.011−0.016)	0.029 (0.023−0.055)	12.08

Remark: * : The LC values were not able to be analyzed because two the lowest concentration tested caused mortality > 50%
CI : *Confidence Interval*
Resistance Factor (RF) were not able to be calculated because the LC values of reference insects were not able to be analyzed

Table 2. Mortality of *Ahasverus advena* collected from three countries after being fumigated with phosphine for 48 h

Origin	Concentration (mg/L) [$\overline{X}$ ± SD (%)] [a]				
	0.005	0.014	0.023	0.031	0.04
Indonesia (Cianjur [reference insects])	46.67 ± 2.3 b	97.33 ± 1.2 a	100.00 ± 0.0 a	100.00 ± 0.0 a	100.00 ± 0.0 a
Indonesia (Bandung)	33.33 ± 3.1 b	96.00 ± 4.0 a	100.00 ± 0.0 a	100.00 ± 0.0 a	100.00 ± 0.0 a
Dominican Republic	68.00 ± 15.1 a	96.00 ± 4.0 a	100.00 ± 0.0 a	100.00 ± 0.0 a	100.00 ± 0.0 a
Ivory Coast (San Pedro)	34.00 ± 7.2 b	43.33 ± 1.2 b	98.67 ± 2.3 a	99.33 ± 1.2 a	99.33 ± 1.2 a

Remark: a : Means followed by similar letters in each column were not significantly different according to LSD test at 5%
$\overline{X}$: *Mean*; SD : *Standard Deviation*
Number of tested insects each concentration : 150

since mortality at the highest concentration (0.04 mg/L) did not reach 50% (Table 3). Considering the data of each individual concentration, mortality of *C. ferrugineus* from Cameroon and Ivory Coast (San Pedro and Abidjan) significantly lower than those from Dominican Republic, Bandung and the reference insects (Tabel 4). This suggested that the population of Cameroon and Ivory Coast (San Pedro and Abidjan) had some degree of resistant. Klementz and Reichmuth (2007) in Germany reported that *C. ferrugineus* collected from the imported cocoa beans from Africa could survive after fumigation at doses of 2, 4, and 6 g/m^3 for 60 h. These findings were also confirmed by Reichmuth *et al*. (2004) which documented that insects on imported cocoa beans from West Africa were resistant to phosphine. These previous studies proved that resistance had occurred on the populations of *C. ferrugineus* from Africa, primarily Cameroon, Ivory Coast (San Pedro and Abidjan). *C. ferrugineus* from Bandung was also resistant to phosphine. In the storage warehouse in Bandung not only save the

Table 3. Resistance of *Cryptolestes ferrugineus* collected from four countries after being fumigated with phosphine

Origin	df	Slope (± SE)	LC_{50} (mg/L) (95% CI)	LC_{99} (mg/L) (95% CI)	RF_{50}	χ^2
Exposure for 20 h						
Indonesia (Biotrop [Reference Insects])	3	5.15 (± 0.30)	0.016 (0.014–0.019)	0.047 (0.035–0.078)	-	9.80
Indonesia (Bandung)	3	3.93 (± 0.28)	0.027 (0.020–0.042)	0.105 (0.057–1.335)	1.68	23.91
Dominican Republic	3	3.51 (± 0.25)	0.020 (0.018–0.022)	0.091 (0.067–0.147)	1.25	3.46
Ivory Coast (San Pedro)	-	-	> 0.04*	-	-	-
Ivory Coast (Abidjan)	-	-	> 0.04*	-	-	-
Cameroon	-	-	> 0.04*	-	-	-
Exposure for 48 h						
Indonesia (Biotrop [Reference Insects])	3	4.34 (± 0.30)	0.013 (0.011–0.014)	0.046 (0.035–0.077)		6.45
Indonesia (Bandung)	3	3.68 (± 0.25)	0.022 (0.016–0.031)	0.093 (0.052–0.806)	1.69	22.05
Dominican Republic	3	4.47 (± 0.31)	0.013 (0.010–0.015)	0.044 (0.032–0.082)	1	8.67
Ivory Coast (San Pedro)	-	-	> 0.04*	-	-	-
Ivory Coast (Abidjan)	-	-	> 0.04*	-	-	-
Cameroon	-	-	> 0.04*	-	-	-

Remark: * : The LC value were not able to be analyzed due to mortality at the highest concentration (0.04 mg/L) was < 50%
CI : Confidence Interval; SE : Standard Error
RF_{50} : LC_{50} of the tested insects/ LC_{50} of the reference insects

Table 4. Mortality of *Cryptolestes ferrugineus* collected from four countries after being fumigated with phosphine for 48 h

Origin	Concentration (mg/L) [$\overline{X}$ ± SD (%)][a]				
	0.005	0.014	0.023	0.031	0.04
Indonesia (Biotrop [Reference Insects])	34.67 ± 4.2 a	47.33 ± 3.1 a	82.67 ± 2.3 a	95.33 ± 2.3 a	99.33 ± 1.2 a
Indonesia (Bandung)	14.67 ± 2.3 b	25.33 ± 7.6 b	38.00 ± 7.2 b	73.33 ± 9.0 b	90.00 ± 7.2 b
Dominican Republic	35.33 ± 3.1 a	47.33 ± 3.1 a	87.33 ± 2.3 a	94.00 ± 3.5 a	100.00 ± 0.0 a
Ivory Coast (San Pedro)	6.67 ± 2.3 c	13.33 ± 2.3 cd	17.33 ± 1.2 d	25.33 ± 2.3 d	29.33 ± 1.2 d
Ivory Coast (Abidjan)	14.67 ± 2.3 b	18.67 ± 1.2 c	25.33 ± 1.2 c	33.33 ± 2.3 c	43.33 ± 1.2 c
Cameroon	7.33 ± 1.2 c	10.00 ± 0.0 d	12.67 ± 2.3 d	16.00 ± 2.0 e	22.67 ± 3.1 e

Remark: a: Means followed by similar letter in each column were not significantly different according to LSD test at 5%
$\overline{X}$: Mean; SD: Standard Deviation
Number of tested insects each concentration: 150

Table 5. Response of *Cryptolestes ferrugineus* collected from four countries after being fumigated at the high concentration of 0.25 mg/L for 48 h

Origin	Number of insects	Mortality ($\overline{X}$± SD, %)[a]	Classification
Indonesia (Biotrop [Reference Insects])	450	100.00 ± 0.0 a	*Susceptible*
Indonesia (Bandung)	450	100.00 ± 0.0 a	*Weak Resistance*
Dominican Republic	450	100.00 ± 0.0 a	*Susceptible*
Ivory Coast (San Pedro)	450	24.22 ± 4.3 b	*Strong Resistance*
Ivory Coast (Abidjan)	450	26.00 ± 4.0 b	*Strong Resistance*
Cameroon	450	8.45 ± 3.3 c	*Strong Resistance*

Remark: [a] : Means followed by similar letters in each column were not significantly different according to LSD test at 5%

cocoa beans originating from the territory of Indonesia but also come from abroad especially from African countries (Cameroon, Ivory Coast, and Ghana). Cocoa beans from Africa has been stored ranged from 3−5 months. Therefore, this resistance development might be due to crossing over between resistant insects from imported cocoa beans and susceptible ones living in that warehouse. Such crossing could generate the strong and weak resistant strains (Collins *et al*., 2000). Weak resistant of *C. ferrugineus* might be dominantly controlled by one main gene, while at least two main genes were responsible for strong resistant trait in which those genes interacted each other and caused the high resistance level (Jagadeesan *et al*., 2015).

Classification of Resistance

Based on the application of phosphine at the high concentration of 0.25 mg/L for 48 h, *C. ferrugineus* from Cameroon and Ivory Coast (San Pedro and Abidjan) were classified into strong resistant, while Bandung population was classified as weak resistant. Discriminating concentration tests were used to provide an initial diagnosis of the likely phosphine resistance phenotype of each strain. The dose (0.25 mg/L) was used to separate weak-resistance from strong resistance insects. Survivors of this test are classified as having 'strong' resistance. (Daglish & Collins, 1999; Collins *et al*., 2002; Lorini *et al*., 2007; Emery *et al*., 2011). As much as 75% of the population of *C. ferrugineus* from Cameroon and Ivory Coast (San Pedro and Abidjan) were resistant to phosphine (Table 5). Furthermore, the Cameroon populations showed more resistant to phosphine than that of Ivory Coast (San Pedro and Abidjan). High resistant of *C. ferrugineus* on grain to phosphine was firstly reported in 1983 from Bangladesh (Mills, 1983) and China (Zeng, 1999). In Australia, *C. ferrugineus* on wheat was reported as strong resistant under phosphine exposure for 48 h with LC_{99} of 37.68 mg/L (Nayak *et al*., 2012).

CONCLUSION

The population of *A. advena* originating from Ivory Coast, Dominican Republic, and Bandung were susceptible to phosphine. The populations of *C. ferrugineus* originally coming from The Dominican Republic was also susceptible to phosphine. However, the populations of *C. ferrugineus* from Cameroon and Ivory Coast (San Pedro and Abidjan) developed high resistance to phosphine, while the population from Bandung was weak resistant to phosphine.

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